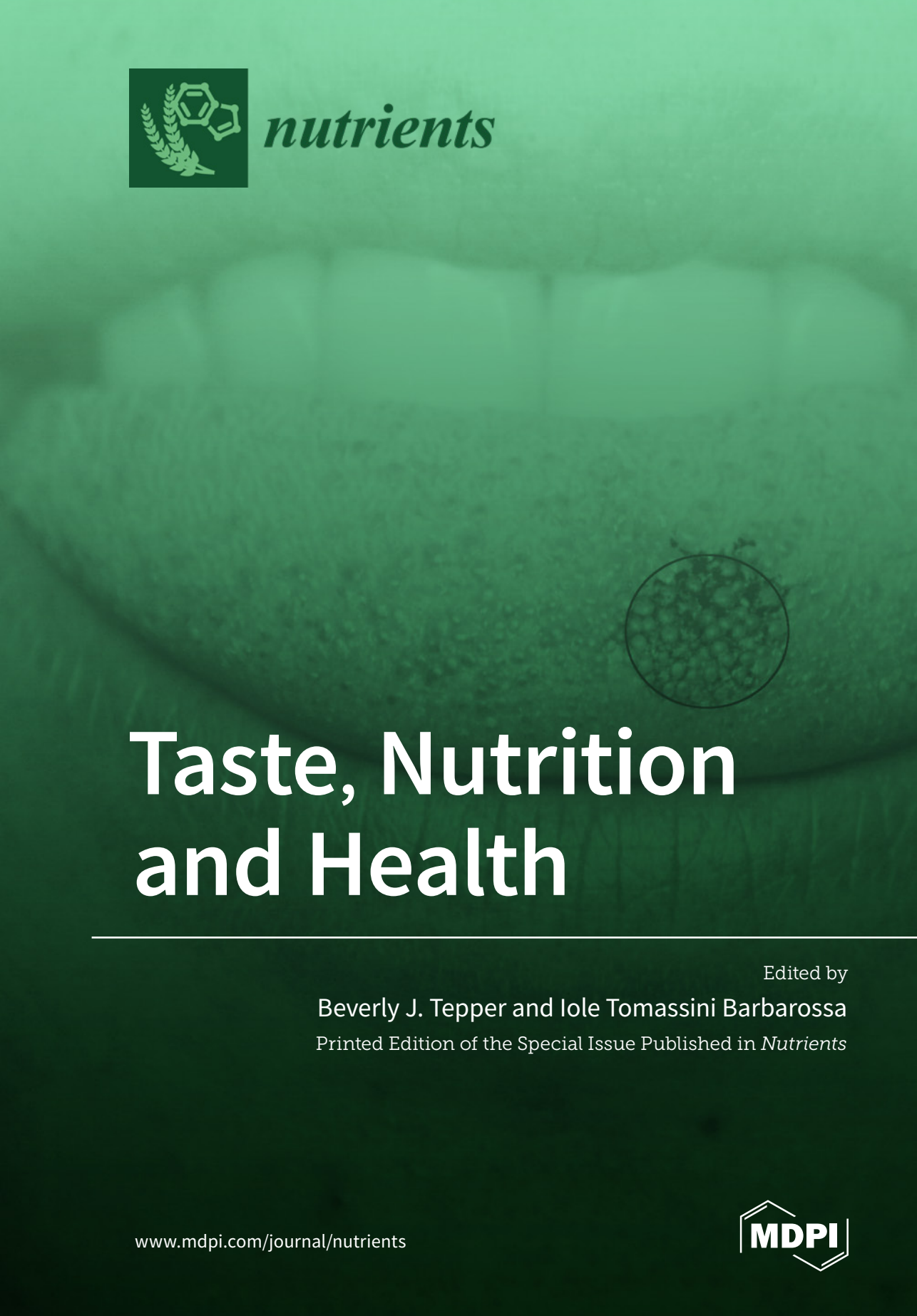

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Taste, Nutrition and Health

Edited by
Beverly J. Tepper and Iole Tomassini Barbarossa

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Cover image courtesy of Iole Tomassini Barbarossa.

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About the Special Issue Editors

Beverly J. Tepper Ph.D., is Professor of Sensory Science at the Department of Food Science, Rutgers, The State University of New Jersey, USA, where she directs the Sensory Evaluation Laboratory. Her research program combines food sensory science with nutritional science and psychology to better understand the links between taste, diet, and health. Specific research areas include the influence of genetic variation in taste perception on the pathways linking oral sensations to food preferences, diet selection, and body weight; the role of salivary proteins in sensory perception and oral health; the influence of personal traits on consumer behavior; and sensory evaluation and consumer testing of natural products and novel food ingredients and technologies. She is also the co-founder and director of the Center for Sensory Sciences & Innovation (CSSI) at Rutgers, where she conducts basic and applied research in partnership with the food industry. Dr. Tepper is a Fellow of the Institute of Food Technologists.

Iole Tomassini Barbarossa is a Full Professor of Physiology at the Department of Biomedical Sciences, University of Cagliari, Italy. During the last 10 years, she has built a strong and internationally recognized research profile, mainly due to her role as the principal investigator in multidisciplinary studies aimed at analyzing the physiology of the sense of taste and its role in food preferences, nutritional status, and human health. By integrating psychophysics, molecular biology, neurobiology, genetics, nutrition, and electrophysiology methods, these studies have focused on the identification of the physiological basis of individual taste variability; the relationships between taste sensitivity, food behavior, and nutritional status; and on modifications of taste perception. Recently, she designed and patented a new technique based on electrophysiological recordings of the bioelectric potentials generated in the taste cells of the human tongue by taste stimulation, thus providing a direct, objective, and quantitative measure of the peripheral taste function.

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Editorial

Taste, Nutrition, and Health

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Abstract: The sensation of flavour reflects the complex integration of aroma, taste, texture, and chemesthetic (oral and nasal irritation cues) from a food or food component. Flavour is a major determinant of food palatability—the extent to which a food is accepted or rejected—and can profoundly influence diet selection, nutrition, and health. Despite recent progress, there are still gaps in knowledge on how taste and flavour cues are detected at the periphery, conveyed by the brainstem to higher cortical levels and then interpreted as a conscious sensation. Taste signals are also projected to central feeding centers where they can regulate hunger and fullness. Individual differences in sensory perceptions are also well known and can arise from genetic variation, environmental causes, or a variety of metabolic diseases, such as obesity, metabolic syndrome, and cancer. Genetic taste/smell variation could predispose individuals to these same diseases. Recent findings have also opened new avenues of inquiry, suggesting that fatty acids and carbohydrates may provide nutrient-specific signals informing the gut and brain of the nature of the ingested nutrients. This special issue on "Taste, Nutrition, and Health" presents original research communications and comprehensive reviews on topics of broad interest to researchers and educators in sensory science, nutrition, physiology, public health, and health care.

1. Sweet Taste

Understanding the role of sweet taste in health and nutrition has been a major focus of chemosensory research for more than 50 years. Although significant strides have been made in this area, a complete understanding of the complex links between sweet taste perception, liking, and intake remains elusive. Tan and Tucker [1] reviewed the current state of knowledge in this area, concluding that current measures of sweet taste perception and liking may have limited capacity to predict dietary behaviours. The characterization of individuals as "sweet likers" or "sweet dislikers" has been a useful concept for understanding person-to-person differences in hedonic reactions to sweetness across a range of intensities. Building on their previous work, Iatridi, Hayes, and Yeomans [2] presented a new methodological approach for fine-tuning sweet-liker/-disliker classifications. These advances are taking place against a backdrop of escalating public health concerns about excess sugar in the diet and are reflected in current dietary guidelines in the United States [3] and many other countries across the globe [4], which now limit daily sugar consumption. To achieve the goal of sugar reduction at the population level, consumers would need to change their behaviours by making different diet choices, selecting sugar-reduced products, or a combination of these activities. Sugar reduction has been an ongoing focus of the food industry. Wee, Tan, and Forde's [5] study of 16 sweeteners provides an up-to-date and comprehensive guide for comparing the potencies of several classes of sweeteners to sucrose, the goal standard. Sweetener classes include, e.g., saccharides and polyols, non-nutritive synthetics (e.g., aspartame, sucralose), and non-nutritive naturals such as stevia.

2. Food Preferences/Individual Differences

Understanding individual differences in food preferences and eating behaviours has important implications for both food research and nutrition monitoring. Many of the contributions in this issue examine individual differences, from a variety of perspectives such as age, gender, culture/ethnicity, and genetic variation. For example, to gain insight into food preferences in a cross-cultural context, Wanich et al. [6] compared liking ratings for foods tasted in the laboratory to general liking responses obtained by questionnaire. Jilani et al. [7] studied a large European family cohort (>12,000 respondents) to establish the validity of a single instrument collecting food preference data from children, adolescents, and adults. The review by Keller et al. [8] presents a new conceptual model and fresh look at sex differences in eating behaviours in children. Two papers address the role of genetic variation in food preferences and choice. De Toffoli et al. [9] examined the interaction between PROP taste sensitivity (a marker for bitter taste) and psychological traits on the selection of astringent, polyphenol-rich foods, while the short review by Robino et al. [10] proposes that other genes and phenotypes (in addition to traditional taste-modifying genes) may play a role in food preferences.

3. Umami and Fat Taste

The role of other taste sensations in nutrition and health remains a vibrant and active area of research interest. Two contributions in this issue focus on fatty acid taste sensations. Sollai et al. [11] utilized a novel technique to measure electrophysiological responses from the gustatory cells of the human tongue following the direct application of oleic acid. They report strong associations between physiological signals and self-reports of fat taste sensations, demonstrating the reliability of this technique. Furthermore, Peterschmitt et al. [12] showed that direct lingual application of long-chain fatty acid to the circumvallate papillae of the mouse activated brain circuits involved in taste signaling, reward, and memory. Together, these studies reveal important features of the gustatory, peripheral, and central mechanisms involved in fat taste that are relevant to both animals and humans.

Finally, Hartley, Liem, and Keast [13] re-examine the notion that umami qualifies as a basic taste. They argue that umami meets most of the criteria for a basic taste—it is elicited by a distinct class of stimuli (e.g., L-glutamate), it activates specific receptor(s), (e.g., T1R1/T1R3), etc., but it does not generate a unique taste quality. They propose a new subclassification called "alimentary taste" for umami, and other taste qualities (such as fat) that may be more important signals for regulating postingestive metabolism than as sensory cues for the presence of specific nutrients in foods.

4. Disease States and Role of the Gut

Alterations in taste or smell are well-known features of a variety of metabolic diseases and pathological states. However, for many of these conditions, data from well-described clinical populations are scarce. In this issue, Singh et al. [14] present comprehensive findings on taste disruptions and oral complaints in patients with Sjögren's syndrome, an autoimmune disease affecting exocrine glands, such as the salivary glands, which results in dry mouth, burning mouth, and poor oral health. Importantly, this study included patients with Sjögren's syndrome, individuals with so-called "sicca" complaints who do not meet the diagnostic criteria for the disease (and are rarely studied), and healthy controls. There is also a critical need to develop food products that help patients with nutritional diseases to adhere to prescribed diets. Proserpio et al. [15] assessed the acceptability of different formulations of low-phenylalanine foods using a check-all-that-apply (CATA) methodology in individuals with phenylketonuria.

Obesity is increasingly characterized as an inflammatory disease arising from gut dysbiosis associated with an obesogenic diet. In the study by Bernard et al. [16], mice chronically fed a high-fat diet exhibited a blunted preference for sucrose that was partially corrected by supplementing the diet with a prebiotic (10% inulin-type fructan). Examination of caecal contents showed a greater abundance of beneficial bacteria in the diet-induced obese mice fed the prebiotic supplement. These

interesting findings suggest that prebiotic supplementation warrants more attention as an aid to the dietary management of obesity.

Lastly, taste receptors are expressed throughout the gastrointestinal tract and are known to release satiety hormones such as GLP-1, CCK, and PYY. In a single-blind, crossover trial, Klaassen et al. [17] delivered a tastant mixture via a naso-duodenal-ileal catheter to healthy participants and measured food intake and satiety from a subsequent meal. However, no differences in outcome measures were observed as a function of duodenal (proximal) or ileal (distal) infusions.

5. Lifestyle Factors

Two papers examine the extent to which lifestyle factors influence taste perception and food preferences in healthy individuals. Using fMRI, Gramling, Kapoulea, and Murphy [18] demonstrate that chronic caffeine consumers and nonconsumers experience differential activation in neuronal areas involved in reward, memory, and information processing when they are exposed to bitter and sweet tastants. Likewise, Feeney et al. [19] showed that in men, habitual physical activity selectively alters taste perceptions. Specifically, active men gave higher intensity ratings to sweet and umami solutions in comparison to nonactive men.

The study by Larsen et al. [20] examined the complex interrelationships between taste and diet in a cohort of chronic smokers who were also overweight or obese. Because obese smokers reportedly use smoking as a means of controlling their appetite and weight [21], gaining greater insights into taste changes and smoking-related dietary behaviors in this population may have important implications for treatment and prevention. Notably, participants also rated a liking for sweet e-juice, which is used to flavor e-cigarettes, a popular alternative to tobacco cigarettes. Using structural modeling, Larsen et al. [20] showed that taste (including e-juice liking) was associated with body mass index (BMI) in chronic smokers through liking of fats/carbohydrates and that smoking-related dietary behaviors (assessed by questionnaire) could influence BMI by a separate pathway. These novel findings could help to inform the development of new smoking intervention strategies.

6. New Product Formulations

This volume would not be complete without addressing consumer acceptance of new products and formulations designed to enhance health and wellbeing. Grapefruit is rich in vitamins, antioxidants, and anti-inflammatory compounds, but is rejected by many consumers due to its bitter taste. Gous et al. [22] developed 36 model grapefruit beverages varying in taste, aroma, flavor, and color to characterize their sensory profiles and to identify the formulations best-liked by consumers. Franks et al. [23] present unique findings showing that the type of water (tap, bottled, or deionized) used to brew tea influences sensory characteristics and nutrient extraction. Color, flavor, and epigallocatechin gallate (EGCG) extraction were higher for teas (especially green tea) made with purified water, but consumer liking was higher for less intensely flavored green tea made with tap water. These findings suggest that the consumer's choice of water source can maximize the flavor or health benefits of tea according to their personal preferences.

7. Olfaction

The determination of the odor detection threshold is a classic technique for assessing smell function, but such methodology is time-consuming and not well suited to diagnostic evaluation in the clinical setting or in the field with a large number of subjects. Using Sniffin' Sticks (odour-impregnated pens) and a Bayesian adaptive algorithm (QUEST protocol), Höchenberger and Ohla [24] established a rapid method with reduced testing duration and less variability between measurements.

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Review

Sweet Taste as a Predictor of Dietary Intake: A Systematic Review

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Abstract: Taste is frequently cited as an important factor in food choice, and while a number of studies have attempted to identify relationships between taste function and dietary intake, a systematic review of these studies has been lacking. This review identified studies that examined associations between taste function or taste perception and dietary intake. The purpose was to determine which taste measure was most closely associated with dietary intake in healthy adults. Studies that measured some component of dietary intake, either acutely or longer-term, were eligible for inclusion. Studies were grouped into three categories: those that measured sensitivity (thresholds), intensity, or hedonic responses to sweet stimuli. Sensitivity and intensity studies demonstrated little association with dietary intake measures. Hedonic measurements were more likely to be associated with dietary intake, especially if sweet likers were analyzed separately from sweet dislikers, but the degree of heterogeneity among stimulus concentrations and dietary measures as well as small sample sizes likely obscured more consistent relationships between hedonic evaluation and dietary intake. Due to the potential for within-day and between-day variability in both taste function and dietary intake, future work should explore obtaining more than one taste measurement before comparing results to longer-term dietary assessments and attempts to standardize methods.

Keywords: sweet taste; psychophysics; nutrition; diet; threshold; intensity; liking

1. Introduction

The sense of taste is commonly referred to as the "gatekeeper" of food intake [1]. This concept is supported by consumer surveys that report food choices are made primarily based on the flavor of the selected foods, with considerations about healthfulness or cost typically rated as less important [2]. Taste is an important component of the chemosensory attributes (taste, smell, chemesthesis or chemical irritation) that comprise flavor [3], and thus, guide food selection and intake. Dietary intake, in turn, influences nutritional status and body composition. Thus, individual differences in taste function and perception may lead to differences in dietary behaviors and risk of chronic disease [4].

Each taste quality has been associated with specific nutrients that are important to health and well-being. For example, sweet taste is commonly thought to help identify sources of carbohydrate, sour taste with the presence of vitamins, salty taste with essential electrolytes, and umami with protein [5]. Bitter taste likely serves as a warning against potentially dangerous compounds [5]. If these purported functions are accurate, then positive associations between taste function and/or preference for these taste qualities and related nutrient intake should exist.

Research regarding taste is typically concerned with one of two questions. First, how well does the system function? Sensitivity testing, which involves determining the absolute minimum concentration of a stimulus that can be reliably detected (detection threshold) or recognized (recognition threshold), is

frequently performed, but perceived intensity measurements of suprathreshold concentrations are also used. Threshold measurements can take several forms, but these tests usually involve presenting the participant with several samples – only one of which contains the stimulus of interest. The participant is required to identify the sample that contains the stimulus. A variety of approaches in terms of the number of samples to present and number of correct answers needed to stop the experiment exist [6]. Intensity measurements typically involve presenting a stimulus to the participant and asking for a rating of the intensity. Scales commonly used include a visual analog scale [7], a category scale [8], or a general Labeled Magnitude Scale [9]. The second question typically assesses a hedonic aspect, such as, how much is the stimulus liked, the preferred stimulus when a participant is asked to compare two or more stimuli of different concentrations, or the optimal stimulus concentration—often determined using an adjustment method where the participant increases or decreases the concentration of the taste quality. All of the taste measures just described are considered to be independent of each other, providing separate but complementary information about how the stimulus is detected and perceived [10].

When research is conducted on a specific taste quality, model stimuli, often consisting of a prototypical stimulus dissolved in deionized water, are typically used. For example, commonly used prototypical stimuli for sweet taste include sucrose or glucose solutions; whereas, sodium chloride solutions comprise the typical salty stimulus. Participants usually swish and then expectorate the liquid samples, but other approaches, including filter paper impregnated with stimuli [11], cotton swabs [12], edible wafers [13], or edible films [14] have been used. The simplicity of model systems allows for attention to be focused on the taste quality of interest with minimal distraction, but the obvious drawback of the model system is that it does not reflect the complex sensory experiences provided by foods and beverages. Thus, the question that arises is: how closely do taste test results using model systems correlate with dietary intake?

Given their simplicity but seemingly limited ecological validity [15], the ability of taste tests using model solutions to adequately predict dietary intake was previously considered limited [16,17]. However, few studies had adequately assessed intake when this question was first considered [16]. The question remains relevant, as recent work has examined how results from taste testing are associated with dietary intake. For example, the proposal of "fat" as another taste quality has led to renewed interest in connecting taste measurements to dietary intake and weight status (for a recent meta-analysis, see [18]). This suggests that relationships between taste measures and intake remain of interest to taste researchers.

In recent years, sugar intake has been proposed as a potential cause of the increasing prevalence of obesity globally [19,20]. The relationship is especially strong between intake of sugar-sweetened beverages and obesity [21]. As a result, recommendations that added sugar in habitual diets should not exceed 10% of total daily energy intake have been made by a number of governmental and non-governmental organizations including the United States Dietary Guidelines for Americans [22], the Australian Dietary Guidelines [23], and the World Health Organization [24]. Mechanistically, scientists posit that sugar consumption is driven by hedonics, i.e., its pleasant sweet taste, and evidence also suggests that sweet taste enhances the liking and wanting of sweet-tasting foods [25]. Some studies further demonstrated that sugar activates the opioid (e.g., nucleus accumbens) and dopaminergic (e.g., ventral tegmental area and right amygdala) reward centers in the brain [26,27], leading to the notion that sugar is 'addictive' and leads to excessive food intake and subsequent weight gain. Together, these mechanistic studies appear to suggest that sweet taste triggers food seeking behaviors and dietary intake. Although a number of individual studies have performed sweet taste testing using model systems and assessed associations with intake, to our knowledge, a systematic review summarizing these findings has not been undertaken. Therefore, the purpose of this review was to determine if psychophysical tests for sweet taste were associated with dietary intake and, if possible, to determine which test is the most closely associated with dietary intake.

2. Materials and Methods

A systematic literature search of the electronic databases PubMed, PsycInfo, Web of Science, and CINAHL was conducted. The search string used in PubMed was ("Taste" (Mesh)) AND ("Diet, Food, and Nutrition" (Mesh)); filters included Adult 19+, English, and Human. These filters were used in the other databases when available. Review articles that were identified were searched to identify articles that the searchers missed. Studies that recruited generally healthy individuals and collected at least one psychophysical measure of sweet taste and reported some sort of dietary intake measure, either acute or long-term were included. There was no restriction on adiposity, that is, all categories of body mass index were accepted. Studies were excluded if the populations were currently or had previously been ill, for example diabetes, alcoholism, or eating disorders; had known changes or deficits in chemosensory function, for example gastric bypass surgery patients; were pregnant; or were smokers. The review protocol was registered with PROSPERO, review #CRD42018111833.

After the initial searches were completed and duplicate entries removed, all potential studies were entered into a master database. Initial screenings by title and abstract were completed by the authors. In the case that a determination to include or exclude could not be made based on the abstract, the full paper was reviewed. The authors discussed questions about inclusion or exclusion until consensus was reached. The authors searched the reference lists of relevant articles to identify potential articles (n = 2) that were missed by the systematic search.

3. Results

In total, 3206 publications were identified and 17 were included in this review (Figure 1). Studies were placed into three categories based on psychophysical method utilized: (1) sensitivity measurements consisting of detection and recognition thresholds (n = 6), (2) intensity measures (n = 8), and (3) hedonic evaluations, namely liking and preference (n = 13). Some studies used more than one method; those that did were examined multiple times. Given the heterogeneity of psychophysical measures [10] and stimuli concentrations [28] as well as differences in stimuli tested (glucose vs. sucrose vs. non-nutritive sweeteners) [29] and dietary intake assessment methods [30], a meta-analysis could not be attempted.

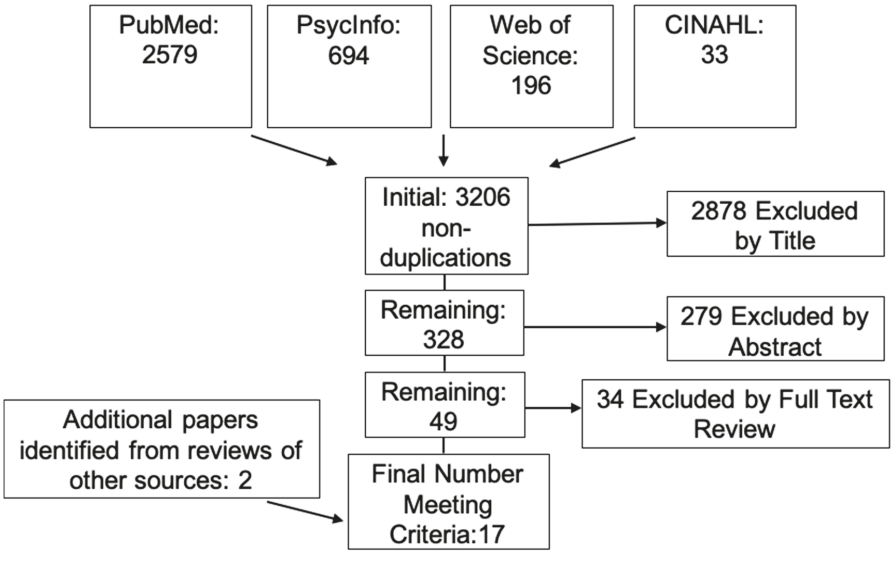

Figure 1. A total of 17 articles meeting the inclusion criteria were identified.

3.1. Sensitivity Testing

A total of six studies examined relationships between taste sensitivity and dietary intake [9,16,29,31–33] (Table 1). Studies varied in terms of the stimuli used, e.g., glucose vs. sucrose vs. non-nutritive sweeteners, the ranges of concentration tested, and the dietary assessment methods employed. Sensitivity was measured based on detection threshold [9,29,31,32], recognition threshold [9,16,29], and/or ability to correctly identify a 9 mM sucrose solution three times in a row using a triangle test [33]; individuals who could perform this task correctly were classified as "highly sensitive". Of the six studies identified, only two observed significant associations between sweet taste thresholds and dietary intake [32,33]. One of the studies (n = 30) was an acute experimental study that reported that individuals who were highly sensitive to a 9 mM sucrose solution consumed significantly less carbohydrate and more non-sweet foods, dietary protein, and protein as a percent of energy at an *ad libitum* feeding opportunity 60 min after exposure to either a sweet, non-sweet (umami), or "no-taste" soup [33]. The use of a 9 mM sucrose solution to establish sweet taste sensitivity is not an approach that was used by any other study in this review, and the validity of this approach has not been established. The second study (n = 56) reported that aspartame threshold was negatively associated with energy intake as assessed by a 7-day food diary [32]. However, the association was very weak, albeit statistically significant, and may have limited implications (beta coefficient = -0.003, $p < 0.0009$); no further association between sucrose threshold and any diet measures were observed. Another study examining non-nutritive sweetener thresholds did not identify diet-taste relationships [29]. Differences in diet assessment methods (FFQ [29] vs. 7-day food diaries [32]) could contribute to these disparate results.

To summarize, most available studies failed to observe a significant relationship between sweet sensitivity and dietary intake, suggesting that testing for sweet taste threshold is not likely to be predictive of dietary intake. The only studies that reported an association found that sweet-sensitive individuals consumed less carbohydrate and more non-sweet foods [33]. The methodological limitations and small samples sizes of these studies also limit the generalizability of the findings.

Table 1. Sensitivity Studies Examining Taste-Diet Relationships.

Author (Year)	Subjects	Taste Test	Sweet Stimuli	Stimuli Concentrations	Dietary Assessment Methods	Key Findings
Mattes (1985) [16]	n = 35 (17 M, 18 F) Age = 18–42 years old	RT	Sucrose	Serial half dilutions of sucrose: 1.2×10^{-5} M to 0.8 M	7-day diet record with predominant taste recorded	Sweet taste threshold and intensity did not correlate with sweet E, CHO, PRO and fat intake.
Martinez-Cordero (2015) [32]	n = 56 (30 M, 26 F) Age = 32.9 ± 7.9 years old	DT	Sucrose Aspartame	Sucrose—14 [ ] from 4.09×10^{-1} M to 1.63×10^{2} M Aspartame—14 [ ] from 0.82×10^{-3} M to 3.27×10^{-1} M Both at 0.2 log dilutions per successive solution	7-day food diaries	Aspartame threshold was negatively associated with E intake (B = −0.003 ± 0.001; $p < 0.0009$). No association between sucrose threshold and dietary intake.
Low (2016) [29]	n = 60 Age = 26.5 ± 1.0 years old	DT; RT	Glucose mono-hydrate Fructose Sucrose Sucralose Erithritol Rebaudio-side A	Varying concentrations for each	Validated FFQ; also assessed consumption of foods and/or beverages sweetened with high-intensity sweeteners	No association between threshold measures and dietary measures.
Smith (2016) [31]	n = 51 (9 M, 42 F) Age = 25 ± 8y	DT	Sucrose	2.1% w/v sucrose Quarter-log step dilutions	24-hour recall	No association between threshold measures and dietary intake.
Han (2017) [33]	n = 30 (16 M, 14 F) Age = 24–34 years old (M), 20–37 years old (F)	Sensi-tivity	Sucrose	9 mM	*Ad libitum* intake after soup preload (one sweet, one umami, one no-taste energy control)	Highly-sensitive consumed more non-sweet foods, PRO, %E from PRO, and %E from fat (after non-sweet soup only) ($p < 0.05$ for all). Highly-sensitive consumed less CHO as %E ($p = 0.02$).
Jayasinghe (2017) [9]	n = 42 (all F) Age = 28 ± 634 years old	DT; RT	Glucose	15, 30, 45, 60, 90, 120, 150, 180 mM	4-day weighed food record Sweet food FFQ Sweet beverage liking questionnaire	No association between threshold measures and dietary intake.

Abbreviations: [ ] concentration, CHO = carbohydrate, DT = detection threshold, E = energy, FFQ = food frequency questionnaire, F = female, M = male, PRO = protein, RT = recognition threshold, w/v = weight for volume.

3.2. Intensity Testing

Eight studies examined relationships between measures of sweet taste intensity and dietary intake [7,9,16,29,34–37] (Table 2). As with the sensitivity studies, stimuli and concentrations tested also varied widely. Only two of the ten studies observed significant relationships [9,29]. The first study (n = 42) reported negative associations between diet and intensity ratings for a 250 mM glucose stimulus [9]. Intensity was negatively correlated with total energy, carbohydrate (starch as well as total sugar, glucose, and fructose), but not sucrose intake. Sweet food intake was also negatively associated with intensity ratings of the 500 mM and 1000 mM samples. In this study, dietary intake was measured both by 4-day weighed food records as well as by an unvalidated sweet food FFQ and a sweet beverage liking questionnaire. The second study (n = 60) reported that intensity ratings for Rebaudioside A and sucralose, both non-nutritive sweeteners, were positively associated with mean total energy intake ($p < 0.01$ for both) [29]. No associations between intensity ratings and other dietary measures, including carbohydrate, sugar, or starch were observed, and no associations with the other sweet stimuli tested (glucose monohydrate, fructose, sucrose, or sucralose) were noted [29]. This study relied on the validated Cancer Council of Victoria Food Frequency Questionnaire [38] to assess dietary intake.

In conclusion, only two studies demonstrated the utility of sweet taste intensity ratings in reflecting dietary intake, and neither study used sucrose—a prototypical sweet taste stimulus. The negative association between sweet taste intensity rating of glucose and energy as well as carbohydrate intake was consistent with the findings from the sensitivity studies that also reported significant negative associations [9,29]. On the other hand, associations with non-nutritive sweeteners (Rebaudioside A and sucralose) were present but positively associated with dietary intake. Further study is needed to understand the underlying mechanisms that contribute to these distinct relationships.

Table 2. Intensity Studies Examining Taste-Diet Relationships.

Author (Year)	Subjects	Taste Test	Sweet Stimuli	Stimuli Concentrations	Dietary Assessment Methods	Key Findings
Mattes (1985) [16]	n = 35 (17 M, 18 F) Age = 18–42 years old	Intensity	Sucrose	5 concentrations ranging from 0.05 M to 0.80 M	7-day diet records	No association between intensity measures and dietary intake.
Holt (2000) [37]	n = 132, Australian 27 M, 42 F Malaysian 29 M, 34 F; Australian 22.8 ± 4.3 years old Malaysian 21.5 ± 1.2 years old	Intensity	Sucrose	2, 4, 8, 16 and 32% v/v	Separate FFQ for the Australian and Malaysian participants	No association between intensity measures and dietary intake.
Sartor (2011) [34]	n = 12 (7 M, 5 F) Age = 26 ± 6 years old	Intensity	Sucrose	0, −0.5, −0.75, −1, −1.25, −1.5, −1.75, −2, −2.25, −2.5, −2.75 log(sucrose) mol/L	14 diet diaries on random days	No association between intensity measures and dietary intake.
Cicerale (2012) [35]	n = 85 (89% F) Age = 21 ± 4 years old	Intensity	Sucrose	200 mM	Food & diet questionnaire Food variety survey 2 × 24-hour food diaries	No association between intensity and any diet measures.
Low (2016) [29]	n = 60 Age = 26.5 ± 1.0 years old (SEM)	Intensity	Glucose mono-hydrate Fructose Sucrose Sucralose Erithritol Rebaud-ioside A	Varying concentrations	Validated FFQ; also assessed consumption of foods and/or beverages sweetened with high-intensity sweeteners	Intensity and dietary intake associations varied by sweetener. Rebaudioside A and sucralose intensity ratings were positively associated with mean total E intake (p < 0.01 for both).
Stevenson (2016) [36]	n = 87 (38 M, 49 F) Age = 21 ± 3 years old (18–31 years old)	Intensity	Sucrose	0.03 M and 0.36 M	26-item Dietary Fat and Sugar questionnaire (DFS) designed to identify variation in saturated fat and added sugar intake	No association between intensity and any diet measures.
Jayasinghe (2017) [9]	n = 42 (all F) Age = 28 ± 6 years old	Intensity	Glucose	125, 250, 500, 1000 mM	4-day weighed food record Sweet food FFQ Sweet beverage liking questionnaire	Intensity at 250 mM or higher correlated negatively with total E, CHO (starch, total sugar, fructose, glucose) but not sucrose intake (p < 0.05 for all). Intensity also negatively associated with total sweet food intake (p < 0.05 for all).
Leong (2018) [7]	n = 100 (50 M, 50 F) Age = 25.7 ± 4.2 years old (M), 25.7 ± 5.1 years old (F)	Intensity	Sucrose	12.0% w/v	2 × 24-hour food recalls	No association between intensity and any diet measures.

Abbreviations: CHO = carbohydrate, E = energy, FFQ = food frequency questionnaire, F = female, M = male, v/v = volume for volume, w/v = weight for volume.

3.3. Hedonic Testing

A total of 13 papers examined relationships between hedonic evaluation and dietary intake [7–9, 16,28,31,34,36,37,39–42]. As before, the concentrations of sweet solution used in these studies varied considerably as did dietary assessment methods (Table 3). In contrast to the sensitivity and intensity studies, all but one [9] used sucrose. Hedonic measurements included determining the preferred concentration out of a range of stimuli [31] or through an adjustment task [16,42] or a rating of how much the stimulus was liked, typically using either a visual analog [7,28,37,40,41], labelled magnitude scale [9,34,36], or likert-style hedonic scales [8,39]. Five of the studies that measured hedonics also classified participants as sweet "likers" or "dislikers" [28,34,37,40,41]. A sweet liking phenotype has been associated with different hedonic responses to sweetness (for a recent review, see [37]), so failure to identify sweet liker phenotype could influence findings. That is, if the study population was comprised predominantly of sweet likers or dislikers, results could be skewed. Therefore, these studies are presented separately from the others. One study analyzed the data with and without sweet liker classification [37], so it is reported twice – both with those studies that did and did not identify sweet likers.

3.3.1. Studies that Determined Sweet Liking Phenotypes

Among the five studies that distinguished between sweet likers and dislikers, the classification methods used to determine sweet liker status varied greatly [28,34,37,40,41]. Classification was performed by hierarchical cluster analysis [28,41]; by preferred concentration cut-off, i.e., favorable ratings above a specific concentration [34,40]; a mean favorable rating over all concentrations tested [41]; and a pattern of increasing hedonic scores [37]. Among these six papers, three observed relationships between hedonics and dietary intake measures [28,37,40]. Among the studies demonstrating associations with sweet liker status and intake, one (n = 418) reported that energy intake from sugar-sweetened beverages was higher among likers compared to dislikers (p = 0.008) based on a beverage food frequency questionnaire [28]. A second study (n = 196) that examined sweet liker and PROP taster status combinations observed that individuals who were both sweet likers and PROP tasters reported consuming more energy from beverages and fiber as measured by two 24-h recalls [40]. The last study (n = 132) reported positive associations between the preferred level of sucrose and frequency of sweet food consumption, intake of refined sugars, and total sugars [37]. Two studies did not observe taste-diet relationships, but the reported sample sizes raise questions about the power of these studies to detect relationships (n = 12 (6 sweet likers) [34] and n = 36 (12 sweet likers)) [41]. Overall, sweet likers appear to consume more energy from sugar-sweetened beverages and more energy from refined and total sugars. It appears that identifying an individual's sweet liking phenotype may increase the likelihood that relationships between hedonic scores and dietary intake will be observed, especially if sample sizes are sufficiently large enough.

3.3.2. Studies that Did Not Determine Sweet Liking Phenotypes

Among the nine studies that did not classify sweet likers, associations between hedonic responses and intake were observed in five [9,16,31,37,42] but not in the other four [7,8,36,39] (Table 3). Preferred sweetness concentration was associated with greater total energy intake [31], carbohydrate intake [31,42], percent of sweet calories consumed [37,42], refined and total sugars [37], and frequency of carbohydrate-rich food selections [42], while one study observed positive associations with liking ratings of glucose at 500 mM and 1000 mM and total energy and carbohydrate (total sugar, fructose, glucose) but not starch and sucrose intake [9]. One study observed a negative association between preferred sweetness concentration and carbohydrate intake [16]. The studies finding associations between hedonic evaluations and dietary intake used one 24-h recall [31], 4-day weighed food records [9], and 7-day diet records [16,42]. Sample sizes for these studies ranged from n = 25 [42] to n = 51 [31]. Studies not observing associations reported sample sizes ranging from n = 17 [8] to n = 100 [7]. In summary, hedonic measures appear to be better correlated with dietary intake, and these relationships are strengthened when sweet likers are analyzed separately.

Table 3. Hedonic Studies Examining Taste-Diet Relationships.

Author (Year)	Subjects	Taste Test	Sweet Stimuli	Stimuli Concentrations	Dietary Assessment Methods	Key Findings
Weizenbaum (1980) [8]	n = 17 (5 M, 12 F) Age = 18.6 y (M), 19.7 years old (F)	Pleasantness	Sucrose	0.01, 0.023, 0.046, 0.1, 0.23, 0.46, 1.0 M	*Ad libitum* intake of salted peanuts and candies after testing	No relationship between pleasantness and amount of food consumed.
Mattes (1985) [16]	n = 35 (17 M, 18 F) Age = 18–42 years old	Preferred concentration of sweetness	Sucrose	Self-adjusted (dilution)	7-day diet records	Preferred concentration of sweet solution negatively correlated ($r = -0.36$, $p = 0.04$) with CHO intake.
Mattes (1986) [42]	n = 25 (all M) Age = 17–34 years old	Preferred concentration of sweetness using an adjustment task	Sucrose	0M & 1.0 M solutions were provided. Subjects modified the samples until the preferred sweetness was reached. Preferred sweetness levels from both the unsweetened and sweetened baseline stimuli were averaged.	7-day diet records	Mean preferred concentration was positively correlated with %CHO intake ($r = 0.637$, $p < 0.001$). Preferred concentration of the 1.0 M sucrose samples were positively correlated with %CHO intake ($r = 0.748$, $p < 0.001$), % sweet calorie intake ($r = 0.504$, $p < 0.001$), and frequency of selection of carbohydrate-rich foods ($r = 0.532$, $p < 0.01$).
Drewnowski (1999) [39]	n = 159 (all F) Age = 27.0 ± 0.7 years old (SEM)	Liking	Sucrose	5 [ ] ranging from 2% to 32% w/v	3-day food records; 171-item food preference checklist	No associations between liking and dietary intake measures, but higher hedonic ratings for sucrose were associated with higher ratings for sugar in tea and many sweet desserts.
Holt (2000) [37]	n = 132, separated into Australian-born Caucasian and Malaysian born; Australian: 27 M, 42 F Malaysian: 29 M, 34 F; Australian: 22.8 ± 4.3 years old Malaysian: 21.5 ± 1.2 years old	Liking	Sucrose	2, 4, 8, 16 and 32% v/v	Separate FFQs for the Australian and Malaysian subjects	Refined sugar intake was higher in sweet likers com-pared to dislikers. No other differences were observed. For all participants, positive associations between the preferred level of sucrose and frequency of sweet food consumption, intake of refined sugars, and total sugars were observed ($p < 0.05$).
Sartor (2011) [34]	n = 12 (7 M, 5 F) Age = 26 ± 6 years old	Pleasantness Preference	Sucrose	Pleasantness 11 [ ]: 0, −0.5, −0.75, −1, −1.25, −1.5, −1.75, −2, −2.25, −2.5, −2.75 log(sucrose) M Preference: 10 random presentations of pairs of 0, −0.5, −0.75, −1 and −1.25 log(sucrose) M	14 diet diaries on random days	No associations between taste measures and dietary intake.
Turner-McGrievy (2013) [40]	n = 196 (85% F) Age = 42.6 ± 11.0 years old	Liking	Sucrose	0.05, 0.10, 0.21, 0.42, and 0.83 M. Participants who liked the 0.83 M sucrose solution the best were classified as sweet likers	2 × 24-hour food recalls	Those who were sweet likers consumed more E from beverages and less fiber ($p < 0.05$).
Methven (2016) [41]	n = 36 (12 M, 23 F, 1 unknown) Age = 26 years old (median)	Liking	Sucrose	3%, 6%, 12%, 24%, 36%	FFQ used by EPIC	Intake did not differ between sweet likers and dislikers.
Smith (2016) [31]	n = 51 (9 M, 42 F) Age = 25 ± 8 years old	Preferred concentration of sweetness	Sucrose	2.1% w/v stock solution Quarter-log step dilutions	24-hour recall	Sweet preference after short-sleep was positively correlated with E intake ($r = 0.31$, $p = 0.043$) and CHO intake ($r = 0.32$, $p = 0.34$), but not after habitual sleep.
Stevenson (2016) [36]	n = 87 (38 M, 49 F) Age = 21 ± 3 years old (18–31 years old)	Liking	Sucrose	0.03 & 0.36 M	26-item Dietary Fat and Sugar questionnaire (DFS) designed to identify variation in saturated fat and added sugar intake.	No association between liking and any diet measures.

Table 3. *Cont.*

Author (Year)	Subjects	Taste Test	Sweet Stimuli	Stimuli Concentrations	Dietary Assessment Methods	Key Findings
Jayasinghe (2017) [9]	$n = 42$ (all F) Age = 28 ± 6 years old	Liking	Glucose	125, 250, 500, 1000 mM	4-day weighed food record; Sweet food FFQ; Sweet beverage liking questionnaire	Sweet taste liking at 500 mM or higher correlated positively with total E, CHO (total sugar, fructose, glucose) ($p < 0.05$ for all) but not starch and sucrose intake.
Garneau (2018) [28]	$n = 418$	Liking	Sucrose	5 [ ] ranging from 0% *w/v* to 13.7% *w/v*	Validated beverage FFQ (BEVQ-15)	Mean E intake from all beverages was higher among likers compared to neutrals ($p = 0.004$). Total E intake by dislikers did not differ from the other groups. E intake from sugar-sweetened beverages was higher among likers compared to dislikers ($p = 0.008$). Neutrals did not differ from the other groups.
Leong (2018) [7]	$n = 100$ (50 M, 50 F) Age = 25.7 ± 4.2 years old (M), 25.7 ± 5.1 years old (F)	Liking	Sucrose	12.0% *w/v*	2 × 24-hour food recalls	No association with liking and dietary intake.

Abbreviations: [ ] = concentration, CHO = carbohydrate, EPIC = European Prospective Investigation into Cancer and Nutrition study, E = energy, FFQ = food frequency questionnaire, F = female, M = male, w/v = weight for volume.

4. Discussion

The sensory properties of food, including taste, play an important role in food selection and intake [2]. Psychophysical studies exploring taste function and perception have sought to determine if responses obtained in these studies can be associated with dietary intake. Given the challenges of assessing dietary intake [43], a proxy measure that is a simple, quick, and reliable predictor of intake would be welcomed.

Of the taste testing methods used—sensitivity testing, intensity measures, or hedonic evaluation—hedonic ratings proved to be superior in their ability to correlate with dietary intake, although these studies also did not report consistent findings. The fact that sensitivity was not a reliable indicator of dietary intake was not unexpected, as others have noted that an individual's sensitivity to a taste quality often fails to predict intake since these exposures can be quite dissimilar to the suprathreshold exposures experienced while eating [16,44]. Intensity measures lacked predictive power as well. One study observed positive associations between dietary intake and hedonic evaluation but not with intensity [37]. Another study reported that intensity evaluations between sweet likers and dislikers did not differ [28]. These results further support the argument that measuring sensitivity, intensity, and hedonic responses provides distinct but complementary information about the taste sensations experienced by an individual [10], but that, based on the available data, hedonic evaluation may provide a more reliable indication of dietary intake.

Further, among the studies that classified sweet likers and dislikers, three of the five studies reported that sweet likers were more likely to demonstrate associations between dietary intake measures and hedonic evaluations. Sweet likers are typically classified by increasingly favorable hedonic responses to increasingly sweeter stimuli [45]. Thus, the positive associations between hedonic responses and intake of sugar sweetened beverages and sugar intake make intuitive sense. The two studies [34,41] that failed to see associations between hedonic responses and intake in sweet likers had small sample sizes of sweet likers ($n \leq 12$). Intriguingly, while the methods used to assess sweet liking phenotype differed, results were consistent across studies. This agrees with others who reported that among these methods, no single classification approach demonstrated superiority [45].

The differences in both taste and diet measurements likely contribute to the discrepancies reported. First, a discussion of the taste measurement differences. The stimuli and concentrations used will have a direct impact on results. While different nutritive sweeteners were noted to have detection and recognition thresholds as well as intensity scores that were correlated with each other, actual values differed [46]. This is unsurprising, as different sugars have different potencies; sucrose, for example, is sweeter than glucose at the same concentration [47]. Further, the human sweet receptor responds to many compounds besides mono- and disaccharides, including amino acids, proteins, and non-nutritive sweeteners [48]. Sucrose and glucose are presumed to be the best stimuli to correlate with dietary intake, but this has not been tested, and one study reported that the threshold for the non-nutritive sweetener aspartame was negatively associated with energy intake, unlike sucrose [32]. The concentrations of the sweet stimulus presented to a participant can also influence taste results. Smaller differences between successive concentrations will allow for more precise determination of the taste threshold, but additional trials add to participant burden and increase the risk of fatigue. There is no standardized procedure for determining the difference in concentration between one stimuli and the next. The range of concentrations presented to participants in order to determine sweet liker/disliker phenotypes also varied by study [28]. It is conceivable that some individuals could be classified as sweet likers with one set of concentrations and sweet dislikers if the concentrations presented were higher. This is especially true if sweet liker phenotype is determined by the response to one concentration. Thus, if individuals were misclassified, results could change.

In terms of dietary assessment, it is well known that self-reported dietary information is subject to over- and under-reporting [49]. Over- or under-reporting could obscure taste-diet relationships. In addition, due to the high degree of variability in intake from one day to the next, depending on the nutrient of interest, many days of intake in the form of diet diaries or records must be recorded [50].

For example, at minimum, two weeks of intake records are needed to estimate average energy intake in an individual, which is impractical for many studies, and accuracy declines over time [51]. This number falls to three days when estimating energy intake for groups of people [50]. Even with this reduction, dietary record keeping can be burdensome for participants [43] and items consumed can be poorly estimated or forgotten entirely.

There are two main approaches to reduce participant burden when assessing dietary intake. These include the 24-hour diet recall, where participants are asked to remember what they ate during the previous day rather than recording it as each food and beverage is consumed, or a food frequency questionnaire (FFQ) [43]. The 24-h recall allows dietary information to be recorded at one time point, but accurate information collection relies on trained staff and suffers from recall bias [43]. FFQs employ a checklist approach, where participants can indicate how much and/or how often they consume certain foods. The main drawback of this approach is that the ability to accurately remember and quantify intake is severely compromised [43]. While both approaches are valuable, diet diaries are considered to be more accurate measures [43].

Based on the studies examined, there was no clearly superior method of dietary assessment that was more likely to identify taste-diet relationships. For the sensitivity studies, among the studies observing relationships, one utilized an acute intake measurement, i.e., consumption following a pre-load [33], while the other used 7-day food diaries [32]. Studies not observing relationships between taste sensitivity and dietary intake relied on 4-day weighed food records [9], food frequency questionnaires [9,29], 24-hour recall [31], and 7-day food diaries with predominant taste recorded [16]. For intensity, studies that observed relationships between taste and diet used 4-day weighed food records as well as an unvalidated sweet food FFQ and a sweet beverage liking questionnaire [9] and a validated FFQ not used by any other of the studies included in this review [29]. Studies failing to find associations between intensity measures and diet used two 24-h food recalls [7], multiple (3–14) day diet records [16,34,35,39], *ad libitum* intake of specific test foods [8], and various food frequency questionnaires [35,36,39]. Studies measuring hedonic responses that observed associations used multiple day (3–7) food records [9,16,42], 24-hour recalls [31,40], and food frequency questionnaires [9,28]. Studies that did not find associations used multiple day (3–14) food records [34,39], food frequency questionnaires [36,41], 24-h recalls [7], and food preference surveys [39]. At this time, it is not possible to make a recommendation for one dietary assessment method over the other.

The majority of the studies relied on a one-time measure of taste response and attempted to map this response to dietary intake that spanned over days or months—a further limitation of the literature. Taste responses can vary throughout the day [52] or across days [31], posing problems in terms of test-retest reliability [53]. Day-to-day variability in both taste responses and dietary intake could obscure more immediate or acute relationships. One study noted that taste-diet relationships were observed after a night of sleep that lasted less than 7 h but saw no relationships after a night of longer sleep [31]. Sleep or other confounding variables may obscure taste-diet relationships. One of the two studies that did assess acute intake observed that sweet taste sensitivity correlated with a greater amount of non-sweet foods, protein, and protein as a percent of energy consumed by highly sensitive participants, and those participants also consumed less carbohydrate as a percent of energy [33]. The other study that assessed acute intake observed no relationships between intensity and hedonics [8]. The selection of the foods available for *ad libitum* intake could influence intake; thus, in addition to the different taste measures, it is difficult to compare these studies. Further exploration of whether taste measures are superior predictors of acute intake compared to longer-term intake needs to be undertaken.

There are several limitations to this review. As with all systematic reviews and meta-analyses, the inclusion criteria dictate the findings. While all studies were considered, taste testing studies are at high risk of bias due to the reliance on non-random selection of subjects and failure or inability to blind researchers and participants to the test stimuli or purpose of the study. The decision to focus solely

on sweet taste limits generalizability to other taste qualities. The heterogeneity of taste testing and dietary assessment methods makes definitive conclusions difficult. Further work examining taste-diet relationships in children and populations with chronic conditions should be undertaken.

5. Conclusions

In conclusion, only a small proportion of available studies reported significant associations between taste sensitivity, intensity, and hedonics with dietary intake. However, of those that reported significant associations, sensitivity and intensity measurements (sensory function) were negatively associated with intake, while liking and preferred concentration measurements (hedonics) were positively associated with intake in all but one study. Measures of taste liking and preference appear to provide relatively superior insight into dietary behaviors compared to sensitivity and intensity measures. Future considerations regarding standardizing methods are imperative.

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Quantifying Sweet Taste Liker Phenotypes: Time for Some Consistency in the Classification Criteria

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Abstract: Taste hedonics is a well-documented driver of food consumption. The role of sweetness in directing ingestive behavior is largely rooted in biology. One can then intuit that individual differences in sweet-liking may constitute an indicator of variations in the susceptibility to diet-related health outcomes. Despite half a century of research on sweet-liking, the best method to identify the distinct responses to sweet taste is still debated. To help resolve this issue, liking and intensity ratings for eight sucrose solutions ranging from 0 to 1 M were collected from 148 young adults (29% men). Hierarchical cluster analysis (HCA) revealed three response patterns: a sweet-liker (SL) phenotype characterized by a rise in liking as concentration increased, an inverted U-shaped phenotype with maximum liking at 0.25 M, and a sweet-disliker (SD) phenotype characterized by a decline in liking as a function of concentration. Based on sensitivity and specificity analyses, present data suggest the clearest discrimination between phenotypes is seen with 1.0 M sucrose, where a liking rating between −15 and +15 on a −50/+50 scale reliably distinguished individuals with an inverted U-shaped response from the SLs and the SDs. If the efficacy of this approach is confirmed in other populations, the discrimination criteria identified here can serve as the basis for a standard method for classifying sweet taste liker phenotypes in adults.

Keywords: sweet taste; hedonics; sweetness; taste test; individual differences; classification method

1. Introduction

Hedonic responses to taste stimuli are dissociable construct from motivation or a desire to eat (i.e., "liking" vs. "wanting") as proposed by Berridge [1], and these responses influence dietary intake [2–4]. Elsewhere, a conceptual model linking sensation to intake via affective/hedonic responses has also been proposed [5]. Under these models, it is highly plausible that interpersonal variations in hedonic responses to sweet taste—in conjunction with genetic and epigenetic inputs, environmental forces, and other acquired individual characteristic—may contribute to variations in the susceptibility for obesity and obesity-related diseases. For almost half a century, observations of distinct individual liking patterns to sweet taste stimuli have repeatedly been made, thereby challenging the widespread belief that sweetness is universally highly liked. Witherly and colleagues, for example, speculated that humans exhibit up to four distinguishable responses to various sweetened beverages [6], which, as was also illustrated later by Drewnowski [7], could be described as a rise in liking with increasing sweetener concentration followed by a decline (Type I), a rise and then a plateau (Type II), a monotonic decline (Type III), and a non-systematic change in liking (Type IV).

Since the pioneering work of Pangborn [8], sensory scientists using simple sucrose solutions and multiple different scaling methods in laboratory settings have similarly identified at least four

different sweet taste liker phenotypes. As summarized in Figure 1, the associated response patterns are characterized by either a positive slope, a horizontal ("flat") slope, an inverted U-shape, or a negative slope. Simpler schemes also exist, where participants are dichotomized into sweet likers (SLs) and sweet dislikers (SDs). The SL phenotype (sometimes reported as the Type II phenotype) generally refers to liking for ever-higher sweetness (e.g., in References [9,10]) and accounts for 48.5% of the published literature [11]. In contrast, the SD phenotype, which shares a very similar distribution (48.2%) with the SL phenotype [11], has been defined differently across various studies: it can describe either as a monotonically decreasing liking as sucrose concentration increases (e.g., in References [12,13]), or a liking for moderate levels of sweetness, which is graphically presented as an inverted U (e.g., in Reference [14]) and sometimes also called Type I phenotype (e.g., in References [15,16]). To note, a few studies identifying both subtypes of the SD response pattern classified them into a single group reported as SD phenotype, as well (e.g., in References [17,18]).

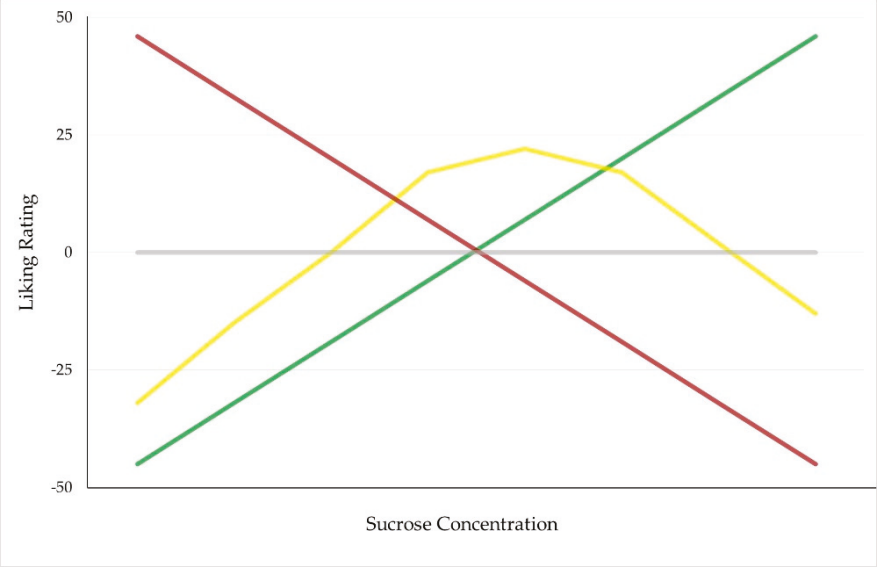


Figure 1. Graphical representation of the most commonly reported sweet taste liker phenotypes. The green line corresponds to a phenotype characterized by a rise in liking with increasing sucrose concentration (e.g., sweet liker phenotype), yellow line illustrates an inverted U-shaped hedonic response as a function of sucrose concentration (e.g., inverted-U phenotype), grey line represents an insensitive response to changes in sucrose concentration, and red line corresponds to a phenotype characterized by a decline in liking as sucrose concentration increases (e.g., sweet disliker phenotype). Adapted with permission from Reference [11].

Accordingly, an important question to be addressed is how these distinct hedonic responses to sweet taste can be defined and identified. Among 71 studies we recently reviewed [11], four main phenotyping methods (each relying on different classification criteria) were identified: the visual or algorithmic interpretation of hedonic responses from multiple sucrose concentrations (Method 1a and Method 1b, respectively), the "highest preference using ratings" method that dichotomizes participants based on whether they like the highest sucrose concentration tested the most (Method 2), the "average liking above mid-point" or "positive/negative liking" method where liking ratings are compared to one or two predefined cut-off scores (Method 3), and the "highest preference via paired comparisons" method that categorizes participants based on which sucrose concentration they prefer the most (Method 4). As detailed in our recent review [11], Method 2 and Method 3 suffer from arbitrariness

associated with the strength of the taste stimuli and/or the classification rating thresholds, and both methods are prone to misclassification. The dependence on visual inspection in Method 1a raises the potential for subjective interpretation, and Method 4 involves a choice paradigm based on preference rather than liking per se.

Considering these methodological challenges, along with the ongoing debate over the role of sugar intake as a factor in obesity [19–22], there is strong need for a more precise and consistent method to identify sweet taste phenotypes. The numerous prior studies that have investigated the presence of different sweet taste liker phenotypes and their potential relationship to dietary intake (e.g., in References [14,18,23]) or to body mass index (BMI: e.g., in References [13,16,24–26]) have used widely different methods to define phenotypes; presumably, this has contributed to the inconsistencies reported across studies. Accordingly, in our recent review [11], we suggested that a rapid and reliable phenotyping method is needed to facilitate comparisons across future studies. In our review, we proposed that an optimal sucrose concentration be identified that best separates distinct sweet taste liker phenotypes, in terms of sensitivity and specificity. In 2015, Asao et al. [27] piloted this idea in order to discriminate SLs from SDs. However, as commonly happens with small pilot studies, their sample size likely affected the phenotyping process, potentially leading to an underestimation of the true number of distinct response patterns, a limitation the authors noted in their report. Further, the total number of stimuli they used was rather large [27], raising additional issues of fatigue, adaptation, and inattentiveness. Finally, their participants were tested after they had fasted for an average of 12.1 h [27], which may influence the appetitiveness of the stimuli.

The present study aimed to extend the approach used by Asao et al. [27] while also eliminating some of the methodological issues mentioned above toward a goal of defining a new standardized phenotyping method. We had three aims. First, we identified different sweet taste liker phenotypes statistically. Second, we analyzed these phenotyping data to identify a single sucrose concentration where an application of one or two specific cut-off liking scores ensures the most reliable and replicable definition of each of the identified phenotypes. Last, potential relationships between the motivational state and baseline characteristics of our participants with these sweet taste liker phenotypes were explored.

2. Materials and Methods

2.1. Participants

A total of 148 non-diabetic participants aged 18–34 were recruited from students and staff at the University of Sussex between September and December 2017 (Table 1). Cohort size was determined by the suggested minimum of 100 participants in our recent methodological review for the successful identification of the main sweet taste liker phenotypes [11], which was further increased to adjust for the expected underrepresentation of the SD phenotype in our young adult population. Inclusion criteria comprised being medication free (other than oral contraception), smoking less than five cigarettes a week, and having no history of diagnosed eating disorders. Individuals with a current respiratory illness or having recently (less than two weeks) undergone a dental procedure, those being on a weight loss or a medically induced special diet, and women with an irregular menstrual cycle were also excluded. At enrollment, participants gave their written informed consent for inclusion in the study, but they were naive to the study's hypothesis until they had completed all tasks (debriefing provided). The University of Sussex Science and Technology Cross-Schools Research Ethics Committee approved the protocol on the 22 September 2017 (ER/VI40/1), and the study was conducted in accordance with the 1964 Declaration of Helsinki.

Table 1. Participant characteristics.

	Total	Sweet Taste Like Phenotype [1,2]		
		Sweet Liker	Inverted U-Shaped	Sweet Disliker
	***n* = 148**	***n* = 46**	***n* = 73**	***n* = 27**
Gender, N (%)				
Woman	105 (70.9)	33 (71.7)	48 (65.8)	22 (81.5)
Man	43 (29.1)	13 (28.3)	25 (34.2)	5 (18.5)
Ethnicity, N (%)				
Caucasian	112 (75.7)	39 (84.8)	53 (72.6)	19 (70.4)
Asian	14 (9.4)	2 (4.3)	9 (12.3)	3 (11.1)
Other	22 (14.9)	5 (10.9)	11 (15.1)	5 (18.5)
Dieting, N (%)				
Once or more times in the past	52 (35.6)	15 (32.6)	23 (31.9)	12 (46.2)
Never	94 (64.4)	31 (67.4)	49 (68.1)	14 (53.8)
Added sugar in drinks/cereals, N (%)				
More when being younger	72 (48.6)	18 (39.1)	39 (53.4)	14 (51.9)
Same as when being younger	27 (18.2)	11 (23.9)	9 (12.3)	7 (25.9)
Never	49 (33.1)	17 (37.0)	25 (34.3)	6 (22.2)
Age range (median) in years	18.2–34.0 (20.2)	18.3–32.8 (19.8)	18.2–34.0 (20.2)	18.2–34.0 (20.9)
BMI range (median) in kg/m^2	17.8–32.4 (22.1)	17.9–29.1 (23.0)	17.8–32.4 (21.6)	18.2–30.3 (22.7)

BMI, body mass index; Q1, 25th percentile; Q3, 75th percentile. All frequencies reported refer to valid percentages. [1] Participants demonstrating erratic responses to sweet stimuli ($n = 2$) were excluded from this analysis. [2] $p > 0.05$ for all between group comparisons performed with chi-square or Kruskal Wallis tests.

2.2. Taste Test

2.2.1. Taste Stimuli

To ensure sufficient individual ratings for the development of hedonic curves while trying to minimize confounding effects of adaptation [28] and sensory specific satiety [29], the taste test consisted of seven different aqueous sucrose solutions (0.03125, 0.0625, 0.125, 0.25, 0.5, 0.67, and 1 M) and one water blank, replicated in two separate blocks, for a total of 16 tastings.

The particular concentration range tested was equivalent to sucrose solutions between 1.07% and 34.23% (*w/v*) based on density at 20 °C [30], and were chosen to reflect four different considerations: (1) previously reported effects of age on sucrose recognition thresholds [31–33]; (2) the most commonly used sucrose concentrations in prior relevant studies (reviewed in Reference [11]); (3) the sweetness typically encountered in sugar-sweetened beverages [34]; and (4) a compromise between equal log spacing and serial dilution for sample preparation.

All sweet stimuli were prepared at least 24 hours in advance by dissolving food-grade sucrose in mineral water at room temperature. Solutions were stored at 4 °C until used. On the experimental day, solutions were allowed to warm up to room temperature prior to presentation, and were presented as 10 mL samples in transparent 60 mL glass cups labelled with random three digit codes. For the solute and rinsing, we used a commercial mineral water with the lowest dry residue concentration available at the time (Volvic, Danone Waters London and Ireland Ltd., London, U.K.).

2.2.2. Rating Scales

Participants evaluated liking and intensity for each stimulus using a horizontal visual analogue scale (VAS) end-anchored with "dislike extremely" (scored −50) and "like extremely" (scored +50) and a vertical generalized labeled magnitude scale (gLMS) with properly positioned descriptors ranging from "no sensation" (scored 0) to "strongest imaginable sensation of any kind" (scored +100), respectively. To ensure within and between-subjects compliance, training for both scales was provided. The practice session for VAS involved rating liking for a series of non-food items, while training in the use of gLMS was applied by evaluating responses to noise and light [35].

On the basis of Cabanac's theory regarding possible enhancement of stimulus value by internal state ("alliesthesia" [36]), two series of VAS appetite ratings [37] were completed before the first and after the second taste test block. All ratings were collected using the Sussex Ingestion Pattern Monitor (SIPM version 2.0.13, University of Sussex, Falmer, U.K.), a computer-based system developed to record and score rating data.

2.2.3. Procedure

The taste test was conducted approximately 2 h after breakfast (between 09.30 am and 12.30 pm depending on each participant's personal routine). Participants were also asked to abstain from smoking, chewing gum, and tooth brushing for the 2 h prior to testing; no restrictions applied to water consumption. During both taste test blocks, a "sip and spit" protocol was followed: participants were instructed to place the entire 10-mL solution in their mouth, swirl it around for 10 s, and expectorate it. They then rated their liking and sweetness intensity before rinsing their mouth with water and proceeding to the next sample. Stimuli were presented in randomized order with participants blinded to the concentration of sucrose tasted each time. After the taste test was complete, demographic (date of birth, sex, and ethnicity) and lifestyle characteristics ("Have you ever been on a diet in order to lose weight?" with possible answers "Yes, one or more times in the past" or "Never," and "Did you usually add more sugar in your coffee, tea or cereals when you were younger?" with possible answers "Yes, I used to add more sugar in my coffee, tea or cereals when I was younger," or "No, I add the same sugar as I did in the past," or "Never added sugar in my coffee, tea or cereals") were collected.

2.3. Anthropometry

To minimize any possible interactions between the sensory ratings and anthropometric measures, participants revisited the laboratory for a separate early morning session (08:30–10:30) for anthropometry; this visit was scheduled between two days and two weeks after the taste test. Height was measured to the nearest 0.1 cm using a stadiometer and weight to the nearest 0.1 kg using a calibrated body composition analyzer (MC-780MA P, TANITA, Tokyo, Japan). Standardized procedures were followed, including wearing light clothing and no shoes [38].

2.4. Statistical Analysis

Our primary goals were to (a) algorithmically identify the different sweet taste liker phenotypes in our study cohort and (b) to determine the specific sucrose concentration and associated cut-off score(s) for liking ratings that most reliably allowed for the identification of those distinct phenotypes. Assumptions of normality were tested prior to the main statistical analyses using visual inspection (histograms, Q-Q plots, and bloxplots), and summary statistics (skewness and kurtosis *z*-scores computed by dividing skewness or kurtosis values with the associated standard errors). *Z*-scores (absolute values) larger than 1.96 were indicative of a normal distribution. All ratings are reported as means and standard errors (normally distributed), while medians and ranges are used for age and BMI (not normally distributed); categorical characteristics are expressed as percentages.

Interclass correlation coefficients (ICCs) were calculated to assess test–retest reliability of liking ratings over the two taste test blocks. Given our experimental design, an average measures absolute agreement two-way mixed-effects model was selected [39]. Per the guidelines, an ICC value less than 0.5 indicates poor reliability, values between 0.5 and 0.75 reflect moderate reliability, and values between 0.75 and 0.9 indicate good reliability [40].

As the first step to achieve the principle aim of the current study, an agglomerative hierarchical cluster analysis (HCA) was performed and meaningful groups (clusters) of participants who shared similar liking patterns within each group but were heterogeneous in the between-group contrasts were identified. The mean liking ratings from the eight replicated concentrations in the two taste test blocks were treated as the dimensions for the HCA. The squared Euclidean distance between pairs of cases or clusters and the between-groups (average) linkage method were selected to assist with the merging

process [41]. The final decision on the true number of clusters in our dataset was dictated graphically by interpreting the scree plot of coefficients of the agglomeration schedule we designed (Office Excel 2013 for Windows, Microsoft, Washington, DC, USA) and then applying this information ("stopping rule") to the dendrogram provided by the statistical software on the HCA output [41].

We then implemented a two-by-two cross tabulation function to estimate the dyads of sucrose concentration and liking score with the highest sensitivity and specificity in predicting the three distinct sweet taste liker phenotypes. In each two-by-two cross tabulation table, the phenotyping results emerged when a specific dyad of sucrose concentration and liking score was used as the classification criteria for the identification of the sweet taste liker phenotype under investigation were contrasted with the associated phenotyping results suggested by the HCA. The number of true positives (e.g., classified as SL by both the dyad tested and the HCA) and the number of true negatives (e.g., not classified as SL by both the dyad tested and the HCA) indicated the sensitivity and specificity attached to that particular dyad of sucrose concentration and liking score, respectively. Reported liking ratings for stimuli from 0.03125 M to 1.0 M sucrose and potential cut-off values ranging between −20 and +20 in 5-point increments were tested for their prediction value. A K-1 series of sensitivity-specificity tests were conducted, where k represents the number of main clusters previously identified in the HCA.

To test the hypothesis that the sucrose concentration (within subject factor) and the initial clusters or subsequent sweet taste liker phenotypes (between subject factor), as well as their interaction, affect liking and intensity ratings of the presented sweet taste stimuli, two-way mixed ANOVAs with Greenhouse-Geisser correction were carried out. We also employed separate one-way ANOVAs to contrast liking and intensity (both mean ratings and ratings across each of the eight concentrations) by sweet taste liker phenotype. In cases of violation of the equal variances assumption, Brown–Forsythe tests were applied, instead [42]. Post hoc Fisher's least significant difference (LSD) and Games-Howell tests were used as appropriate to further understand the nature of specific paired comparisons.

Nonparametric (Mann–Whitney) tests for the previously reported not normally distributed continues variables (age and BMI) and Pearson's chi-square tests for the categorical variables (gender, ethnicity, dieting history, and habitual use of table sugar) were used to investigate for differences in participant characteristics across the distinct sweet taste liker phenotypes. To explore whether there were also gender differences in measures of interest, additional chi-square tests were performed. Phi symmetric measures instead of Pearson's results are reported in cases of cells with an expected count less than 5.

To ensure participants' compliance with the taste test protocol, changes in hunger and thirst before and after delivering the taste test were explored using paired *t*-tests. We also calculated multiple linear regressions to investigate the degree to which pre- and post-test hunger and thirst predicted liking and intensity ratings across the study sample. The influence of pre- and post-test levels of hunger and thirst was further explored using either one-way ANOVAs or Brown–Forsythe tests [42] to detect differences across the distinct sweet taste liker phenotypes.

The extent to which our method for the identification of the distinct sweet taste liker phenotypes agrees with those in previous literature (see Introduction for details) was assessed by Cohen's Kappas and 95% confidence intervals (CIs) based on the "Estimate $\pm$ 1.96 $\times$ Standard Error" formula [43]; participants exhibiting an inverted U-shaped response were excluded from this analysis due to the bimodal nature of the phenotyping results elicited by Method 2 and 3. The relevant frequency distributions were also estimated. For the comparison with Method 2 participants who rated the highest sucrose concentration, namely the 1 M solution, as the most pleasant were considered as SLs, whilst all remainder liking patterns were classified into the SD phenotype [44,45]. The agreement with Method 3 was tested using the 0.5 M sucrose solution and the corresponding neutral cut-off hedonic score of 0 (zero) as the classification criteria to discriminate SLs from SDs [23].

Unless otherwise stated, data were analyzed using SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA). An alpha level of 0.05 was set as the threshold for statistical significance and all performed statistical tests were two-tailed.

3. Results

3.1. Participant Characteristics

Participant characteristics are summarized in Table 1; three (two women and one man) failed to report to the laboratory for both sessions. As a whole the cohort tested here was relatively young and lean (*Mdn* = 20.2 years and *Mdn* = 22.1 kg/m^2, respectively) and was mainly comprised of women (70.9%); most self-identified as Caucasian (75.7%). Nearly half of the participants reported that they currently add less sugar in their drinks and cereals than when they were younger, and one in three had been on a diet for weight loss at least once in the past. Overall, the women were slightly younger than the men (*Mdn* = 21.1 years for men and *Mdn* = 20.1 years for women; $U = 1454.5$, $Z = -3.263$, $p = 0.001$), and had a lower average BMI (*Mdn* = 23.4 kg/m^2 for men and *Mdn* = 21.6 kg/m^2 for women; $U = 1475.5$, $Z = -2.861$, $p = 0.004$). This was expected, as it reflects the typical differences in BMI between men and women and the differences in BMI across different age groups in the U.K. [46].

3.2. Taste Test

Test-retest reliability analysis comparing liking ratings across the two taste test blocks indicated moderate to good reproducibility based on the ICC cut-offs suggested by Portney and Watkins [40] for all but the 0.125 M solution (Figure 2). The two highest sucrose concentrations (0.67 and 1.0 M), and water were associated with the strongest agreement between the two repetitions. As expected, there was a main effect of concentration on liking across all participants with significantly different mean hedonic scores reported for different solutions ($F(2.12, 312.15) = 10.65$, $p < 0.001$, $\eta p^2 = 0.068$).

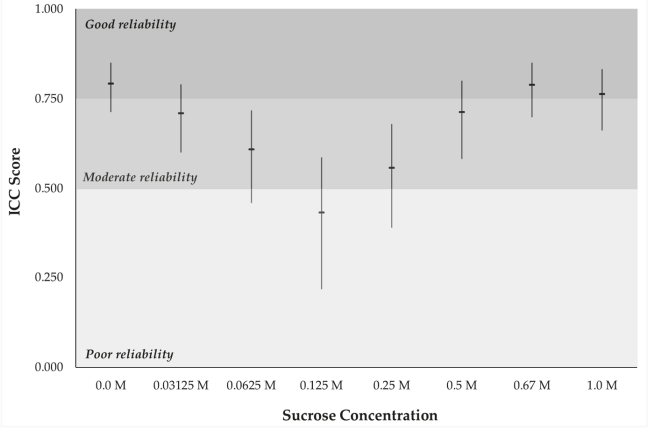


Figure 2. Interclass correlation coefficient (ICC) scores (95% confidence interval) for liking ratings from the two taste test blocks across the different taste stimuli.

3.2.1. Identifying Distinct Responses to Sweet Taste: HCA

HCA resulted in ten subgroups of distinct responses to sweet taste with a significant effect of cluster on liking ($p < 0.001$ for all eight sucrose concentrations and effect sizes ranged from 0.22 for the 0.125 M solution to 0.80 for the 1.0 M solution). Three main clusters that accounted for 92% of the study sample were observed. Cluster 1 ($n = 44$) and cluster 3 ($n = 22$) described hedonic response patterns consistent with the sweet liker (SL) and sweet disliker (SD) phenotypes. Both trends were particularly evident for solutions with added sucrose above 0.125 M. Notably however, almost half of the study sample fell into cluster 2 ($n = 70$), where liking increased modestly with concentration up to an intermediate level of sucrose (0.25 M) and then decreased as the concentration continued to increase. Remarkably, participants who were classified into cluster 2 rated both the lowest ($M = 1.0$, $SEM = 0.76$

for 0.03125 M) and the highest ($M = -1.5$, $SEM = 1.44$ for 1.0 M) sucrose concentration as neutral; that is, they neither liked them nor disliked them ($t(69) = 1.46$, $p = 0.148$ for the paired comparison between the lowest versus the highest concentration).

Regarding the 12 participants classified into one of the remaining clusters (clusters 4 to 10), plotting liking as a function of concentration revealed that participants in cluster 9 ($n = 2$) and those in cluster 10 ($n = 3$) followed a classical SL and a SD liking pattern, respectively. Their ratings from the eight different sucrose concentrations resulted, however, in steeper liking curves ("extreme" responses) than those in our main SL and SD clusters, which explains why they emerged as separate groups during the clustering procedure. Indeed, before we applied the "stopping rule" as appropriate (see Section 2.4 for details), participants grouped into clusters 9 and 10 and those grouped into clusters 1 and 3, respectively, had been considered as homogenous only subsequent to the inverted U-shaped phenotype merged with the SL phenotype. Likewise, an inverted U-shaped response corresponding to corresponding to that of cluster 2 was observed for participants classified into cluster 4 ($n = 2$), cluster 7 ($n = 2$), and cluster 8 ($n = 1$): among the heterogeneous mean liking ratings to those of cluster 2, a different optimal sweetness (0.5 M for cluster 4 and 0.67 M for cluster 8) and a higher rating for the breakpoint concentration of 0.25 M sucrose ($M = 8.9$, $SEM = 1.15$ for cluster 2 and $M = 28.5$, $SEM = 4.50$ for cluster 7, $t(70) = -2.84$, $p = 0.006$) stand out. Two single cases of erratic responses were also identified and eliminated from further analysis (cluster 5 and cluster 6).

3.2.2. Identifying Distinct Sweet Taste Like Phenotypes: New Classification Criteria

With regard to the specific sucrose concentration and liking thresholds that best discriminated between the three main clusters, the 1 M solution and liking scores of −15 or lower for the identification of SDs and +15 or higher for the identification of SLs were associated with the lowest number of false negative classifications (90.9 and 97.7 percentage sensitivity for SDs and SLs, respectively) and the lowest number of false positive classifications (93.9 and 93.5 percentage specificity for SDs and SLs, respectively). The results are shown in Tables 2 and 3.

Table 2. Sensitivity and specificity checks to discriminate sweet dislikers (cluster 3) from the rest of sweet taste liker phenotypes.

Liking Cut-Off Scores	Sucrose Concentration (M)							
	0.25		0.5		0.67		1.0	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
−20	13.6	100.0	36.4	100.0	45.5	99.1	81.8	96.5
−15	13.6	100.0	54.5	97.4	68.2	95.6	90.9 *	93.9 *
−10	27.3	99.1	63.6	94.7	77.3	92.1	95.5	87.7
−5	50.0	94.7	77.3	93.0	95.5	86.0	100.0	77.2
0	59.1	89.5	90.9	86.8	100.0	76.3	100.0	68.4

Percentages (%) with an asterisk (*) indicate the dyad of sucrose concentration and liking cut-off score with the highest combined sensitivity and specificity for the prediction of the sweet disliker phenotype across all dyads tested.

We then applied these classification criteria individually to participants who were assigned to the remaining clusters. The revised grouping (SL phenotype: $n = 46$; 31.5%, inverted U-shaped phenotype: $n = 73$; 50%, SD phenotype: $n = 27$; 18.5%) was in agreement with the classification suggested by the visual interpretation of the shape of the relevant liking curves in all participants except those initially classified into cluster 4. Those participants met the new SD phenotype criteria rather the criteria associated with the inverted U-shaped response pattern. A closer inspection of their hedonic responses revealed that they actually had rated all sucrose solutions as neutral or unpleasant. In addition, integrating the very small clusters into the main groups of responses reduced overfitting and allowed for the subsequent statistical analyses required.

Table 3. Sensitivity and specificity checks to discriminate sweet likers (cluster 1) from the rest of sweet taste liker phenotypes.

Liking Cut-off Scores	Sucrose Concentration (M)							
	0.25		0.5		0.67		1.0	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
0	95.5	26.1	100.0	40.2	100.0	55.4	100.0	64.1
5	79.5	43.5	100.0	54.3	97.7	63.0	100.0	77.2
10	56.8	67.4	100.0	67.4	97.7	76.1	97.7	89.1
15	38.6	84.8	88.6	79.3	88.6	87.0	97.7 *	93.5 *
20	20.5	88.0	63.6	87.0	79.5	96.7	84.1	97.8

Percentages (%) with an asterisk (*) indicate the dyad of sucrose concentration and liking cut-off score with the highest combined sensitivity and specificity for the prediction of the sweet liker phenotype across all dyads tested.

Confirming the diverse nature of the sensory responses to sweet taste among participants classified into the three main sweet taste liker phenotypes, overall liking and intensity significantly varied across these newly defined distinct groups, $F(2, 56.21) = 89.44$, $p < 0.001$ for liking and $F(2, 77.95) = 5.74$, $p = 0.005$ for intensity. A main effect of sucrose concentration ($F(4.44, 635.19) = 8.53$, $p < 0.001$, $\eta p^2 = 0.056$), as well as a strong interaction effect between sucrose concentration and phenotype ($F(8.88, 635.19) = 78.65$, $p < 0.001$, $\eta p^2 = 0.524$) on liking were also found. As shown in Figure 3, follow-up analysis indicated that participants with an inverted U-shaped response liked the three lower sucrose concentrations at a similar level when compared with both SLs and SDs. When liking ratings of those stimuli were separately contrasted between the two extreme phenotypes, we found that SLs rated them as less pleasant than SDs did. Liking for the 0.125 M sucrose solution did not differ between groups, whereas liking ratings for the rest of the sweet taste stimuli significantly differed by cluster ($p < 0.001$ for most paired comparisons).

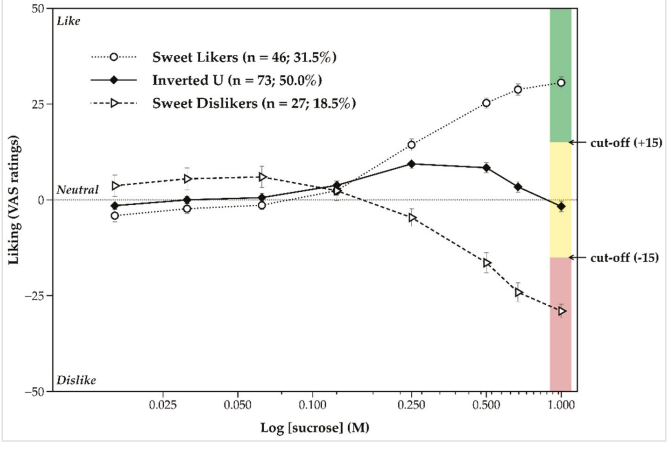


Figure 3. Liking ratings (mean ± standard error of the mean) as a function of sucrose solutions by the three sweet taste liker phenotypes. Ratings were averaged across the two taste test blocks. The response pattern for the sweet liker phenotype is displayed with a dotted line, the response pattern of inverted U-shaped phenotype with a solid line, and the response pattern of sweet disliker phenotype with a dashed line. Different colors denote the different ranges of liking ratings for 1 M sucrose which, according to the relevant sensitivity and specificity checks (see Tables 2 and 3 for details), could be used for the reliable discrimination between the three distinct sweet taste liker phenotypes: green color corresponds to the range of liking ratings for 1 M sucrose representing sweet likers, yellow color indicates the hedonic response spectrum to 1 M sucrose characteristic of the inverted U-shaped phenotype, and red color corresponds to the range of liking ratings for 1 M sucrose for sweet dislikers.

We next sought to examine the perceived variations in sweetness for the different stimuli between the three sweet liker phenotypes. Paired comparisons between the intensity ratings for each successive concentration and the intensity ratings for the previous indicated that participants were clearly able to distinguish between the different sucrose concentrations ($p = 0.002$ for water and 0.03125 M, and p's < 0.001 for all remainder pairs). Rated intensity also increased as sucrose concentration increased across all three sweet taste like phenotypes, $F(2.32, 336.30) = 535.25$, $p < 0.001$, $\eta p^2 = 0.787$ (Figure 4). SDs overall perceived the taste stimuli as sweeter ($M = 23.3$, $SEM = 1.62$) than both SLs ($M = 17.2$, $SEM = 0.73$; $p = 0.001$) and participants classified in the inverted U-shaped phenotype ($M = 19.2$, $SEM = 0.96$; $p = 0.015$). No interaction effect between concentration and sweet taste like phenotype on intensity was, however, observed, $F(4.67, 333.68) = 521.10$, $p = 0.082$, $\eta p^2 = 0.027$.

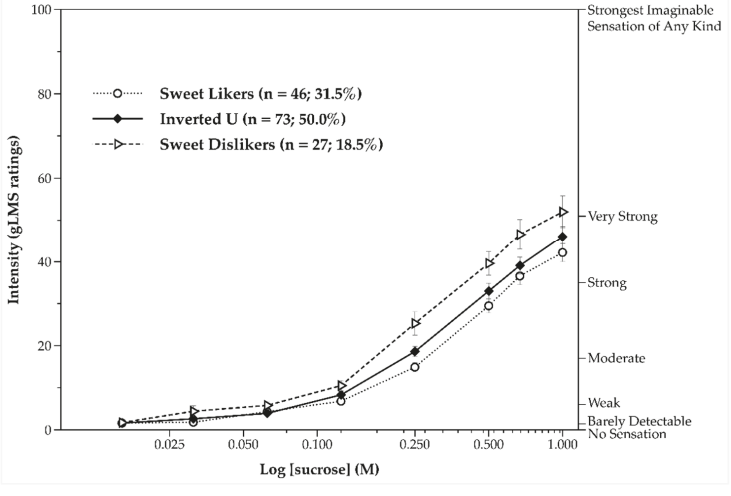


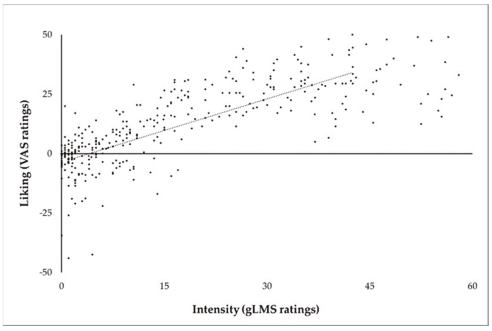
Figure 4. Intensity ratings (mean ± standard error of the mean) as a function of sucrose solutions by the three sweet taste liker phenotypes. Ratings are averaged across the two taste test blocks. The intensity curve of the sweet liker phenotype is displayed with a dotted line, the intensity curve of the inverted U-shaped phenotype with a solid line, and the intensity curve of the sweet disliker phenotype with a dashed line.

To explore whether the identified sweet taste liker phenotypes were merely indirect consequences of differences in perceived intensity rather than true differences in hedonics per se, liking ratings were also plotted as a function of intensity separately for the three main clusters. As shown in Figure 5a–c, no such indication was found.

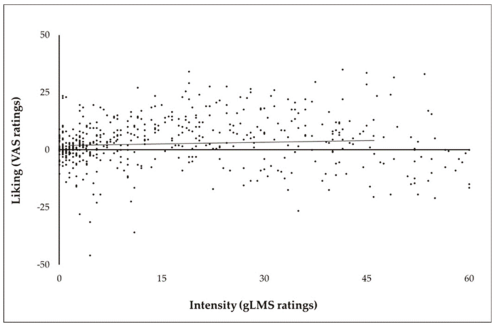
3.2.3. Pre- and Post-Test Levels of Hunger and Thirst

Pre-test levels of hunger ($M = -7.5$, $SEM = 2.11$) and thirst ($M = 0.3$, $SEM = 1.68$) confirmed participants' compliance with the taste test preparation instructions, whereas the increase in hunger ($t(147) = -3.25$, $p = 0.001$) and decrease in thirst ($t(147) = 2.32$, $p = 0.022$) over time was also in line with the effects of the "sip and spit" and "mouth rinsing with water" parts of the taste protocol. Neither hunger nor thirst ratings before taste test block 1 or after taste test block 2 predicted liking ($F(2, 145) = 2.065$, $p = 0.130$ for pre-test levels of hunger and thirst; $F(2, 145) = 0.607$, $p = 0.546$ for post-test levels of hunger and thirst) or intensity ($F(2, 145) = 1.041$, $p = 0.356$ for pre-test levels of hunger and thirst; $F(2, 145) = 0.403$, $p = 0.669$ for post-test levels of hunger and thirst) across the study sample. When ratings of hunger and thirst were examined against the three distinct sweet taste liker phenotypes, non-significant differences were found ($F(2, 143) = 2.410$, $p = 0.093$, and $F(2, 143) = 0.094$, $p = 0.910$ for pre-test levels of hunger and thirst, respectively; $F(2, 76.22) = 0.986$, $p = 0.378$, and $F(2, 143) = 0.107$,

p = 0.899 for post-test levels of hunger and thirst, respectively). These data clearly show that the group differences in sweet liking cannot be attributed to the observed changes in hunger or thirst.



(**a**)



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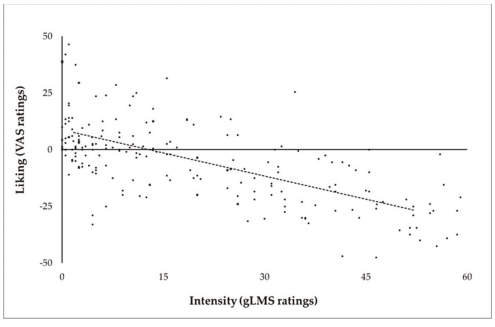

(**c**)

Figure 5. Individual ratings of liking as a function of perceived intensity for the sweet taste stimuli in (**a**) sweet likers, (**b**) individuals exhibiting an inverted U-shaped hedonic response, and (**c**) sweet dislikers. Lines represent the average ratings across individuals classified within each phenotype.

3.3. Participant Characteristics by Sweet Taste Liker Phenotype

Possible variations in participant characteristics relative to sweet taste liker phenotype were also examined. Gender ($\chi^2(2, N = 146) = 2.39$, $p = 0.302$), ethnicity ($\phi = 0.152$, $p = 0.496$), dieting history ($\chi^2(2, N = 144) = 1.84$, $p = 0.400$), habitual use of table sugar ($\phi = 0.194$, $p = 0.240$), age ($H(2) = 2.60$,

p = 0.273) and BMI (H(2) = 0.67, p = 0.717) did not differ between groups. All associated values by phenotype are summarized in Table 1.

3.4. Comparison to Existing Classification Methods

When Method 2 (rating the 1 M sucrose solution or not as the most pleasant) and Method 3 (rating the 0.5 M sucrose solution higher than 0 or not) were used to distinguish the different sweet taste liker phenotypes, the proportions of SD and the SL were respectively overestimated: 113 participants were classified as SDs according to Method 2 and 108 as SLs according to Method 3. Compared with our phenotyping method, in both cases, the majority of those participants (56.6% of SDs in Method 2 and 53.7% of SLs in Method 3) exhibited an inverted U-shaped response. Focusing on Method's 2 phenotypic classification, all 27 participants classified as SDs using our method were also identified as SDs using Method 2. Regarding the SL phenotype, 22 out of 46 participants initially fell into the SL phenotype were classified as SDs using Method 2. Those 22 participants liked the 1 M sucrose solution significantly lower than the previous concentration (M = 25.3 for 1 M versus M = 30.6 for 0.67 M, p = 0.014), while no significant difference was observed when compared with the third higher sucrose concentration (M = 25.3 for 1 M versus M = 28.4 for 0.5 M, p = 0.222). The kappa coefficient was accordingly low at 0.447 (95% CI: 0.286 to 0.608). In contrast, the agreement with Method 3 was good with a Kappa coefficient at 0.879 (95% CI: 0.764 to 0.993). All SLs identified using our method were also classified as SLs by Method 3. The two phenotyping approaches were also in line regarding the SD phenotype: only four SDs using our method were discordantly classified as SLs using Method 3. Those participants had a mean liking for the 0.5 M barely over the neutral point (M = 1.1) and their liking rating for the 1 M, which was our concentration of choice for distinguishing sweet taste liker phenotypes, was as low as −28.7. A graphical representation of the level of consistency/disagreement among the methods compared here is provided in Figure 6.

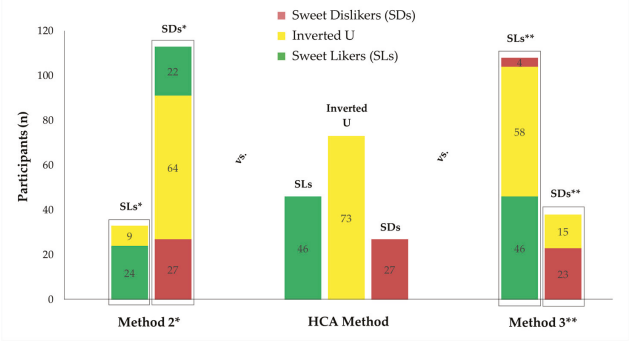

Figure 6. Comparison of the distribution of sweet taste liker phenotypes in our study sample when different classification methods were used. Method 2 (rating the 1 M sucrose solution or not as the most pleasant) and Method 3 (rating the 0.5 M sucrose solution higher than 0 or not) were, by definition, limited to a two-response group phenotyping outcome (binomial distribution), while HCA method (rating the 1 M sucrose solutions higher than +15, lower than −15, or between −15 and +15) allowed for the identification of three distinct sweet taste liker phenotypes. 133 participants (77.4%) versus 27 (18.5%) were classified as SDs and 108 participants (74.0%) versus 46 (31.5%) were classified as SLs when Method 2 and Method 3 were contrasted with the method we proposed here (HCA method), respectively. Different colors of the stacked columns and the associated data labels (numbers) correspond to the number of participants classified into the phenotype of the same color when the HCA method was used. Data labels (numbers) within each column add up to the total number of participants classified into the phenotype illustrated at the upper end of the relevant column. Asterisks (*/**) denote alternatives to our definition for SLs and SDs. SDs, sweet dislikers; SLs, sweet likers.

4. Discussion

4.1. General Findings

The present report describes how hedonic responses to taste stimuli of varied sweetness can be algorithmically interpreted using HCA, and clustered into groups that represent similar sweet-liking patterns. For the current dataset, consistent differences in liking ratings across the eight sucrose solutions were found, which then allowed a clear characterization of participants as SLs, those with an inverted U-shaped response, or as SDs. Another key feature of the study was the subsequent identification of the 1 M aqueous sucrose solution and the VAS-based cut-off liking scores of −15 and +15 as the statistically reliable criteria to efficiently categorize individuals into these three different sweet taste liker phenotypes.

4.2. HCA Selection Advantages

Regarding our decision to use HCA for the identification of different sweet taste liker phenotypes, this was principally driven by the need for a statistically robust and unbiased merging of individuals into groups. Indeed, using an advanced statistical clustering technique allowed the three sweet taste liker phenotypes to emerge, whereas this would have been difficult to discern using more traditional visual inspection methods, particularly if the inspector was assuming a simple dichotomous mode. HCA is also based on hedonic responses across multiple stimuli rather than based on an arbitrarily selected single liking rating or the average value of hedonic scores of different stimuli. Accordingly, most elements of subjectivity and arbitrariness noted in the other phenotyping methods discussed earlier were controlled for. When we re-analyzed our current data using other widely used methods (defined as Methods 2 and 3 in the introduction, and in our recent review [11]), many participants were misclassified relative to the cluster analysis performed here, as the bimodal phenotyping approach in those methods assumes a priori that there are only two distinct response patterns. Critically, the HCA analysis shown here, as well as other recent studies [9,13], all suggest that response patterns for sweet stimuli are better described by three distinct phenotypes. Regarding the observed overestimation of SDs by Method 2 and of SLs by Method 3, this was a consistent feature of those methods in our recent evaluation of the impact of different sweet taste liker classification approaches [11]. In contrast, discriminating participants between the different sweet taste liker phenotypes based on a single sucrose concentration and predetermined cut-off liking scores as used in Method 3, led to the least misclassifications, further supporting the utility of such a phenotyping approach.

4.3. Phenotyping Results

Our findings confirm some [8,9,13,47,48] but not all, studies using phenotyping methods that allowed for a non-dichotomous identification of sweet-liking patterns. Indeed, in some published reports, participants with an inverted U-shaped response were considered as outliers [12,15,17], whilst elsewhere they were treated as homogeneous with the SDs [49–51]. Here, the generated icicle plot of our statistical output (not shown) revealed that during the final stages of the clustering process, SLs merged with those from the inverted U-shaped phenotype before SDs joined them both, uncovering a greater resemblance of the SL rather than of the SD phenotype to the inverted U-shaped response group. It is then plausible to assume that eliminating or misclassifying this intermediate phenotype is problematic and possibly obfuscates potential relationships between sweet taste liker phenotypes and health outcomes of interest. We also noticed that the sucrose concentration associated with the highest liking in the inverted U-shaped response group (i.e., the 0.25 M), was in line with the concentration observed in most previous work [15–18,27,52,53], although lower values have also been reported [8,14,48,54]. Practically speaking, this commonly identified 0.21–0.3 M range of sucrose concentration threshold in individuals who like intermediate levels of sweetness is lower than the sugars composition of the commercially available sweetened beverages [34]. This may potentiate the argument for reexamining the utility of sugar-tax policies [55]. The multisensory aspects of tasting

real-life products should not, however, be disregarded [56], as well as the possible attenuating or enhancing effects of other flavor components on perceived sweetness in complex products [57–60]. As sagely noted by Pangborn, "a change in one ingredient can cause multiple physical-chemical interactions which alter several sensory attributes simultaneously: appearance, aroma, texture, taste etc." [61] (p. 65).

Turning now to the frequency distribution of the identified sweet taste liker phenotypes, one third of our participants were classified as SLs, a proportion consistent with observations by others who also used HCA as their phenotyping method of choice [9,13,14]. Conversely, results in Asao et al. [27] and Kim et al. [62] indicate that this sweet-liking pattern accounted for roughly 50% of their study samples. Two possible explanations can be considered. First, the absence of a monotonically negative slope implies that individuals in both cohorts generally exhibited stronger liking for sweetness. Notably, in Kim et al. [62], two thirds of those classified in the inverted U-shaped phenotype rated 0.7 M as the most liked, a sucrose concentration breakpoint twice as high as the concentration we identified. Second, in those studies, sweet-liking was assessed under extreme motivational states with participants' hunger [27,62] and/or satiety [62] being manipulated. Critically, when the same Korean researchers replicated their study using a more typical pretest protocol (i.e., refraining from eating for one to two hours prior to the taste test), their measures generally correspond with the data shown here. Focusing on the frequency distribution of the monotonically negative slope regardless of the SD label, our findings disagree with previous observations. For example, of the 650 age diverse adults tested by Garneau et al. [13], only 55 exhibited decreasing liking as concentration increased. Presumably, this is due to the relatively low sucrose concentrations they used; indeed, the highest concentration they used (0.40 M) fell near the concentration breakpoint we identified for our inverted U-shaped phenotype. In contrast, SDs in Kim et al. [9] were approximately as frequent as SLs and as participants in the inverted U-shaped phenotype (31.7, 32.5, and 35.8%, respectively). Nonetheless, they reported that, for the purposes of the study, two distinct clusters were treated as a single sweet-liking pattern representing the SD phenotype, with no further information provided; each of those clusters accounted for 10 and 21.7% of the total sample, respectively [9].

Here, despite the similar liking ratings of the lowest and the highest sucrose concentration by participants classified into the inverted U-shaped phenotype, perceived sweetness varied considerably when intensity ratings of those stimuli were contrasted. Therefore, this type of response cannot be attributed to reduced sensitivity to taste stimuli or from differences in recognition thresholds; rather, it appears to reflect a distinct liking pattern. Figure 5a,c indicated that this is also true for the SL and the SD phenotype, since inclusion of intensity ratings in the liking plots generated the expected liking patterns. In previous research, any differences in sweetness intensity between participants, when reported, were interpreted independent of the associated phenotyping results (e.g., in References [45,63,64]). The few studies that have contrasted sweetness intensity between the defined sweet taste liker phenotypes have had mixed outcomes: some studies report greater overall sweetness intensity in SDs than in SLs and/or than in other phenotypes in line with what we observed here [12,15,49,65], but the majority found no differences in sweet taste perception [10,13,16,66–71]. These inconsistencies could arise from several factors including the phenotyping methods and the stimuli concentrations used in these studies. Many of the most relevant studies did not, however, specifically report differences in sweetness intensity between their defined sweet taste liker phenotypes, limiting meaningful contrasts between our findings and prior work.

4.4. Recommended Criteria for the Identification of Distinct Sweet Taste Liker Phenotypes

Except for a pilot experiment [27], this is the first study suggesting specific criteria for the identification of the distinct sweet taste liker phenotypes that could be considered as both statistically robust and easy-to-apply. One core element of the proposed simpler approach is the administration of a single sucrose concentration that allows for both a less time-consuming and resource-demanding assessment process and for elimination of potential issues from the contrast effects which are

"hard-wired" to longer protocols [72]. Within the taste literature, this in a not a novel concept. In 1980, Lawless addressed the need to identify an efficient classification method that could be used to rapidly screen large cohorts in terms of bitter taste phenotypes for phenylthiocarbamide (PTC) and 6-n-propylthiouracil (PROP), i.e., thiourea tasters and nontasters [73]. After using multiple approaches within the same study cohort, he concluded intensity ratings (on a 7-point scale) for a single antimodal concentration of PTC or PROP presented in a two-series taste test allowed for a rapid and reliable separation of the tasters from the nontasters [73].

Despite using a similar analysis to that of Asao et al. [27], we concluded that approximately twice the concentration of sucrose, compared to the concentration they proposed, is required to deliver the highest sensitivity and specificity in the discrimination between distinct sweet taste liker phenotypes. A small sample size, dichotomous grouping, and participants' pre-test fasting state in the earlier pilot experiment [27] raise questions about the broader applicability of the concentration (0.598 M sucrose) recommended in their study. Indeed, other studies using multiple sweet taste stimuli identified concentrations ranging from 0.83 M (e.g., in References [66,74–78]) to 0.99 M (e.g., in References [79–81]). Moreover, the 0.6 M sucrose solution referred in Tuorila et al. [23] was actually shortlisted from their previous work where two additional lower concentrations were tested but not any higher [82]. Finally, the replication in our sample of the proposed by Asao and colleagues' U-shaped association between sucrose concentration and reproducibility of the liking ratings across the repeated blocks of the taste test [27] may also bear critically upon sweet-liking protocols based on intermediate concentrations. Indeed, taste measures for about 40% of the adult sample in Garneau et al. [13] indicated indifferent responses to a range of stimuli between 0 M and 0.4 M sucrose.

Considering the comparatively less sophisticated and less restrictive concepts of the VAS compared to the labelled magnitude or Likert-type scales, the decision to record liking on an analogue scale further strengthens our classification criteria proposal. In particular, VAS-based ratings are independent of the range of prior sensory experiences and of the assumption that the same descriptors (labels) reflect equivalent meaning across different responders [83,84]. That said, in our lab, we have repeatedly observed that participants find VAS to be more straightforward than gLMS, although when we directly contrasted the two scales in a sample of young educated adults, VAS and gLMS yielded similar results [17]. Additionally, VAS is appropriate for recording the multi-dimensional continuum of human responses that a fixed pre-coded format does not by principle permit [85]. Clearly, no scaling approach is perfect: the "anchor effect" phenomenon (centering bias) characterized by less use of the extreme response has been associated with most rating scales, the VAS included [72]. Overall, we propose that utilizing VAS for sweet-liking assessment when phenotyping protocols are applied to groups of diverse characteristics is likely to come with the least challenges.

4.5. Controlling for Protocol Conditions

Although previous research presents an inconclusive picture [16,62,86], some studies report an effect of hunger [10,87,88] and thirst [89] on liking for sweet taste stimuli. It was thereby critical to ensure that recorded sensory responses were not driven by participants' motivational state and that the motivational state did not differ between the contrasted sweet taste liker phenotypes. Analysis of the pre- and post-test levels of hunger and thirst across our study sample and between-groups confirmed this was not so.

The nature of changes in levels of hunger and thirst over the test period (increased and decreased by 15.2% and 10.1%, respectively) also indicated little or no likely influence of post-ingestive effects of sucrose on the sensory-related measures [90], suggesting the "sip and spit" protocol worked as expected. Notably, Running and Hayes [91] observed no significant differences in the rated intensity of a 0.5 M sucrose solution when "sip and spit" and "sip and swallow" protocols were contrasted. Nonetheless, the differences in the density of taste buds [92] and in the associated saliva [93] across the different regions of the oral cavity and the known role of gastrointestinal tract's sweet taste receptors in metabolic regulation [94,95], suggest a need for both explicit instructions and subsequent compliance

checks in sensory evaluations, particularly when a wide range of concentrations or a relatively strong solution are being tested.

4.6. No Effect of Sweet Taste Liker Phenotype on Participant Characteristics

Analysis of this young healthy sample found no effect of sweet taste liker phenotype on the few demographic, lifestyle, and anthropometric characteristics we examined. First, the frequency distribution of the SL phenotype did not differ between women and men. With the exception of the multi-ethnic cohort of Thai et al. [53], lack of sex differences in sweet-liking is consistent with previous published work focusing on sweet taste liker phenotypes generated from simple sucrose solution-based taste tests and where women and men were represented equally [27,49,52,64,66,77]. In his recent review, Spence [96] argues that individual differences rather than sex differences might be the most important influence on shaping our taste worlds, particularly when the hedonic aspects of taste are studied. Animal models provide equivocal results on sucrose sensory properties by sex [97]. These findings fail to support Katz's theory of "gendered eating patterns" generated by either evolution or, according to others, by cultural norms [98], as well as baseline reports from the NutriNet-Santé study where, remarkably, men and not women liked sweet tastes more [99]. It is worth stressing though that sensory data in the French cohort were collected indirectly using "Pref-Quest," a proxy of laboratory-based taste tests that measures recalled liking for different taste modalities via asking questions on selective food items and eating habits [100]. In the present work, we also failed to observe an effect of age on hedonic responses to sweet taste. This stands in direct contrast to the fairly consistent effect of age on sweet-liking whenever children or adolescents were compared with adult populations [101–104], and may be due to the relatively restricted age range tested here. To note, in some [13,16,74,76,78,105–108] but not all [13,81,109–111] studies testing middle-aged or older adults, SDs and those with an inverted U-shaped response outnumbered SLs. Critically, methodological limitations that may lead to possible overestimation of the SD phenotype in prior studies cannot also be overlooked [11].

Other factors worth exploring with regard to humans' responses to sweet taste are dieting and BMI. Regarding attempts to investigate how being on a weight loss diet affects classification into the distinct sweet taste liker phenotypes, evidence has been loose and is drawn on research on sweet-liking either as a continuous measure (e.g., in References [112–114]) or assessed via questionnaires instead of laboratory-based taste tests [99]. As discussed in a recent review, bariatric surgery is also likely to augment gustatory sensitivity to sweet taste and to attenuate relevant hedonic responses post-operatively [115]. In our study, being a former dieter was more apparent in SDs. This may seem counterintuitive to the sensory specific satiety theory (decline in pleasantness for a food stimulus subsequent to consumption compared with the uneaten [29]), but could be backed up within the hedonic hunger context (motivation to consume palatable foods in the absence of food deprivation [116]). Nonetheless, no explicit information on the timing, duration, or mode of the dietary regime or the extent of weight loss and weight regain was collected. Additionally, considering the small size of this particular subgroup and the subsequent lack of significance, caution is advised in interpreting this observation until replicated. BMI, on the other hand, did not differ across the three sweet taste liker phenotypes. Although one can argue that this was due to the limited range of BMI in our sample, our finding was consistent with a sizable body of published evidence [13–15,17,24,49,53,66,69,76,106,117–119]. It is also of note that some early reports testing individuals of greater BMIs showed that obese were more often classified into the SD phenotype than normal-weight participants [16,26,54,120,121]; only one study of 12 participants has provided suggestive evidence for the opposite association [25].

4.7. Potential Mechanisms

Different mechanisms may account for the observed variations in affective responses to sweet taste, and fundamental biology likely plays a part. Sweet tasting substances activate various neural circuits

including some associated with dopamine-linked reward centers in the prefrontal cortex [122–124]. This activation accommodates the urge to meet physiological needs such as the central nervous system's energy supply (e.g., in Reference [125]). Internal state-specific factors ("homeostasis") have also been implicated in explaining the variation of hedonic responses to sweet taste as a function of deprivation state. In this context enhanced sweet-liking in fetuses [126,127] and infants [128–130] may relate to the increased needs for energy during the stages of rapid growth [131]. Likewise, Coldwell and colleagues reported that SL adolescents had higher levels of a bone growth factor compared with their SD peers [49]. Similarly, negative gustatory alliesthesia, which refers to diminishing liking as a response to internal energy abundance (as in satiety or obesity) [36], has been proposed to contribute to the apparent inverse relationship between BMI and sweet-liking.

Later advances have implicated taste genetics with sweetness, both directly and indirectly. TAS1R2 and TAS1R3 taste receptor genes have directly been linked to sweet taste perception [132–134]. The heterodimeric protein encoded by these genes is expressed in taste receptor cells in the oral cavity, providing the mechanism by which sweet taste occurs [135]; subsequently, these receptors have also been found in extra oral tissues [123]. Salivary glucose levels and salivary protein profile have recently identified as additional potential determinants of sweet taste perception [136]. Finally, some reports suggest that differences in the density of structures that house taste cells (i.e., fungiform papillae) may explain differences in suprathreshold taste intensity, including sweetness [92,137], although others account conflict with this explanation [138–141].

4.8. Limitations

The present study has some limitations that require further confirmatory analyses in different populations to allow the proposed method to be applied universally. First, we had a gender-imbalanced sample of young adults primarily from European Caucasian ancestry. Past literature has partly identified more SLs than SDs when direct contrasts between younger and older adults were performed [16,26,47,77]. Whether sweet taste liker phenotypes vary by ethnic group is, however, not yet well understood [18,23,49,53,76,107]. Nevertheless, due to the higher risk of many non-Caucasian ethnic groups and of older versus much younger individuals in developed countries for non-communicable diseases [142], this research area is worthy of further investigation. Our findings may also not translate to populations with a different habitual intake of sugar. Studies in the U.S., for example, suggest a slightly higher daily intake of free sugars [143] compared with U.K.-based cohorts [144], whereas the recommended daily allowance [145] is also double the U.K. recommendations [146]. On the basis of the conflicting evidence surrounding the influence of exposure in sweet-tasting foods on hedonic responses to sweetness [147,148], this limitation may leave particular populations vulnerable to any possible interplay between sweet-liking patterns and eating patterns and therefore much still need to be learned. Moreover, women and men in our sample were not of a representative BMI for their age-matched group [46]. Whilst this is presumably a caveat for the generalizability of our results, the reader is advised to consider that, as noted earlier, both in our study and elsewhere, BMI did not differ by sweet taste liker phenotype. Still, the fact that the observed proportion of SDs was relatively low, although it was expected from phenotyping results from prior studies using HCA (see Section 4.3 for details), it also means that group contrasts need to be treated with some caution. Finally, no phenotyping method is beyond limitations. The one inherent in using HCA is the lack of a formal "stopping rule" in the clustering process; the researcher is called to indicate the number of stages displayed in the agglomeration schedule that need to be eliminated from further merging and then manually incorporate this decision on the generated dendogram [41].

5. Conclusions

The present study confirms that the expression of sweet-liking is not universal but responses to sweet taste stimuli vary considerably across people. What is new is the statistical determination of some robust but concurrently usable classification criteria for the identification of the different

sweet taste liker phenotypes in a large-scale study. Despite limitations arising mainly from participant characteristics, there is good reason to believe that our approach might still be widely applicable as HCA-based liking patterns between our U.K. based study and those by American [13] and Korean [9] researchers largely align. Conceivably, the potential of a broader use of the psychophysical comparisons we delivered herein in epidemiological studies and clinical trials could have a fruitful impact on research associated with health and wellbeing. Accordingly, we may now have appropriate tools to finally address a longstanding issue first Mattes noted over 30 years ago, that is: "The question remains whether individual responsiveness to sweet taste can tell us anything about the individual, his or her physiological or nutritional status, or the likely patterns of food selection." [149].

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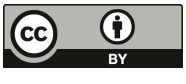

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A Comparison of Psychophysical Dose-Response Behaviour across 16 Sweeteners

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Abstract: Reduction or replacement of sucrose while maintaining sweetness in foods is challenging, but today there are many sweeteners with diverse physical and caloric compositions to choose from. The choice of sweetener can be adapted to match reformulation goals whether these are to reduce calories, lower the glycaemic response, provide bulk or meet criteria as a natural ingredient. The current study sought to describe and compare the sweetness intensity dose-response, sweetness growth rate, sweetness potency, and potential for calorie reduction across 16 different sweeteners including sucrose. Sweetness growth rate was defined as the rate of change in sweetness intensity per unit of sweetener concentration. Sweetness potency was defined as the ratio of the concentration of a sweetener to that of sucrose at equivalent sweetness intensity, whereas the potential for calorie reduction is the caloric value of a sweetener compared to sucrose at matched sweetness intensities. Sweeteners were drawn from a range of nutritive saccharide (sucrose, dextrose, fructose, allulose (D-psicose), palatinose (isomaltulose), and a sucrose–allulose mixture), nutritive polyol (maltitol, erythritol, mannitol, xylitol, sorbitol), non-nutritive synthetic (aspartame, acesulfame-K, sucralose) and non-nutritive natural sweeteners stevia (rebaudioside A), *luo han guo* (mogroside V). Sweetness intensities of the 16 sweeteners were compared with a sensory panel of 40 participants (n = 40; 28 females). Participants were asked to rate perceived sweetness intensity for each sweetener series across a range of concentrations using psychophysical ratings taken on a general labelled magnitude scale (gLMS). All sweeteners exhibited sigmoidal dose-response behaviours and matched the 'moderate' sweetness intensity of sucrose (10% w/v). Fructose, xylitol and sucralose had peak sweetness intensities greater than sucrose at the upper concentrations tested, while acesulfame-K and stevia (rebA) were markedly lower. Independent of sweetener concentration, the nutritive sweeteners had similar sweetness growth rates to sucrose and were greater than the non-nutritive sweeteners. Non-nutritive sweeteners on the other hand had higher potencies relative to sucrose, which decreases when matching at higher sweetness intensities. With the exception of dextrose and palatinose, all sweeteners matched the sweetness intensity of sucrose across the measured range (3.8–25% w/v sucrose) with fewer calories. Overall, the sucrose–allulose mixture, maltitol and xylitol sweeteners were most similar to sucrose in terms of dose-response behaviour, growth rate and potency, and showed the most potential for sugar replacement within the range of sweetness intensities tested.

Keywords: sweeteners; sugar reduction; psychophysical dose-response; sweetness growth rate; sweetness potency

1. Introduction

Sweetness is a key driver of liking in food products and a heightened liking for sweet tastes has been associated with increased intakes of foods with added sucrose [1]. The rising incidence of obesity and type-2 diabetes has been linked with excessive sucrose intake, and fuelled the need for reducing added sucrose in food products [2,3]. Countries such as the United Kingdom and Singapore have pledged to cut sucrose to either 5% free sugars in foods [4] or a 25% sucrose reduction from the current levels [5], namely through reducing added sucrose, using non-nutritive sweeteners and public health education. Sweetness intensity is associated with liking and reducing sucrose can negatively impact the hedonic appeal of a product and consumer acceptance of reformulated products, thereby limiting the widespread reduction of sucrose to achieve these public health goals. Non-nutritive sweeteners can be used to maintain product sweetness, while reducing the negative health impact of excessive sucrose intake, including increased body weight and risk of type-2 diabetes and cardiovascular diseases [6–8]. Sweet taste intensity has been shown to be associated with sucrose content of a product, but not with its energy content [9,10] thus creating an opportunity to reduce energy whilst matching sweet taste intensity and liking through the use of lower calorie sweeteners. As such, there has been a rising trend in the use of non-nutritive sweeteners such as sucralose and aspartame, in line with an increasing consumer demand for reduced-calorie foods. In the United States, 1 in 4 consumers include non-nutritive sweeteners in their diets based on a 24-hour diet recall [11]. This may be an effective strategy to improve public health, and a recent meta-analysis has shown that transition to lower-energy sweeteners in place of sucrose leads to reduced energy intake and body weight in both children and adults, as energy reductions associated with the intake of these sweeteners is often not fully compensated for during subsequent eating episodes [7].

Synthetic non-nutritive sweeteners like aspartame and sucralose are still the most widely consumed due to their low cost, quality of their sweet taste and calorie-free advantage, although their long-term metabolic impacts are still being investigated [8]. In addition to reduced sucrose and calories, in recent years there has been a rise in consumer demand for 'natural' and clean-label ingredients [11]. As a result, many food manufacturers have shifted towards the use of natural sweeteners such as plant-based glycoside extracts from stevia (rebaudioside, stevioside) and monk fruit (*luo han guo*; mogroside V). Alternative sugars such as the rare sugar D-psicose (allulose) and the isomerized sucrose isomaltulose (palatinose) have also gained interest due to their natural source, clean sweet taste and post-ingestive anti-glycaemic effects [12–14]. Polyol sweeteners are a group of sugar alcohols that have been reported to have excellent sweetness quality [15], fewer calories than sucrose and can also act as bulking agents in low sucrose foods, giving them an advantage over several non-nutritive sweeteners [16,17]. To date, the sweetness intensity and dose-response behaviour of many of these more recent sweeteners such as allulose, palatinose, a sucrose–allulose mixture and *luo han guo*, have not been compared alongside sucrose and other sweeteners.

Dose-response relationships have previously been reported for a range of different commercial sweeteners using, for example, the magnitude estimation or spectrum scaling method standardised with reference sucrose solutions [18–21]. This method obtains relative perceived sweetness intensity values but the comparison to other studies as is highly dependent on the reference solution, scaling method and extent of participant training [21,22]. More recently, psychophysical approaches have compared perceived sweetness intensity using ratings made on the general labelled magnitude scale (gLMS) [23–25]. This technique allows for relative comparisons of perceived sweetness intensity between sweeteners across concentrations, and can be useful for determining sweetening capabilities of a novel sweetener in relation to sucrose and other sweeteners [24].

The current study sought to characterise the perceived sweetness intensity of a wide range of different sweeteners to sucrose using a contemporary psychophysical approach. Based on the change in sweetness intensity across a range of concentrations, the dose-response behaviour of each sweetener was compared for their sweetness growth rate, sweetness potency, and potential to support calorie reduction at an equivalent sweetness intensity. These sweetness characteristics can be used as indices

to gauge the sweetness and concentration-dependency of a sweetener in relation to sucrose. The sweetness growth rate is the Stevens' power law exponent in psychophysical terms, and is defined as the slope of the psychophysical relationship describing the rate of change in sweetness intensity with the rate of change in concentration [19]. Sweetness potency was defined as the ratio of the concentration of a sweetener to that of sucrose at an equivalent sweetness intensity [24], whereas the potential for calorie reduction is the caloric value of a sweetener compared to sucrose at matched sweetness intensities. These characteristics were examined across the selected sweeteners to compare sweetener suitability when attempting to reduce or replace sucrose.

2. Materials and Methods

2.1. Materials

A wide range of different sweeteners was selected to represent a diverse sample of common commercially available sweeteners. The sweeteners used in this study were sucrose (SIS, NTUC Fairprice Supermarket, Singapore), dextrose monohydrate (Suntop Enterprise, Singapore), fructose (Suntop Enterprise, Singapore), allulose (D-psicose; Matsutani Co., Osaka, Japan), palatinose (isomaltulose; Beneo, Singapore), xylitol (Roquette, Lille, France), sorbitol (Suntop Enterprise, Singapore), mannitol (Roquette, Lille, France), erythritol (iHerb, Perris, CA, USA), acesulfame-K (Celanese, Irving, Texas, USA), sucralose (Tate & Lyle, McIntosh, AL, USA), aspartame (Suntop Enterprise, Singapore), *luo han guo* extract (50.6% mogroside V; Hunan Huacheng Biotech Inc., Hunan, China) and stevia (68.0% rebaudioside A; Suntop Enterprise, Singapore). The sucrose–allulose mixture was prepared as a 1:1 blend of sucrose and allulose by weight. Table 1 summarises these sweetener properties including energy density, glycaemic index and bulk properties.

Table 1. Characteristics of the 16 sweeteners used.

Sweetener	Kcal (kcal/g)	Glycaemic Index	Provides Bulk	High Potency	Natural
Acesulfame-K	0.0	0		✓	
Allulose	0.2	0	✓		✓
Aspartame	4.0	0		✓	
Dextrose	3.4	100	✓		✓
Erythritol	0.2	0	✓		✓
Fructose	3.7	19–23	✓		✓
Luo han guo	0.0	0		✓	✓
Maltitol	2.7	36	✓		
Mannitol	1.5	0	✓		
Mixture ‡	2.1	-	✓		✓
Palatinose	4.0	32	✓		✓
Sorbitol	2.5	9	✓		
Stevia (RebA)	0.0	0		✓	✓
Sucralose	0.0	0		✓	
Sucrose	4.0	60	✓		✓
Xylitol	2.5	13	✓		

‡ 1:1 sucrose−allulose mixture (weight basis). A ✓ indicates that the sweetener belongs to the respective category.

2.2. Participants

Forty healthy adult participants (12 males and 28 females; mean age: 26 ± 6 years) were recruited from the campus of the National University of Singapore (NUS) and surrounding areas. Participants were pre-screened for eligibility, basic taste sensitivity and recruitment criteria including being aged between 21 and 50 years old, normal weight (body mass index (BMI) 20–25 kg/m^2), non-smoker, non-denture wearer, no self-reported sinus, taste or smell dysfunction, not currently following a special diet, no specific food dislikes, allergies or intolerances and not phenylketonuric, diabetic or pregnant. Eligible participants provided informed consent and were compensated for their time. This study (reference: 2017/00787) was approved by the Domain Specific Review Board of the National

Healthcare Group, Singapore and complies with the Declaration of Helsinki for research involving human subjects.

2.3. Training and Test Procedure

All participants underwent a total of 5 one-hour sessions on separate days, including 1 training session and 4 test sessions. During training, participants familiarised themselves with rating perceived sweetness intensity using the general Labelled Magnitude Scale (gLMS), based on a previously published approach [25]. Participants were asked to rate the overall taste intensity for seven imagined and/or recalled sensations described verbally including the warmth of lukewarm water and the pain from biting their tongue. Thereafter, participants were presented with four basic taste samples and asked to rate sweet (sucrose), salty (NaCl), sour (citric acid) and bitter (caffeine) to ensure they understood how to use the line scale and practice making ratings using the gLMS.

During each test session, participants rated the sweetness intensities of four sweetener sets, with eight samples for each sweetener set. The order of sweetener presented was randomised and balanced across participants and test sessions using a William's (Latin Square) design. The order of sample presentation within each sweetener set was also randomised. Participants rated a total of 16 sweetener sets over 4 test sessions. For each sweetener set, there is a water sample, six different concentrations of the sweetener (Table 2), and a warm-up sample with a duplicate concentration to one of the samples (sample 4/5; Table 1). The warm-up sample was presented at the beginning to reduce first order effects (data not included in analysis). The concentrations used in this study are by weight basis (% *w*/*v*) presented in Table 2, and the same concentrations expressed in molarity (mmol/L) are provided in the supplementary material (Table S1). For ease of interpretation of the calorie reduction potential and application to product re-formulation, the dose-response behaviour of the sweeteners was expressed on a weight basis in the current study, in line with previous reports [18,21,24]. Therefore, discussions made in this study are based on weight of sweeteners and not by molarity.

Table 2. Concentrations tested for each of the sweetener set by weight basis (% *w*/*v*).

Sweetener	Abbreviation	Concentration (% *w*/*v*)						
		Sample 1	Sample 2	Sample 3	Sample 4, 5	Sample 6	Sample 7	Sample 8
Acesulfame-K	(ACE)	0	0.0100	0.0219	0.0478	0.105	0.229	0.500
Allulose	(ALL)	0	3.80	5.50	8.00	11.7	17.1	25.0
Aspartame	(ASP)	0	0.0100	0.0197	0.0390	0.0770	0.152	0.300
Dextrose	(DEX)	0	3.80	5.50	8.00	11.7	17.1	25.0
Erythritol	(ERY)	0	3.80	5.50	8.00	11.7	17.1	25.0
Fructose	(FRU)	0	3.80	5.50	8.00	11.7	17.1	25.0
Luo han guo	(LHG)	0	0.0100	0.0197	0.0390	0.0770	0.152	0.300
Maltitol	(MAL)	0	3.80	5.50	8.00	11.7	17.1	25.0
Mannitol	(MAN)	0	3.80	5.50	8.00	11.7	17.1	25.0
Mixture ‡	(MIX)	0	3.80	5.50	8.00	11.7	17.1	25.0
Palatinose	(PAL)	0	3.80	6.30	10.6	17.7	29.8	50.0
Sorbitol	(SOR)	0	3.80	5.50	8.00	11.7	17.1	25.0
Stevia	(STE)	0	0.00400	0.00830	0.017	0.0352	0.0727	0.150
Sucralose	(SCL)	0	0.0100	0.0204	0.0415	0.0844	0.172	0.350
Sucrose	(SUC)	0	3.80	5.50	8.00	11.7	17.1	25.0
Xylitol	(XYL)	0	3.80	5.50	8.00	11.7	17.1	25.0

‡ 1:1 sucrose–allulose mixture (weight basis).

All data were collected using Compusense Cloud software as part of the Compusense Academic Consortium (Compusense Inc., Guleph, ON, Canada), in sensory booths under red lights which conform to international standards for the design of test rooms [26]. Participants were instructed to take the sample (15 mL) in their mouth, hold it for 5 s, and rate the sweetness intensity before expectorating. Between samples, participants were instructed to rinse their mouth with filtered water during the mandatory 45-second inter-stimulus interval, to reduce carryover between samples. Solutions were prepared at least 24 h prior to sensory testing using filtered water and stored at refrigeration temperature. The concentration ranges chosen were based on previously published

results for each sweetener [23,27,28], and to reflect the sweetness intensities encountered in commercial food and beverage products. Preliminary testing was done to confirm that the sweetness intensities rated for each sweetener were comparable to one another.

2.4. Psychophysical Scaling

Perceived sweetness intensity was rated using a general labelled magnitude scale (gLMS) [22,29,30]. The scale is partitioned by descriptors: no sensation (0), barely detectable (1.5), weak (6), moderate (17), strong (35), very strong (52) and strongest imaginable sensation (100). Individual scaling behaviours for gLMS ratings were standardized within participants using a previously published weight comparison modality matching task [25,31]. All participants were asked to make intensity ratings using the gLMS across a series of different weight stimuli while holding the container on the palm of their non-dominant hand. There was a significant correlation between the overall sweetness ratings and overall mean heaviness ratings ($r = 0.472$, $p < 0.05$). Assuming the intensity ratings of sweetener samples and the heaviness of the bottles were unrelated, the significant correlation indicated that gLMS ratings were due to individual scale-use rather than differences in sweeteners, and thus required standardization across participants. For each participant, a personal standardization factor was obtained using the grand mean for heaviness ratings across weights and participants divided by the individual's average heaviness ratings. The sweetness intensity rankings for each participant were then multiplied by their individual personal standardization factor to correct for idiosyncratic differences in scale use.

2.5. Mathematical Modelling and Data Analysis

2.5.1. Dose-Response Curves

Dose-response curves were fitted using the software Origin Pro 8.1 (OriginLab, Northampton, MA, USA) with the Hill equation for sigmoidal curves:

$$\mathrm{R} = \mathrm{R_{min}} + \frac{\mathrm{R_{max}} - \mathrm{R_{min}}}{1 + 10^{(\log \mathrm{EC_{50}} - \mathbf{C}) \times \mathrm{HillSlope}}} \quad (1)$$

where R is the predicted sweetness intensity, and C is the sweetener concentration expressed in % w/v. $\mathrm{R_{min}}$ is the minimum sweetness which was constrained to zero, and $\mathrm{R_{max}}$ is the predicted maximum sweetness achievable. The midway point between $\mathrm{R_{min}}$ and $\mathrm{R_{max}}$ is EC50, and the slope of the linear portion of the model is the Hill slope [23]. The fitted parameters are summarised in the supplementary material (Table S2).

2.5.2. Sweetness Growth Rate

Dose-response curves were also converted to log sweetness intensity vs. log concentration plots, which were originally derived from the power law function $R = \mathrm{kC^n}$ in the linear form:

$$\log \mathrm{R} = n \log \mathrm{C} + \log \mathrm{k} \quad (2)$$

where R is the predicted sweetness intensity, C is the sweetener concentration expressed in % w/v, n is the sweetness growth rate (slope of the line or Stevens' power law exponent), and k is the constant (intercept). The sweetness growth rate provides an overall average index of the rate of change for sweetness intensity with change in sweetener concentration. A sweetener with a steep slope (>1) increases in their perceived intensity at a rate that is faster than changes in concentration, whereas for flatter slopes (<1), greater increases in sweetener concentration are required to produce an equivalent change in sweetness intensity. The log k (intercept) values also refer to the log sweetness intensity of the sweetener at a concentration of 1% w/v.

2.5.3. Sweetness Potency

Sweetness potency is the ratio of the concentration of sucrose to that of a sweetener at equivalent sweetness intensities (Equation (3)). A sweetness potency of >1 indicates that a smaller concentration of a sweetener is required to achieve the same sweetness intensity at a particular sucrose concentration and, therefore, the sweetener could be considered as 'more potent' than sucrose. Sweetness potency is often quoted as a single value e.g., 'acesulfame-K is 120 times sweeter than sucrose', however this value should always be reported with the concentration of sucrose at which it was calculated, since sweeteners often have different sweetness growth rates to sucrose.

$$\text{Sweetness Potency} = \frac{\text{Concentration of sucrose}}{\text{Concentration of sweetener at equi} - \text{sweetness intensity to sucrose}} \quad (3)$$

2.5.4. Statistical Analysis

A two-way analysis of variance (ANOVA) was run to confirm absence of first-order and carryover effects. A one-way repeated measures ANOVA analysis was used to test the effect of sweetener type and effect of concentration and statistical significance was set at 5% ($\alpha = 0.05$). Post hoc pairwise comparisons, using Bonferroni corrections, were used to compare differences in sweetness intensity scores across sweeteners (16 levels) and concentration of sweeteners (6 levels) using the statistical analysis software SPSS (IBM SPSS Statistics for Windows, Version 22.0, IBM Corporation, Armonk, NY, USA).

3. Results

The dose-response for all sweeteners are illustrated on semi-log curves (Figure 1A–C) and fitted with the Hill equation (Equation (1)) with $r^2 \geq 0.95$ for all sweeteners. The fitting parameters are listed in Table S2. Repeated-measures ANOVA confirmed that all sweeteners exhibited a concentration dose-dependency for sweetness intensity ($F_{5,39} = 142.12$, $p < 0.001$). Sweetener type had a significant effect on sweetness intensity as concentrations increased ($F_{15,39} = 18.05$, $p < 0.001$) and this was confirmed as a significant interaction between concentration and sweetener type ($F_{75,39} = 4.20$, $p < 0.001$).

3.1. Concentration Dose-Response of Sweeteners

The sweetness intensity of sucrose ranged from 'barely detectable' (3) to 'strong' (33) on the gLMS for the concentration range of 3.8 to 25% *w*/*v*. Nutritive saccharide and polyol sweeteners sucrose, dextrose, allulose, palatinose, maltitol, sorbitol, mannitol, xylitol and erythritol exhibited sigmoidal dose-response functions. By contrast, fructose displayed a more linear response and had a higher sweetness intensity than sucrose and other nutritive sweeteners, across all sucrose concentrations (Figure 1A,B). The sucrose–allulose mixture, maltitol and xylitol had dose-response curves closely matched to sucrose within the range of 3.8 to 25% *w*/*v* sucrose. The dose-response curve for xylitol was similar to sucrose at lower concentrations but had higher sweetness intensity above 11.7% *w*/*v*. Allulose was perceived as less sweet than sucrose at equivalent concentrations, but when allulose and sucrose were blended in a 1:1 mixture, this blend achieved a near identical dose-response curve to sucrose. Palatinose required the highest concentration to match the sweetness intensity of sucrose, and only produced a noticeable rise in sweetness intensity as the concentration went above 10% *w*/*v*. Dextrose, erythritol, sorbitol and mannitol all had lower sweetness intensities than sucrose across the concentration range tested.

Non-nutritive sweeteners exhibited sigmoidal dose-response functions (Figure 1C) and stevia (rebA) and acesulfame-K had flatter responses at low and high concentrations, where increased concentration produced smaller increments in perceived sweetness intensity. In addition, maximum sweetness for these sweeteners peaked below sucrose at 'moderate' (25). Sucralose had a higher peak sweetness intensity (35) compared to sucrose (33) at the highest concentration, and was higher than

the other non-nutritive sweeteners across equivalent concentrations. Aspartame and *luo han guo* both had similar peak sweetness to sucrose, and their dose-response curves were similar to each other. Their sweetness intensities were weaker than stevia (rebA) at low concentrations (0.01–0.1% *w*/*v*) but stronger at higher concentrations (>0.1% *w*/*v*) when the sweetness intensity of stevia (rebA) plateaued.

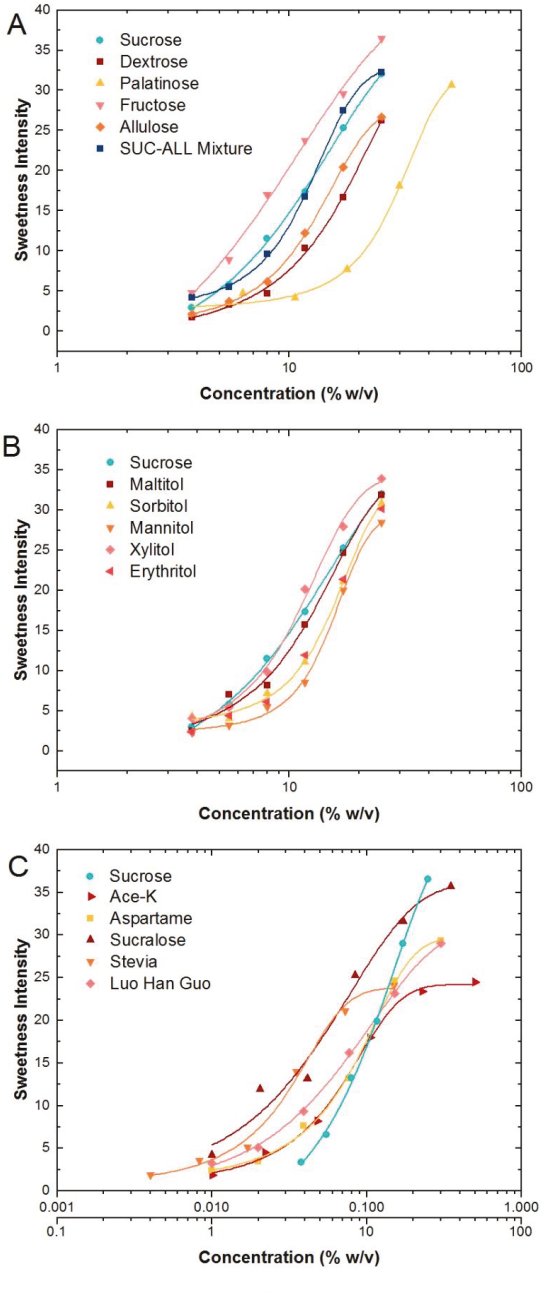

Figure 1. Sweetness intensity with concentration for (**A**) saccharide, (**B**) polyol and (**C**) non-nutritive sweeteners (sucrose is plotted using the secondary x-axis below (0.1–100% *w*/*v*)).

3.2. Comparison of Sweetness Growth Rates across Sweeteners

The sweetness growth rate is represented by the slope of the log-log sweetness intensity concentration curves (% *w*/*v* basis) (Figure 2 and Table 3). Sucrose had a sweetness growth rate of 1.31 whereas saccharide sweeteners (dextrose, palatinose, fructose, allulose, sucrose–allulose mixture,) had sweetness growth rates >1, ranging from 1.08 (fructose) to 1.46 (sorbitol). The bulk polyol sweeteners (sorbitol, xylitol, mannitol and erythritol) had sweetness growth rates with similar slopes to sucrose (~1.3–1.4), indicating a similar growth rate to sucrose such that changes in concentration produce similar changes in sweetness intensity. Palatinose and fructose yielded much flatter sweetness growth rates (slopes ≈ 1) amongst the nutritive sweeteners, with 1.10 and 1.08 respectively. By contrast, non-nutritive sweeteners had compressed sweetness growth rates < 1, ranging from 0.65 (sucralose) to 0.84 (aspartame).

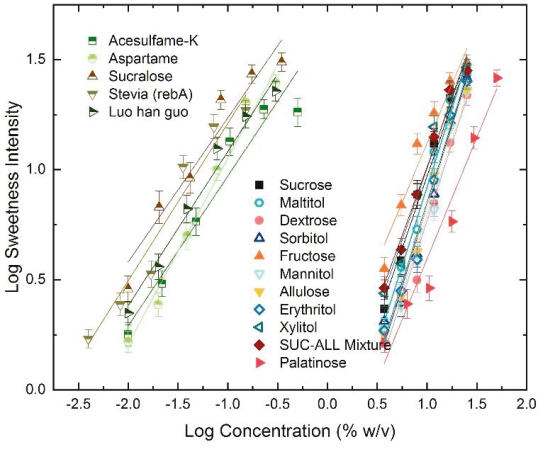

Figure 2. Log sweetness intensity vs. log concentration for 16 sweeteners.

Table 3. Slope and y-intercept values of linear fit between log sweetness intensity and log concentration (% *w*/*v*).

Sweetener.	Slope (*n*)	Y-Intercept (log k)
Acesulfame-K	0.68	1.65
Allulose	1.41	−0.58
Aspartame	0.84	1.90
Dextrose	1.40	−0.63
Erythritol	1.45	−0.6
Fructose	1.08	0.04
Luo han guo	0.70	1.78
Maltitol	1.42	−0.51
Mannitol	1.38	−0.59
Mixture ‡	1.24	−0.23
Palatinose	1.10	−0.51
Sorbitol	1.46	−0.63
Stevia	0.71	1.93
Sucralose	0.65	1.89
Sucrose	1.31	−0.33
Xylitol	1.30	−0.29

‡ 1:1 sucrose–allulose mixture (weight basis).

3.3. Sweetness Potency of Sweeteners Relative to Sucrose

Sweetness potency as well as the concentration of sweetener required to achieve equivalent sweetness intensity to sucrose concentrations at 5%, 10% and 15% *w*/*v* are summarised in Table 4. Saccharide and polyol sweeteners had sweetness potencies <1, with the exception of xylitol (at 15% *w*/*v* sucrose) and fructose. Sweetness potency values for allulose increased from 5% to 15% *w*/*v* sucrose respectively, whereas the sweetness potency for maltitol, xylitol and sucrose–allulose mixture were closer to sucrose across sucrose concentrations, emphasising the similarity of their dose-response functions (Figure 1). Non-nutritive sweeteners had decreasing sweetness potencies at increasing sucrose concentrations. Sucralose has the highest sweetness potency across all sweeteners, but also the largest decline, from sweetness potency of 521 at 5% *w*/*v*, to 201 at 15% *w*/*v* sucrose. Aspartame had the smallest decline in sweetness potency among the non-nutritive sweeteners at higher sucrose concentrations.

Table 4. Concentrations matching for equi-sweetness and sweetness potency of 15 sweeteners to 5%, 10% and 15% *w*/*v* sucrose.

Sweetener	Equi-Sweet Concentrations (% *w*/*v*)			Sweetness Potency		
	5% SUC	10% SUC	15% SUC	5% SUC	10% SUC	15% SUC
Acesulfame-K	0.0293	0.0832	0.170	171	120	88.1
Allulose	7.1	13.3	18.9	0.71	0.75	0.80
Aspartame	0.0290	0.0827	0.134	173	121	112
Dextrose	7.8	15.5	21.6	0.64	0.64	0.69
Erythritol	6.9	13.3	17.8	0.72	0.75	0.84
Fructose	4.0	7.4	11.2	1.25	1.36	1.34
Luo han guo	0.0191	0.0694	0.141	262	144	106
Maltitol	5.6	11.2	15.8	0.93	0.89	0.95
Mannitol	8.6	14.6	18.6	0.58	0.68	0.81
Mixture ‡	5.0	10.7	14.3	0.99	0.93	1.05
Palatinose	12.7	26.4	34.6	0.39	0.38	0.43
Sorbitol	6.3	13.7	17.9	0.80	0.72	0.83
Stevia	0.0144	0.0395	0.0828	348	253	181
Sucralose	0.0096	0.0387	0.0748	521	258	201
Sucrose	5	10	15	1	1	1
Xylitol	5.1	9.9	13.3	0.98	1.01	1.12

‡ 1:1 sucrose–allulose mixture (weight basis).

3.4. Potential for Calorie Reduction across Sweeteners

Figure 3 shows the caloric value across the different nutritive sweeteners at sweetness intensities ranging from weak (6) to strong (35). The equivalent sweetness intensity to sucrose per unit calorie provides a summary of the calorie saving potential across the different sweeteners. With the exception of dextrose and palatinose, all of the other nutritive sweeteners enable calorie saving at an equivalent perceived sweetness intensity to 10% sucrose (indicated by red line on Figure 3). Allulose and erythritol have the lowest energy densities (0.2 kcal/g) and can achieve an equivalent sweetness intensity to 10% sucrose with very few calories (~95% reduction). For example, a product with 10% *w*/*v* sucrose could potentially be reduced from 40 kcal to <5 kcal/100ml by substituting with allulose or erythritol. Mannitol, sucrose–allulose mixture, xylitol, fructose, maltitol and sorbitol provide about 5–20 kcal/100 mL savings in terms of energy required to achieve equivalent sweetness to 10% sucrose.

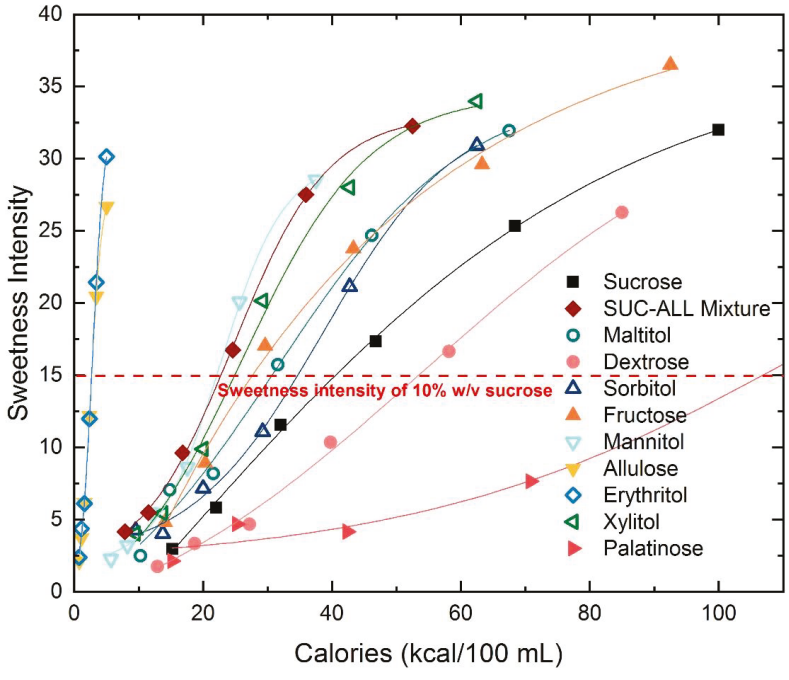

Figure 3. Energy content (kcal/100 mL) of nutritive saccharide and polyol sweeteners to achieve sweetness intensities ranging from weak (6) to strong (35).

4. Discussion

In order to support sugar reduction, sweeteners must first match the sweetness intensity of sucrose across the range of perceived intensities commonly encountered in foods and beverages. From a public health perspective, the reduction in sucrose should also support calorie reduction while maintaining consumer appeal beyond sensory-matching perceived sweetness. In addition to their sweetening capacity, sweeteners that can confer additional functionality such as acting as bulking agents, supporting clean labelling or providing an additional anti-glycaemic effect are also highly desirable. A wide variety of sweeteners are currently available and the present study sought to evaluate the sweetening capabilities of these sweeteners in comparison to sucrose based on their dose-response behaviour, sweetness growth rate, sweetness potency and potential calorie savings at equal sweetness intensities.

All sweeteners exhibited sigmoidal dose-response behaviours although fructose displayed a more linear response across the concentration range tested. This sigmoidal relationship between concentration and perceived intensity is the result of the binding kinetics of the sweetener molecules to taste receptors, which plateaus at higher concentration when receptors become saturated [32,33]. From the dose-response curves, all sweeteners were found to match the perceived sweetness intensity of a 10% w/v sucrose solution, which represents a 'moderate' sweetness associated with 10% sugar that is frequently found in many commercially available sweetened and carbonated beverages (e.g., Arizona Ice Tea 10.1 g/100 mL) [34]. This aligns with similar findings from other studies, where the sweetness intensity of ~10% w/v sucrose was also found to be of 'moderate' intensity with rating scores approximately 15 to 20 on the gLMS [23–25]. Interestingly, the perceived sweetness intensity of the sucrose–allulose mixture (1:1) was nearly identical to that of sucrose by weight basis, although allulose on its own had consistently lower sweetness intensity than sucrose across the concentrations tested. Previous research has demonstrated that the sweetness intensity of binary

mixtures of sweeteners is often an intermediate of the two compounds when tasted alone and at the same total molarity as the mixtures [35,36]. Since the weight of allulose (monosaccharide) is half that of sucrose (disaccharide), the dose-response behaviour of the sucrose–allulose mixture was expected to be between that of sucrose and allulose when expressed in terms of total molarity. Other nutritive sweeteners with smaller molecular weights than sucrose, such as fructose or xylitol, would be relatively even less sweet than sucrose on a molarity basis as compared to weight basis [37]. Nonetheless, for purposes of sweetener application to product re-formulation and interpretation of the calorie reduction potential, the dose-response behaviour of the sweeteners were expressed on a weight basis in the current study, in line with previous reports [18,21,24]. Fructose, xylitol and sucralose were the only sweeteners which had greater peak sweetness intensities than sucrose at the highest concentrations tested, and this has previously been demonstrated across a range of previous studies [23,38,39]. This suggests that 'high-intensity' sweeteners such as aspartame and sucralose may be more accurately described as 'high-potency' sweeteners, as proposed previously by Antenucci and Hayes [23]. The peak sweetness intensities for the non-nutritive sweeteners acesulfame-K and stevia (rebA) were markedly lower than that of sucrose, reaffirming that these high-intensity sweeteners are not necessarily higher in perceived sweetness intensity than sucrose. Further concentration increments of acesulfame-K, stevia (rebA) and sucralose have been shown to produce a further decrease in sweetness intensity [23,24], which was likely due to bitter taste antagonism at higher concentration among these sweeteners [40]. This decrease in sweetness was not observed for any sweeteners at the concentrations used in the current study. The low peak sweetness of acesulfame-K and stevia (rebA) could limit their use in foods where higher sweetness intensities are required. Nevertheless, it is difficult to determine the true peak sweetness achievable unless a plateau in sweetness can be clearly observed [21], on the condition that the intensity scaling method is not limited by a ceiling effect [29,41]. The concentration ranges for the sweeteners were selected prior to the study based on literature and preliminary experiments, although we acknowledge that further concentration increments would likely result in greater perceived sweetness for some sweeteners. In this case, comparing the sweetness growth rate would be a better indicator of the dose-response trajectory rather than the peak sweetness of each sweetener, to understand whether they are likely to match or surpass the sweetness of sucrose.

The sweetness growth rate is the Stevens' power law exponent or slope of the log relationship between changing concentration and the perceived sweetness. It should be noted that the sweetness growth rate obtained in this study is a product of the concentration ranges from which they are derived. These range effects mean that sweetness growth rates can change to be higher or lower depending on the range of concentrations tested, and a higher sweetness growth rate is obtained with a smaller concentration range [42]. Sucrose had a sweetness growth rate of 1.3 which is consistent with previous findings which reported sweetness growth rates of 1.15 to 1.3 [18–20,24]. The sweetness growth rates of sucralose, stevia (rebA), dextrose and mannitol were also found to be comparable to those previously reported [18,24] and collectively these findings highlight that sucrose, and other nutritive sweeteners exhibit sweetness growth rates greater than non-nutritive sweeteners. This appears counterintuitive since only a small quantity of non-nutritive sweetener is required to impart an intense sweetness, which should be perceived as a higher growth rate. However, sweetness growth rates are based on sweetness intensity changes *per* unit log-concentration, and in relative terms greater quantities of non-nutritive sweeteners are required to achieve a proportional increment in perceived sweetness. This may also be due to the emergence of bitter side-tastes and taste–taste antagonism at higher concentrations [40], or different binding mechanisms across sweeteners, which often remain poorly understood [43]. Sweeteners with lower sweetness growth rates to sucrose are capable of matching the sweetness intensity, albeit over a limited concentration range. The implication is that sweetness growth rates should be considered when estimating the predicted sweetness intensity of a sweetener at concentrations beyond those reported in the dose-response curves. For example, sucralose matched the sweetness intensity of sucrose to an upper concentration of 25% w/v, but displayed a smaller growth rate, suggesting that the peak sweetness intensity for sucralose would be lower than that of sucrose.

This is further supported by the flatter dose-response curve for sucralose at higher concentrations (0.172–0.350% w/v).

Sweeteners with the same growth rate as sucrose will increase in perceived sweetness intensity with equal increases per unit concentration. Sweetness potency or relative concentration of sweetener required to produce an equi-intense sweetness to sucrose would, therefore, be similar across a range of different concentrations. By contrast, sweeteners with growth rates that differ significantly from sucrose would have sweetness potencies that vary with sucrose concentration, as demonstrated previously [19,20,24,44]. While sweetness growth rate and sweetness potency are closely related indices, sweetness potency is more often used as a quick indication of the quantity required to achieve an equi-intense sweetness to sucrose at a given concentration. Non-nutritive sweeteners have growth rates significantly lower than sucrose, and therefore their sweetness potency is also highly concentration-dependent. The sweetness potency values reported for non-nutritive sweeteners in the current trial were not fully consistent those reported previously. For example, aspartame was found to be 128 and 185 times more potent than sucrose at 5 and 10% sucrose equivalence by Tunaley, Thomson and McEwan [45], and Cardello et. al. [20] respectively, as compared to the 173 (5% w/v) and 121 (10% w/v) found in our study. Differences were also found for sweetness potencies of stevia (rebA) and sucralose [24]. Sucrose–allulose mixture, xylitol and maltitol have sweetness potencies closest to 1, indicating that the quantities required to achieve an equivalent sweetness intensity on a weight basis are similar to sucrose. This is an important consideration when the replacement sweetener is also required to substitute some of the bulking properties of the removed sucrose. When calorie reduction without the addition of bulk is the main goal of sucrose reduction, low calorie and/or high potency sweeteners may be more effective, particularly among certain products (i.e., beverages) as lower concentrations are required to match sweetness intensity.

With the inclusion of several low-calorie nutritive sweeteners in the study, it is still possible to achieve calorie reduction while maintaining sweetness, even when sweetness potency was not equivalent or higher than sucrose. With the exception of dextrose and palatinose, the nutritive sweeteners profiled supported reductions in total calories to varying extents while meeting the equivalent sweetness intensity of a 10% sucrose solution. Allulose and erythritol in particular have the lowest calories at a sweetness intensity equivalent to 10% sucrose, and could be used to support substantial calorie savings. When allulose was mixed 1:1 to partially replace sucrose, the sucrose–allulose mixture showed very similar sweetening properties to sucrose, while supporting significant reduction in overall calories. Considering the sweetness intensity, sweetness growth rate, sweetness potency and potential calorie reduction together, the sucrose–allulose mixture, maltitol and xylitol were most similar to sucrose, across the concentrations studied. All three sweeteners can provide bulk, support a clean label, reduce total calories for equivalent sweetness intensity and in the case of sucrose–allulose mixture, also impart an additional anti-glycaemic effect post-ingestively [12,14]. When selecting the appropriate sweetener for use in sugar reduction, the sensory, physical, nutrient and metabolic impact of the selected sweetener should be considered, and in some cases sweeteners will have desirable characteristics for some properties but not others. For example, palatinose has an anti-glycaemic benefit which is desirable for products that support the management of glucose homeostasis, but it is required at a greater concentration and energy content to achieve an equi-intense sweetness intensity to sucrose [13]. Non-nutritive sweeteners are calorie-free but may have certain undesirable side-taste attributes, especially at higher concentrations [23,24,40,46] which may limit their usage. With these factors considered, it may be judicious to blend sweeteners with sucrose to optimise the sensory profile and sweetening capacity, and compromise on some elements of the nutrient or metabolic profile. Results from the current study demonstrate that blends like the sucrose–allulose mixture provide encouraging results with excellent sweetness characteristics in line with sucrose, at a fraction of the calories and a potential post-consumption anti-glycaemic benefit.

Findings from the current study are aligned with previously reported differences in sweetness dose–response, growth rate and potency, although some subtle differences were observed in

the absolute values reported. These are likely to be due to differences in methodological approach, individual variability, sweetener source, matrix effects, concentration range used, pH and temperature [20,21,23,25,47]. Our findings are most closely aligned with those previously reported by Antenucci and Hayes which were collected using the same standardised gLMS approach to rate sweetness intensity. This approach minimises ceiling effects and produced comparable intensity ratings for many of the same sweeteners [23]. There is currently no official standardised approach to quantify the perceived sweetness intensity of a sweetener, although the comparability of results would be greatly enhanced if future efforts adopt a consistent objective approach, such as used in the current and previous studies [23–25].

In choosing to focus on sweetness intensity alone, we have not accounted for other temporal and qualitative taste differences between the sweeteners reported elsewhere [46]. In addition, perceived sweetness intensity rated in water does not account for matrix effects or taste–taste interaction that would occur when these same sweeteners and concentrations are used in foods and beverages [48,49]. The current findings present an overview of the psychophysical dose-response behaviour of a wide range of different sweeteners, and provides guidance on the similarity of various sweeteners to sucrose and the likely calorie savings that could be achieved if they are used to reduce or replace sugar. Future research should aim to extend this further by profiling the temporal and qualitative differences between sweeteners and characterising their performance in food and beverages.

5. Conclusions

The current paper characterized the psychophysical dose-response behaviour of 16 sweeteners and identified differences in the peak sweetness, sweetness potency and sweetness growth rate. Sucrose—allulose mixture, maltitol and xylitol exhibited similar psychophysical behaviours to sucrose in terms of peak sweetness intensity, sweetness growth rate and sweetness potency, and showed the greatest potential to match the sweetness of sugar, for significantly fewer calories. Non-nutritive sweeteners offer significant calorie savings, but had lower peak sweetness intensities and lower sweetness growth rates, which may not limit their ability to match sweetness intensity over a wider range of sucrose concentrations. Differences in the psychophysical relationships identified in the current paper should be considered when selecting sweeteners to support sucrose reduction or replacement, and offer significant opportunities to match the perceived sweetness of sugar, while supporting energy density reductions.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/10/11/1632/s1, Table S1: Concentrations tested for each of the sweetener tastant series in molarity (mmol/L), Table S2: Fitting parameters for dose-response of sweeteners using the Hill equation.

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Conflicts of Interest: C.F. has received reimbursements for speaking at conferences sponsored by companies selling food ingredients and nutritional products, and currently serves on the scientific advisory council of a commercial ingredient manufacturer. None of these organizations had any influence on the design or interpretation of the findings in the current study. All other authors declare no conflicts of interest.

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Assessing Food Liking: Comparison of Food Liking Questionnaires and Direct Food Tasting in Two Cultures

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Abstract: Food liking can be directly measured in specialised sensory testing facilities; however, this method is not feasible for large population samples. The aim of the study was to compare a Food Liking Questionnaire (FLQ) against lab-based sensory testing in two countries. The study was conducted with 70 Australian and Thai participants (35 Australian, 35 Thai, mean (SD) age 19 (3.01) years, 51% men). Participants completed a FLQ (consisting of 73 food items Australia, 89 Thai) and then, after tasting the food, rated their liking of a selection of 10 commercially available food items using a nine-point hedonic scale. Both tasks were completed on the same day and were repeated one week later. The reliability of and a comparison between methods was determined using Intra-Class Correlation Coefficients (ICC), and the difference was assessed using an independent sample *t*-test. The results indicate that the test-retest reliability of FLQ and the laboratory-based liking assessment range was moderate (0.40–0.59) to excellent (0.75–1.00). There were significant differences for the FLQ and the laboratory-based liking assessment between countries for three food items: soft drink, instant vegetable soup, and broccoli ($p < 0.01$). However, the data produced from the FLQ reflects the laboratory-based liking assessment. Therefore, it provides representative liking data in large population-based studies including cross-cultural studies.

Keywords: cross-cultural; food liking; sensory; questionnaire; hedonic

1. Introduction

Obesity represents the largest preventable disease worldwide and is a contributor to ill-health outcomes including cardiovascular disease, stroke, type 2 diabetes, hypertension, arthritis, respiratory disorders, and certain cancers [1]. Whilst the causes of obesity are multi-factorial and complex, they are embedded within energy imbalances brought about by psychological, cultural, personal, environmental, lifestyle, and dietary factors which favour excessive energy intake coupled with sedentary behaviour [2]. Energy imbalances due to overconsumption of food are common, especially,

given discretionary foods high in palatable fat, sugar, and salt are increasing in abundance in both developed and developing nations [3,4].

An individual's response to food is multi-dimensional and dynamic. Environment, experience, and physical state are all factors that may influence liking decisions at any point in time [5]. It is the liking or prospective liking of a food that is one of the key drivers of consumption [5,6]. The impact of taste and food preference on food intake is also influenced by age and sex and can be modified by distorted eating behaviours [6–9].

In adults, food flavour has an important influence on food choice [10]. Liking a food's flavour is an important driver of short-term food consumption, as those adults who enjoy the food they are consuming tend to eat more of it [5]. This can result in health issues related to the overconsumption of food [5]. For example, an individual's flavour preferences can have an effect on disease risk by influencing food consumption, particularly the consumption of foods high in fat, sugar, and salt. A study by Duffy et al. [11] demonstrated that the liking of fatty foods was positively correlated with fat intake and the liking for fibre-rich foods was positively correlated with fibre intake. Further, a positive relationship between the liking for fatty foods, body weight, and systolic blood pressure was found. This relationship between food liking and dietary intake was also observed in a large study by Mejean et al. Those with a higher liking for fatty foods had an increased intake of total energy, fat, and certain foods (high in fat) such as meat, butter, desserts, and pastries [12] and a positive relationship between the liking for fatty foods and obesity risk was observed.

There is a paucity of research comparing Food Liking Questionnaires (FLQ) and laboratory-based food acceptance. Cardello and Maller [13] examined the relationship between FLQ and laboratory acceptance testing both using a nine-point hedonic scale with the authors observing higher ratings in the laboratory acceptance testing compared with the FLQ. The authors also found positive, but mostly weak to moderate, correlations between the two methods. In addition, FLQ can be used to explore the relationship between food liking and food consumption. For example, Duffy et al. [14] used a questionnaire to determine food preference and intake to predict the dietary determinants of cardiovascular disease risk factors in 422 US male adults. This study showed that the preference for fatty food, intake of low-fibre food, and alcohol was associated with cardiovascular disease risk factors. Carbonneau et al. [15] developed and validated a food liking questionnaire which aimed to predict the influence of food liking on food and energy intake. A significant correlation was observed between liking scores in the FLQ and self-reported frequency of food consumption ($r = 0.19–0.39$, $p < 0.05$). French et al. [16] have used a self-report measure of the liking and wanting of high-fat food (among other measures such as the three-factor eating questionnaire) to investigate the association with energy intake and individual differences in eating behaviours. This study demonstrated a significant association between eating behaviours and energy intake. Furthermore, Pallister et al. [17] used a liking questionnaire in a UK twin cohort study to evaluate its usefulness to investigate the interaction between genetics and the liking of different fruits, vegetables, meats, and different tastes.

The liking of a specific food or set of foods primarily reflects the cultural environment in which an individual is brought up in and their individual experiences with such food [18]. However, the globalization of the food supply and the increase in disposable income has resulted in a diet where more products are derived from animals and energy dense sources, and the proliferation of Western-style highly palatable foods such as hamburgers, soft drinks, and pizza has created multiple problems in both developed and developing nations [19]. Such issues appear to be increasing in developing countries and include an increased prevalence of overweight and obese children and adolescents [20]. There appears to be a dramatic transition of food consumption patterns in a number of developing nations [21].

Exploring the relationship between the liking of food and dietary intake has not been widely studied; however, as indicated in the aforementioned studies, liking of a food appears to be one of the key factors influencing intake. To enable us to effectively use and interpret a FLQ it is first necessary to determine if a FLQ can appropriately measure food liking when compared to more

established laboratory assessment methods in our two cultures population groups that have never been investigated. Therefore, the aim of this study is to compare a food liking questionnaire with food liking measured in a laboratory setting in a sample of Thai and Australian adults.

2. Materials and Methods

2.1. Participants

Seventy Australian and Thai non-smoking participants (35 Australian, 35 Thai, with a mean (SD) of 19.9 (3.0) years), 51% of which were male in both countries, were recruited to take part in the study. Australian participants were recruited from undergraduate courses at Deakin University, Melbourne, Australia using a range of strategies. Posters were distributed around campus and advertising material was distributed by study personnel to potential participants at locations around the campus. Presentations were also made to first-year undergraduate students enrolled in courses offered within the School of Exercise and Nutrition Sciences at Deakin University. Lastly, the study was advertised through social media. Thai participants were recruited via presentations to first-year undergraduate students enrolled in the Faculty of Science and Technology courses at Rajamangala University of Technology Tawan-ok, Thailand.

Participants were eligible if they were non-smokers, aged over 18 years, in good health, and had no allergies to any foods or food products, as determined through self-report using a short screening questionnaire prior to testing. Ethics approval was obtained from the Human Research Ethics Committee at Deakin University (HEAG-H 102_2016) and all participants who agreed to participate in the study provided written informed consent.

2.2. Procedure and Design

Data for the laboratory-based liking assessment were collected using the Compusense® Cloud (Guelph, ON, Canada) for Australian samples. Hard copy paper questionnaires were used for the Thai sample. Participants were tested in individual booths with white lights and controlled air conditions in the sensory laboratories located at both the Centre for Advanced Sensory Science located within Deakin University and the Faculty of Science and Technology located within Rajamangala University of Technology. Participants were asked to refrain from eating, drinking (except room temperature water), brushing their teeth, and chewing gum for one hour prior to testing.

The participants first completed the food liking questionnaire (FLQ), then tasted and rated their liking of a selection of ten commercially available food items listed on the FLQ, see Table 1. The food items were selected to be representative of the commonly consumed foods within each food group. For example, for the FLQ group 'soft drink' orange Fanta was used in the laboratory testing, and for 'potatoes chips' Smith original chips were used for the Australian testing and Lay's original for Thai. For both the questionnaire and laboratory-based liking assessment, liking was measured using a nine-point hedonic scale. This scale consists of a series of nine verbal categories representing degrees of liking from 'dislike extremely' to 'like extremely'. For subsequent quantitative and statistical analysis, all verbal categories were converted to numerical values: 'like extremely' was coded as '9', 'dislike extremely' as '1'. Participants rated their imagined (for FLQ) and experienced hedonic response for the food items on a nine-point hedonic scale. Both tasks were completed on the same day and all tests were repeated one week after the original session.

Table 1. Food items on the questionnaire and commercial foods.

Food Items on Questionnaire	Commercial Foods
Sweet biscuit	Chocolate biscuit (Arnott's Tim Tam original)
Soft drink	Orange soft drink (The Coca-Cola Company)
Vegetable soup	Instant vegetable soup (Continental Homestyle vegetable)
Potato chips	Potato chips (for Australia: Smith; for Thai: Lay's original)
Ice cream	Ice cream (for Australia: A2 milk classic vanilla ice cream; for Thai New Zealand Natural Premium Ice Cream classic vanilla ice cream)
Butter	Butter (Beautifully Butterfully butter, salt block)
Broccoli	Broccoli (boiled, fresh)
Apple	Apple puree (Sweet Valley)
The heat/burn of a spicy meal	Chilli sauce (Mars Food Australia hot chilli (under the MasterFoods brand)) and Tom yum soup (Roi Thai tomyum soup with coconut milk)

2.3. Food Liking Questionnaire

A modified version of a FLQ from Duffy et al. [11] was adapted for culturally relevant Australian and Thai foods. The Australian version of the questionnaire contained 73 food items, and the Thai questionnaire contained 89 food items. As many foods as possible were kept consistent between the Australian and Thai questionnaires to allow for a direct comparison. Examples of foods used in both questionnaires included: beef, cornflakes, potato chips, strawberries, pizza, and chocolate. The Australian questionnaire included the following culturally specific foods: Kentucky Fried Chicken (KFC) and rotisserie chicken. The Thai questionnaire included the following culturally specific foods: chilli dip, fermented fish, foods that have coconut milk/oil, spicy curry, Tom yum, sticky rice, Thai dessert made from egg yolk and sugar, fruit in thick syrup, and sweet test fruits. Both FLQs contained the instruction "*if you have never eaten a particular food, or never experienced one of the listed items, please rate the item as 'neutral'*". The Thai questionnaire was translated directly into Thai, see Table 2, by the lead author, a Thai researcher based in Australia, and was reviewed by one co-author, a Thai researcher based in Thailand, to ensure cultural appropriateness and accuracy.

Table 2. The nine-point hedonic scale direct translation in to Thai.

English	dislike extremely	dislike very much	dislike moderately	dislike slightly	neither like or dislike	like slightly	like moderately	like very much	like extremely
Thai	ไม่ชอบมากที่สุด	ไม่ชอบมาก	ไม่ชอบปานกลาง	ไม่ชอบเล็กน้อย	ยอกไม่ได้ว่าชอบหรือไม่ชอบ	ชอบเล็กน้อย	ชอบปานกลาง	ชอบมาก	ชอบมากที่สุด

2.4. Statistical Analyses

Statistical analyses were carried out using SPSS version 25.0 (IBM Corporation, Armonk, NY, USA). In order to detect a minimum of one unit mean difference on the nine-point hedonic scale between Australian and Thai samples with 80% power and a standard deviation of 1.5, a sample size of 35 per group was needed. Three different sets of Intra-Class Correlation Coefficients (ICC) were calculated. The first set of ICC was used to determine the comparability between test and re-test results of the FLQ. The second set of ICC was used to determine the comparability between test and re-test results of the laboratory-based liking assessment. The third set of ICC was used to determine the comparability between the results of the FLQ and the laboratory-based liking assessment. The ICC values were interpreted as poor (<0.40), moderate (0.40–0.59), good (0.60–0.74), and excellent (0.75–1.0) [22]. An independent sample *t*-test was used to compare the food liking groups between countries. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Test-Retest Food Liking Questionnaire and Laboratory-Based Liking Assessment Reliability

The level of ICC between test–retest of FLQ are reported in Table 3. Reliability for all the food items in FLQ was in the moderate range (0.40–0.59), except for broccoli which was in the excellent range (0.75–1.0).

The level of ICC between test–retest of the laboratory-based liking assessment, ranged from 0.55 to 0.85, as shown in Table 3. The degree of reliability was excellent (0.75–1.00) for instant vegetable soup and broccoli, good (0.60–0.74) for Tim Tam, orange Fanta, ice cream, apple puree, chilli sauce, and Tom yum soup, and moderate (0.40–0.59) for potato chips and butter.

3.2. Food Items Liking Comparability between the Questionnaire and Laboratory-Based Liking Assessment

The level of ICC between the FLQ and the representative food in the laboratory-based liking assessment ranged from 0.22 to 0.82, see Table 4. The degree of comparability was excellent (0.75–1.00) for broccoli; good (0.60–0.74) for potato chips and butter; and moderate (0.40–0.59) for sweet biscuits, soft drink, vegetable soup, and ice cream. The degree of comparability was poor (<0.40) for apple puree, chilli sauce, and Tom yum soup. When the analyses were repeated for men and women separately in Australian and Thai samples, the degree of comparability of FLQ and laboratory-based liking assessment was similar (data not shown).

3.3. Using the Food Liking Questionnaire to Discriminate between Thai and Australian Cultures

Independent sample *t*-tests were used to compare FLQ and a laboratory-based liking assessment between Australian and Thai samples, see Table 5. Statistically significant mean differences ($p < 0.05$) were observed in three food items: soft drink (mean difference (MD) −1.47 Australian vs. Thai), vegetable soup (MD = 1.58), and broccoli (MD = 1.36). Furthermore, the laboratory-based liking assessment found statistically significant differences in an additional four food items compared to the FLQ: Tim Tams (MD = 0.63), potato chips (MD = −0.542), apple puree (MD = 2.11), and chilli sauce (MD = 1.70).

Table 3. Comparing mean score between test and re-test and reliability of Intra-Class Correlation Coefficients (ICC) of food liking questionnaire and laboratory-based liking assessment.

Food Items in FLQ	Test (n = 70)	Re-Test (n = 70)	95% CI of the Difference	ICC	Food Items in Sensory Laboratory Testing	Test (n = 70)	Re-test (n = 70)	95% CI of the Difference	ICC
	M ± SD	M ± SD				M ± SD	M ± SD		
Sweet biscuit	7.50 ± 1.47	7.64 ± 1.51	−0.47, 0.19	0.58	Tim Tam	8.01 ± 1.50	8.33 ± 1.05	−0.54, −0.09	0.73
Soft drink	6.30 ± 2.19	6.51 ± 2.01	−0.68, 0.25	0.57	Orange Fanta	7.14 ± 1.65	7.31 ± 1.46	−0.48, 0.14	0.66
Vegetable soup	6.41 ± 1.92	6.31 ± 2.00	−0.36, 0.56	0.51	Instant vegetable soup	5.24 ± 2.43	5.23 ± 2.30	−0.37, 0.40	0.77
Potato chip	7.44 ± 1.28	7.73 ± 1.28	−0.57, 0.00	0.56	Potato chips	7.83 ± 1.25	7.80 ± 1.17	−0.23, 0.29	0.59
Ice cream	8.16 ± 1.29	8.01 ± 1.17	−0.18, 0.46	0.41	Ice cream	8.06 ± 1.21	8.16 ± 1.15	−0.34, 0.14	0.64
Butter	6.39 ± 1.78	6.46 ± 1.93	−0.52, 0.38	0.48	Butter	5.99 ± 1.81	6.40 ± 1.84	−0.83, 0.00	0.55
Broccoli	6.60 ± 2.09	6.41 ± 2.37	−0.19, 0.56	0.75	Broccoli	5.93 ± 2.52	5.89 ± 2.51	−0.29, 0.37	0.85
Apple	7.56 ± 1.11	7.54 ± 1.00	−0.24, 0.27	0.50	Apple puree	5.27 ± 2.47	5.67 ± 2.43	−0.83, 0.03	0.72
The heat/burn of a spicy meal	6.61 ± 2.05	6.46 ± 2.10	−0.33, 0.64	0.52	Chilli sauce	3.79 ± 2.45	4.00 ± 2.32	−0.69, 0.26	0.65
					Tom yum soup	4.07 ± 2.32	4.20 ± 2.57	−0.58, 0.32	0.71

Note: Classification of ICC value: <0.40 = poor; 0.40–0.59 = moderate; 0.60–0.74 = good; 0.75–1= excellent. Standard Deviation (SD), Confident Interval (CI).

Table 4. Comparing mean score and reliability (ICC) between food liking questionnaire and laboratory-based liking assessment.

Food Items in FLQ	M ± SD (n = 70)	Food Items in Sensory Laboratory Testing	M ± SD (n = 70)	95% CI of the Difference	ICC
Sweet biscuit	7.57 ± 1.33	Tim Tam	8.17 ± 1.20	0.22, 0.61	0.43
Soft drink	6.41 ± 1.86	Orange Fanta	7.23 ± 1.42	0.41, 0.72	0.59
Vegetable soup	6.36 ± 1.70	Instant vegetable soup	5.24 ± 2.23	0.37, 0.70	0.56
Potato chip	7.59 ± 1.13	Potato chips	7.81 ± 1.08	0.59, 0.82	0.73
Ice cream	8.09 ± 1.04	Ice cream	8.11 ± 1.07	0.31, 0.66	0.51
Butter	6.42 ± 1.60	Butter	6.19 ± 1.61	0.46, 0.75	0.62
Broccoli	6.51 ± 2.09	Broccoli	5.91 ± 2.42	0.72, 0.88	0.82
Apple	7.55 ± 0.92	Apple puree	5.47 ± 2.28	−0.02, 0.43	0.22
The heat/burn of a spicy meal	6.54 ± 1.81	Chilli sauce	3.89 ± 2.17	0.06, 0.49	0.29
		Tom yum soup	4.14 ± 2.26	0.09, 0.51	0.31

Note: Classification of ICC value: <0.40 = poor; 0.40–0.59 = moderate; 0.60–0.74 = good; 0.75–1 = excellent.

Table 5. Comparison between the food liking questionnaire and laboratory-based liking assessment rating between Australian and Thai samples

Food Item in FLQ	Australian $n = 35$	Thai $n = 35$	95% CI of the Difference	t	Food Items in Sensory Laboratory Testing	Australian $n = 35$	Thai $n = 35$	95% CI of the Difference	t
	M ± SD	M ± SD				M ± SD	M ± SD		
Sweet biscuit	7.94 ± 0.82	7.20 ± 1.53	−0.50, 0.67	0.29 n.s.	Tim Tam	8.48 ± 0.60	7.85 ± 1.53	0.07, 1.18	2.25 **
Soft drink	5.67 ± 1.74	7.14 ± 1.68	−2.29, −0.65	−3.58 **	Orange Fanta	6.58 ± 1.40	7.87 ± 1.12	−1.89, −0.67	−4.23 ***
Vegetable soup	7.15 ± 0.87	5.57 ± 1.95	0.86, 2.30	4.37 **	Instant vegetable soup	6.18 ± 1.37	4.28 ± 2.51	0.93, 2.86	3.926 ***
Potato chips	7.57 ± 1.23	7.60 ± 1.03	−0.57, 0.51	−0.10 n.s.	Potato chips	7.54 ± 1.13	8.08 ± 0.96	−1.04, −0.04	−2.15 **
Ice cream	8.14 ± 0.80	8.02 ± 1.23	−0.38, 0.61	0.45 n.s.	Ice cream	8.00 ± 1.07	8.21 ± 1.07	−0.72, 0.29	−0.83 n.s.
Butter	6.42 ± 1.62	6.41 ± 1.59	−0.75, 0.78	0.03 n.s.	Butter	6.50 ± 1.51	5.88 ± 1.66	−0.14, 1.37	1.61 n.s.
Broccoli	7.18 ± 1.14	5.82 ± 2.57	0.40, 2.23	2.84 **	Broccoli	6.48 ± 1.73	5.32 ± 2.85	0.02, 2.28	2.04 *
Apple	7.65 ± 0.87	7.44 ± 0.96	−0.22, 0.65	0.97 n.s.	Apple puree	6.52 ± 1.89	4.41 ± 2.15	1.14, 3.08	4.36 ***
The heat/burn of a spicy meal	6.52 ± 1.36	6.54 ± 2.19	−0.88, 0.85	−0.03 n.s.	Chilli sauce	4.74 ± 2.22	3.04 ± 1.76	0.74, 2.65	3.54 ***
					Tom yum soup	4.44 ± 2.34	3.87 ± 2.17	1.14, 1.60	0.97 n.s.

Note: Definitions—Standard Deviation (SD), mean (M), number of participants in each group (n). * p values are for the comparison food liking rating between Australian and Thai samples were determined using independent sample t-tests. Significance indicated the * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and n.s. indicates not significant at $p > 0.05$.

4. Discussion

The objective of this study was to compare a FLQ with a laboratory-based liking assessment of ten representative foods in Australian and Thai settings, in order to determine whether the FLQ will be a suitable measurement tool in large-scale studies to compare food liking across cultures. The test–retest reliability of the FLQ and laboratory-based liking assessment were found to be moderate to excellent for both the FLQ and laboratory-based liking assessment, with an ICC range of 0.41–0.85 [22].

When comparing the FLQ with a laboratory-based liking assessment of individual food items, the comparability was moderate or high for seven food items (ICC range 0.43–0.82). However, comparability was poor for three food items: liking for apple measured on the FLQ compared with apple puree in the laboratory testing (ICC 0.22), the heat/burn of a spicy meal measured on the FLQ compared with chilli sauce (ICC 0.29), and Tom yum soup (ICC 0.31) in the laboratory taste testing. These comparability results may be explained by the differences in the food items assessed. The laboratory-based liking assessment asked subjects to rate their liking of a number of specific foods immediately after tasting. Conversely, the FLQ asked subjects to rate their liking of a number of foods without tasting the foods and this method may be more of a reflection of past experiences and memories of the food items [5,18]. Laboratory food testing provides a direct measure of liking of the food as consumed [13], with little influence of memory [5,18]. For example, a liking of apple may not equate to a liking of apple in puree form and a liking of the heat burn of a spicy meal, may not translate to a liking of chilli sauce eaten independently of the whole meal. These three food items deviated the most between the FLQ representative food and the actual food tasted in the laboratory and this may explain the poor comparability between methods. Taken as a whole, the results obtained indicate that while the FLQ appears an effective measurement tool to determine the liking across general food items in larger-scale studies, a laboratory-based sensory assessment may be necessary for measuring liking of the specific food products and laboratory testing will be required for direct product comparison. Similar results have been observed in previous studies comparing FLQ and a laboratory-based liking assessment. Deglaire et al. [23] reported on the reliability of utilising a food liking questionnaire to assess liking for salty, sweet, and fatty foods in a large population study. Deglaire observed that the questionnaire-based assessment of food liking was a robust method to collect liking data from large population studies. However, the authors noted that a liking value based on laboratory testing gives a direct measure of a liking value of the perceived flavour of the foods that are actually tasted, as opposed to a questionnaire where the liking value is based on the subject's memories or experience. Cardello and Maller [13] noted that the liking response on the questionnaire was driven more on experience or memory of food, whereas the laboratory-based liking was based on the actual tasting of food samples.

The current results, combined with those of Duffy et al., Deglaire, and Cardello and Maller [11–13] indicate that for larger population studies, using a questionnaire to assess food liking is an appropriate data collection tool as it is reliable and is comparable with laboratory taste testing. In addition, the questionnaire has a significant benefit when assessing links of food liking with diet and anthropometry in that it has the potential to provide a representative view of an individual's liking of a broad range of food groups, compared with what an individual may rate for a specific tasted food. This may provide a benefit when assessing links of food liking with diet and health indices.

One of the aims of our ongoing research program is to explore cultural differences in food liking in similarly aged sample populations. Therefore, we explored the ability of the FLQ to distinguish food liking between two sample populations. The FLQ was able to detect significant differences in food liking observed between Australian and Thai subjects using both FLQ and the laboratory-based sensory assessment. This indicates that the FLQ is able to discriminate between cultural differences in food liking, providing further confidence in its usefulness in exploring cultural differences in large studies. There was a significant difference in food liking identified in the FLQ and the laboratory-based liking assessment for three food items, including soft drink, vegetable soup, and broccoli. However, the laboratory testing found differences in an additional four food items compared to the FLQ.

These items include Tim Tams, potato chips, apple puree, and chilli sauce. The differences in the liking of these four food items might be due to a greater familiarity with these foods within Australia and less familiarity within Thailand. For example, Thai participants' liking ratings during the laboratory testing of Tim Tams and apple puree were significantly lower compared to the Australian subjects. This finding supports other research studies that show a lack of familiarity may influence the liking of a food [24–28].

The present study has a number of limitations that should be noted. The reliability between the FLQ and laboratory-based liking assessment was completed in participants from Australia and Thailand using foods specific to their culture. Therefore, the results may not be generalizable to other population groups. Further, only a representative number of food items were used in the laboratory-based liking assessment.

The present study used a relatively small sample size, which was based on an estimated difference of one unit on a nine-point hedonic scale. As such, the present study can be seen as the first step in our understanding of the relationship between the food liking questionnaire and direct sensory testing in a cross-cultural sample and sets the stage for larger studies. It is important to note that the present study did not aim to develop a method to replace the sensory evaluation of taste liking; rather, it aimed to propose an alternative method to obtain a general liking of food when an actual measurement is not possible.

5. Conclusions

The findings of this study demonstrated that the FLQ reflects the liking ratings in laboratory taste-testing and is an appropriate method to investigate food liking in large population groups including cross-cultural studies. The test-retest reliability of the FLQ and laboratory taste-testing were also assessed. This study concludes that the FLQ is also able to detect differences in liking between the Australian and Thai populations. Laboratory-based sensory testing remains the recommended method for direct product comparison.

Author Contributions: U.W., D.S, L.R., D.G.L., S.M. and R.K. conceived and designed the study; U.W. conducted the experimental methods; U.W., L.R. and M.M. conducted the statistical analyses; U.W., L.R., D.G.L., S.C., M.M. and R.K. interpreted the data; U.W. wrote the manuscript; all authors reviewed the manuscript.

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Relative Validity of a Food and Beverage Preference Questionnaire to Characterize Taste Phenotypes in Children Adolescents and Adults

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Abstract: To assess the relative validity of our food and beverage preference questionnaire we investigated the association between sweet and fatty taste preference scores (assessed using a food and beverage preference questionnaire) and sweet and fatty food propensity scores (derived from a food frequency questionnaire). In I.Family, a large European multi-country cohort study, 12,207 participants from Cyprus, Estonia, Germany, Hungary, Italy, Spain and Sweden, including 5291 adults, 3082 adolescents, and 3834 children, completed a food and beverage preference questionnaire with 63 items. Cumulative preference scores for sweet and fatty taste were calculated from the single item ranking ranging from 1 to 5. The relative consumption frequency of foods classified as sweet and fatty was used to calculate the corresponding consumption propensities, a continuous variable ranging from 0 to 100. We conducted regression analyses to investigate the association between sweet and fatty taste preference scores and sweet and fatty food propensity scores, respectively, separately for adults, adolescents ≥12 years, and for children <12 years. The overall sweet taste preference score was positively associated with the sweet food consumption propensity score ($\beta = 2.4$, 95% CI: 2.1;2.7) and the fatty taste preference score was positively associated with the fatty food consumption propensity score ($\beta = 2.0$, 95% CI: 1.8;2.2). After stratification for age (children <12 years, adolescents ≥12 years, and adults), the effect remained significant in all age groups and was strongest in adolescents and adults. We conclude that our food and beverage preference questionnaire is a useful instrument for epidemiological studies on sensory perception and health outcomes and for the characterization of sensory taste phenotypes.

Keywords: taste preference questionnaire; validation; European children; adolescents; adults

1. Introduction

Methods to accurately measure sensory taste perception are often laborious. A high degree of standardization of the assessment method is a prerequisite for valid results. In large multi-country epidemiological studies it is often not feasible to conduct sensory perception tests according to the standards that can only be reached in a laboratory setting. As an alternative, taste preference questionnaires have previously been applied to investigate the association between taste preferences and health outcomes [1–3]. Only one questionnaire, originally developed for French adults, has so far been developed for a large epidemiological study [4]. While the first part of the questionnaire assesses standard taste preferences, it also assesses the liking of seasoning and whole dishes, for example, in later parts. As this questionnaire is quite elaborate and includes many items that are specific to France, it might not be suitable for children and adolescents in general and also not for use throughout Europe. To date, no study has applied a food and beverage preference questionnaire in both children and their parents at the same time.

The purpose of this study was to analyze the validity of data obtained using a food and beverage preference questionnaire (FBPQ) developed in the context of I.Family, a large pan-European study comprising children, adolescents, and adults. Determining the validity of the questionnaire will help further the investigation of the association between taste preferences and health outcomes. To this end we developed a FBPQ for children, adolescents, and adults and applied it in I.Family. As a measure of the relative validity of the questionnaire, we analyzed the association between self-reported sweet and fatty taste preferences assessed by the FBPQ and self-reported food consumption frequencies of sweet and fatty foods assessed by a food frequency questionnaire (FFQ).

2. Materials and Methods

I.Family is a large multi-country longitudinal cohort study and the follow-up study of the IDEFICS (Identification and prevention of Dietary- and lifestyle-induced health Effects in Children and infantS) study [5,6]. Between March 2013 and April 2014, all children that participated in the IDEFICS study were invited to a follow-up examination together with their siblings and parents. In the sensory taste perception module, we included all participants aged six years and above. This resulted in a study sample of 12,207 participants from Cyprus, Estonia, Germany, Hungary, Italy, Spain, and Sweden who completed the food and beverage preference questionnaire and provided all co-variables.

Each study center obtained ethical approval from its local institutional review board (e.g., Cyprus National Bioethics Committee, Nicosia, Cyprus; Tallinn Medical Research Ethics Committee, Tallinn, Estonia; Ethics Committee of the University of Bremen, Bremen, Germany; Egészségügyi Tudományos Tanács, Pécs, Hungary; Azienda Sanitaria Locale Avellino Comitato Etico, Avellino, Italy; Regionala Etikprövningsnämnden i Göteborg, Gothenburg, Sweden; Comité Ético de Investigación Clínica de Aragón, Zaragoza, Spain). Parents gave written informed consent for themselves and for their young children (up to 11 years). Adolescents 12 years and older gave their own written informed consent. All children were informed orally and gave their oral consent to participate in the study.

2.1. Food and Beverage Preference Questionnaire

Based on two existing tools, we developed an FBPQ suitable for assessment in children from the age of six years. One of the tools was administered to children and tested for reliability [7] and the other was administered to adults and internally validated [4]. A detailed description of the FBPQ has been published elsewhere [8]. The questionnaire consisted of 63 pictures of single foods (e.g., banana, spinach), mixed foods (e.g., hot dog, kebab), condiments (e.g., jam, mayonnaise) and drinks (e.g., coke, lemonade). A pre-test was conducted in every country to ensure the feasibility of all food items across countries and to find out how long participants would require to complete the questionnaire. The

estimated time for the completion of the food and beverage preference questionnaire was 7 min. Each subject ranked his/her own preference for the taste of the respective food or drink on a 5-point Likert (smiley-) scale, with 1 meaning "do not like at all" and 5 meaning "like very much". Subjects could indicate if they did not know or had never tasted a given food item.

Sensory Taste Preference Score

Cumulative preference scores for sweet, fatty, salty, and bitter tastes were calculated from the single item rankings, as described in a previous publication [8]. First, we excluded foods that were rated by less than 75% of the participants. Further, data of participants with more than 20 missing or "Never tried/Don't know" answers were excluded. In principle, "Never tried/Don't know" responses were set to "missing". A latent variable exploratory factor analysis was then conducted [9] to assess the associations between foods and beverages. The age- and sex-specific factor analysis was conducted for the strata boys <12 years, girls <12 years, boys ≥12 years, girls ≥12 years, and adults ≥18 years. A food or drink item was considered to belong to a particular factor if the factor loading was greater than 0.30 on the specific factor. Thereafter, a content analysis was conducted to assign the factors to the taste modalities sweet, salty, fatty, and bitter (Table 1). Food and drink items with no load on one or more of the factors were not included in further analyses.

Table 1. Food and drinks representing the four taste modalities [8].

	Boys <12 Years	Girls <12 Years	Boys ≥12 Years	Girls ≥12 Years	Fathers	Mothers
Sweet						
Milk chocolate	X	X	X	X	X	X
Chocolate bar	X	X	X	X	X	X
Lemonade	X	X		X	X	X
Coke	X	X	X	X	X	X
Diet coke	X		X	X	X	X
Donut	X		X	X	X	X
Jam	X	X	X	X		X
Honey	X	X	X	X	X	X
Plain croissant		X	X	X	X	X
Chocolate croissant	X	X		X	X	X
Cornflakes	X	X	X	X	X	X
Chocolate crispies		X	X	X	X	X
Chocolate spread	X	X	X	X	X	X
Banana	X	X		X	X	
Fruit yoghurt	X	X	X	X	X	X
Yoghurt	X		X	X	X	X
Fruit juice	X	X	X	X	X	X
Chocolate pudding		X	X			
Gateau			X		X	X
Ice tea					X	
Ice cream					X	X
Water					X	
Wholemeal bread					X	

Table 1. *Cont.*

	Boys <12 Years	Girls <12 Years	Boys ≥12 Years	Girls ≥12 Years	Fathers	Mothers
Salty						
Salt			X			
Salted nuts	X	X	X	X	X	X
Salted pistachios	X	X	X	X	X	X
Savoury biscuits	X	X	X	X	X	X
Salty sticks	X	x	X	X	X	X
Olives					X	
Feta					X	
Fatty						
Hamburger	X	X	X	X	X	X
Hot Dog	X	X	X	X	X	X
Fried chicken	X		X	X	X	X
Steak	X			X	X	
French fries	X	X	X	X		X
Chips	X	X	X	X		X
Sausage	X	X	X	X	X	X
Salami	X			X	X	X
Butter	X	X	X	X	X	X
Mayonnaise	X		X	X	X	X
Milk		X		X	X	
Cream			X	X	X	X
Mashed potatoes			X			
Kebab				X	X	X
Nachos				X		X
Chili sauce				X		X
Bitter						
Broccoli	X	X	X	X	X	X
Spinach	X	X	X	X	X	X
Lettuce	X		X			
Olives	X		X			X
Lasagne			X			
Red cabbage					X	X
Sprouts					X	X
Asparagus					X	X
Grapefruit						X
Steak						X

Taste preference scores were calculated separately for each stratum, based on the mean liking of the foods and drinks assigned to each of the 4 taste modalities. To this end, the sum of the ratings for the foods and drinks was calculated and divided by the number of foods and drinks that were included in the specific taste modality group. In the present analysis, only the sweet and fatty taste preference scores were considered.

2.2. Food Frequency Questionnaire

In I.Family, each participant completed the adapted version of the validated [10,11] and reproducibility tested [12] FFQ used in the IDEFICS study. Parents completed the FFQ for children

below the age of 12 years as it has been shown that they might be unreliable reporters of their diet [13]. Parents and adolescents 12 years and older reported on their own diet. The FFQ contained 59 items including 19 fatty items (fried potatoes, whole fat milk, whole fat yoghurt, fried fish, cold cuts/sausages, fried meat, fried poultry, fried eggs, mayonnaise and mayonnaise based products, cheese, chocolate- or nut-based spread, butter/margarine on bread, oil, nuts and seeds, salty snacks, savoury pastries, chocolate-based candies, cake/pudding/cookies, and ice cream) and 16 sweet items (fresh fruit with added sugar, fruit juices, carbonated sugar sweetened drinks, sugar sweetened drinks not carbonated, sweetened coffee, sweetened tea, sweetened or sugar added breakfast cereals, sweetened and/or flavored milk, sweetened and/or flavored yoghurt, jam, honey, chocolate or nut based spreads, chocolate-based candies, non-fat candies, cake/pudding/cookies, and ice cream). The response categories were "1–3 times a week", "4–6 times a week", "1 time/day", "2 times a day", "3 times a day" and "Never/less than once a week". The obtained answers were converted into weekly consumption frequency, ranging from 0 to 28. The weekly consumption frequencies of named fatty foods, sweet foods, and of all foods included in the FFQ were summed up. The sweet and fatty food propensity scores in terms of the relative consumption frequency of named sweet or fatty foods were calculated. This was done by dividing the consumption frequency of the sweet and fatty foods by the consumption frequency of all foods included in the FFQ multiplied by 100. This resulted in a continuous variable, indicating the sweet and fatty food propensity scores ranging from 0% to 100% [14,15]. Thus, the scores reflect the proportion of sweet and fatty foods in the whole diet. For example, a sweet propensity score of 25 indicates that 1/4 of all food items consumed in one week were foods high in sugar. As the ultimate aim of the IDEFICS Study and I.Family was to investigate risk factors for child and adolescent health, such as obesity and to describe the obesogenic food environment and healthy or unhealthy food choices, the FFQ was designed to measure consumption frequency of obesogenic foods, which primarily contain sugar and fat. Hence, the FFQ was designed to allow the expression of the consumption frequency of sweet and fatty foods rather than of bitter and salty foods and may be used to describe the tendency to choose sweet or fatty food alternatives over foods lower in sugar or fat. Whenever the FFQ contained a sweetened and an unsweetened alternative of a food group (e.g., sweetened vs. unsweetened milk products or sweetened fruits vs. unsweetened fresh fruits), the sweetened alternative was included in the food consumption propensity score. This was done to describe the behavior to choose the sweetened alternative over the unsweetened alternative.

2.3. Questionnaires and Anthropometry

Self-completion questionnaires were used to assess age, sex, country of residence, and highest level of education. For each parent we categorized the highest educational level acquired according to the International Standard Classification of Education (ISCED), ranging from 1 (low education) to 8 (high education) [16]. For the present analysis, the education level was grouped into three categories; "low education" (ISCED level 0–2), "medium education" (ISCED level 3–5) and "high education" (ISCED level 6–8). The family affiliation was assessed using a kinship interview. Parents completed questionnaires for themselves as well as for their children under the age of 12 years. Adolescents 12 years and older completed the questionnaire on their own.

The height and weight of all participants were measured in a fasting state. The body mass index (BMI) was calculated for all participants and converted into age-and sex-specific z-scores for all children and adolescents [17]. For adults, the cut off of 25 kg/m^2 was chosen to classify parents as overweight, including obese [18].

2.4. Statistical Analysis

We calculated the characteristics of the study sample separately for adults, adolescents ≥12 years, and for children < 2 years. For further descriptive analysis we categorized the sweet and fatty taste preference score into 4 categories separately for sweet and fatty. In category 1 we assigned all taste preference scores ranging from 1 to <2, in category 2 all taste preference scores ranging from 2 to <3,

in category 3 all taste preference scores ranging from 3 to <4 and in category 4 all taste preference scores ranging from 4 to 5. After this, we calculated the mean, standard deviation, and lower and upper quartiles (p25, p75) for the sweet food propensity score (stratified by sweet taste preference score categories), as well as for the mean fatty food propensity score (stratified by fatty taste preference score categories) separately for adults, adolescents ≥12 years, and for children <12 years. We conducted regression analyses to investigate the association between sweet and fatty taste preference scores and sweet and fatty food propensity scores, respectively, separately for adults, adolescents ≥12 years, and for children <12 years. This we did separately for adults, adolescents ≥12 years, and for children <12 years. In the regression model we included the sweet and fatty taste preference scores as non-categorized independent variables, whereas the sweet and fatty food propensity scores were considered as the dependent variables. We adjusted all models for sex, age, BMI (for children and adolescents: BMI z-score), highest education level, and country of residence as fixed factors and family affiliation as a random factor. As associations between weight status and food intake have been described in previous studies [19,20], it is important to conduct stratified analyses. Thus, we investigated data not only in the full sample (including all individuals), but also separately for underweight and overweight/obese participants. Further stratified analyses were performed according to sex, country, and education level.

All regression analyses were carried out using PROC MIXED (SAS version 9.3). Effect estimates were presented with the corresponding 95% confidence intervals (95% CI) and *p*-values.

3. Results

The study sample consisted of 5291 adults, 3082 adolescents, and 3834 children. The mean age of the total sample was 24.8 years and 40% were overweight or obese. The highest proportion of participants was from Cyprus (21.5%) and the smallest from Spain (6.7%). The mean sweet and fatty taste preference scores for the total sample were 3.8 for each of the tastes. The mean sweet food propensity score was 21.3 and the mean fatty food propensity score was 24.2. More detailed characteristics can be found in Table 2.

In Table 3 the mean sweet and fatty food propensity scores within each sweet and fatty taste preference category are displayed separately for adults, adolescents ≥12 years, and for children <12 years. The results show that the consumption of sweet and fatty foods generally increased as the sweet and fatty taste preference scores increased, respectively. It is only in children that the mean sweet and fatty food propensity scores in the lowest sweet and fatty preference score categories were higher than in the other sweet and fatty preference score categories, respectively.

Table 3 shows the effect estimates of the association between sweet and fatty taste preference scores and sweet and fatty food propensity scores, respectively, separately for adults, adolescents ≥12 years and for children <12 years. A positive association could be seen for sweet and fatty taste in adults (sweet: β = 3.1, 95% CI: 2.7; 3.5, fatty: β = 2.3, 95% CI: 2.0; 2.6) and adolescents (sweet: β = 3.0, 95% CI: 2.3; 3.6, fatty: β = 2.9, 95% CI: 2.4; 3.4). The association in children was weaker but still positive (sweet: 0.8, 95% CI: 0.3; 1.2, fatty: β = 0.5, 95CI: 0.1; 0.9). In the overall sample, the sweet taste preference score was positively associated with the sweet food propensity score (β = 2.4, 95% CI: 2.1; 2.7) and the fatty taste preference score was positively associated with the fatty food propensity score (β = 2.0, 95% CI: 1.8; 2.2). Further, the Table S1 (see supplement) shows the effect of all included co-variables on the sweet and fatty food consumption frequencies.

Table 2. Characteristics of the study sample.

	Adults *n* = 5291	**Adolescents *n* = 3082**	**Children *n* = 3834**	**Total *n* = 12,207**
	Mean (SD) (*p*25; *p*75)	**Mean (SD) (*p*25; *p*75)**	**Mean (SD) (*p*25; *p*75)**	**Mean (SD) (*p*25; *p*75)**
Age	42.4 (5.8) (38.4; 46.2)	13.6 (1.0) (12.8; 14.0)	9.6 (1.6) (8.8; 10.8)	24.8 (15.9) (11.0; 41.0)
Sweet food propensity score	18.3 (11.5) (9.4; 25.5)	24.5 (11.1) (16.5; 31.1)	22.9 (10.3) (15.7; 29.0)	21.3 (11.3) (13.0; 28.4)
Fatty food propensity score	22.0 (9.1) (15.6; 28.2)	24.5 (9.1) (18.3; 30.1)	27.0 (8.9) (21.2; 32.5)	24.2 (9.3) (17.8; 30.2)
Sweet preference score	3.5 (0.7) (3.0; 4.0)	4.0 (0.6) (3.6; 4.4)	4.1 (0.6) (3.4; 4.6)	3.8 (0.7) (3.3; 4.4)
Fatty preference score	3.5 (0.8) (3.0; 4.0)	4.0 (0.6) (3.6; 4.4)	4.1 (0.6) (3.8; 4.6)	3.8 (0.7) (3.3; 4.4)
	N (%)	**N (%)**	**N (%)**	**N (%)**
Female	3490 (66.0)	1584 (51.4)	1896 (49.5)	7242 (57.0)
Overweight/obese	2988 (56.5)	857 (27.8)	1046 (27.3)	4891 (40.1)
All countries				
Cyprus	1151 (21.8)	691 (22.4)	781 (20.4)	2623 (21.5)
Estonia	761 (14.4)	478 (15.5)	580 (15.1)	1819 (14.9)
Germany	834 (15.8)	466 (15.1)	550 (14.4)	1850 (15.2)
Hungary	988 (18.7)	434 (14.1)	512 (13.4)	1934 (15.8)
Italy	720 (13.6)	589 (19.1)	682 (17.8)	1991 (16.3)
Spain	346 (6.5)	168 (5.5)	306 (8.0)	820 (6.7)
Sweden	491 (9.3)	256 (8.3)	423 (11.0)	1170 (9.6)

Abbreviations: *n* = number, SD = standard deviation, *p* = percentile.

Stratified analyses by country, sex, weight status, and education level showed that the association between taste preference scores and food propensity scores remained stable within the strata (Table 4). A few differences could be observed, however. When stratifying the regression analyses by sex, no differences were observed between female and male adolescents and adults. Among children, on the other hand, the association was stronger in girls than in boys. When stratifying the regression analyses by country, the associations were again present and positive in all countries for adolescents and adults. For children, the associations were not positive for sweet in Sweden and Hungary and for fatty in Italy, Estonia, and Sweden. When stratifying by weight status and education level, the same patterns as in the full sample could be seen. The association was present and positive in adolescents and adults. While the association was still positive for children, for the fatty taste it was no longer significant for overweight children and children with parents with low/medium or high education.

Table 3. Mean sweet and fatty food propensity scores within sweet and fatty taste preference score groups and β estimates for the association between sweet and fatty taste preference scores and sweet and fatty food propensity scores.

	Adults	**Adolescents**	**Children**	**Total**
Sweet preference score category (range)	**Sweet propensity score (mean (SD)) (*n*)**			
1 (1–<2)	13.7 (12.1) (109)	17.7 (5.7) (6)	23.6 (11.9) (12)	14.8 (12.2) (127)
2 (2–<3)	15.3 (10.7) (1108)	21.2 (11.3) (181)	20.3 (9.7) (188)	16.6 (10.9) (1477)
3 (3–<-4)	18.3 (11.3) (2785)	23.1 (10.9) (1167)	22.3 (10.1) (1169)	20.3 (11.1) (5121)
4 (4–≤5)	21.1 (11.8) (1289)	25.8 (11.0) (1728)	23.5 (10.4) (2464)	23.6 (11.0) (5481)
β (95% CI) [1,2]	3.1 (2.7;3.5)	3.0 (2.3;3.6)	0.8 (0.3;1.2)	2.4 (2.1;2.7)
p-value	$p < 0.0001$	$p < 0.0001$	$p = 0.001$	$p < 0.0001$
Fatty preference score category (range)	**Fat propensity score (mean (SD)) (*n*)**			
1 (1–<2)	15.7 (8.0) (185)	16.8 (11.8) (6)	27.7 (10.2) (14)	16.5 (8.8) (205)
2 (2–<3)	19.7 (8.8) (1066)	20.1 (9.3) (183)	26.0 (9.2) (168)	20.5 (9.1) (1417)
3 (3–<4)	22.3 (8.9) (2553)	23.0 (8.8) (1156)	26.7 (9.1) (1087)	23.5 (9.1) (4796)
4 (4–≤5)	23.8 (9.1) (1487)	26.1 (8.9) (1737)	27.2 (8.7) (2565)	26.0 (9.0) (5789)
β (95% CI) [1,2]	2.3 (2.0;2.6)	2.9 (2.4;3.4)	0.5 (0.1;0.9)	2.0 (1.8;2.2)
p-value	$p < 0.0001$	$p < 0.0001$	$p = 0.02$	$p < 0.0001$

CI: Confidence interval; [1]: Sweet and fatty preference scores entered the regression models as continuous variable; [2]: Regression models were adjusted for sex, age, BMI, highest education level, and country of residence as fixed factors and family affiliation as random factor.

Table 4. Stratified results of the association between sweet and fatty taste preference and sweet and fatty food propensity scores (β estimates and 95% CI).

	Adults	**Adolescents**	**Children**
	Sweet food consumption score β (95% CI)		
Male and Female	3.1 (2.7;3.5)	3.0 (2.3;3.6)	0.8 (0.3;1.2)
Male	2.8 (2.0; 3.6)	2.7 (1.8; 3.6)	0.4 (−0.1; 1.3)
Female	3.1 (2.6; 3.6)	3.2 (2.3; 4.2)	0.8 (0.2; 1.5)
Under-/normal weight	3.2 (2.6; 3.9)	2.7 (1.9; 3.5)	0.6 (0.1; 1.2)
Overweight/Obese	2.9 (2.3; 3.4)	3.4 (2.2; 4.7)	1.0 (0.01; 2.0)
Low/medium education level	2.9 (2.3; 3.5)	3.2 (2.3; 4.2)	1.0 (0.3; 1.7)
High education level	3.1 (2.6; 3.7)	2.6 (1.7; 3.6)	0.6 (−0.0; 1.2)

Table 4. *Cont.*

	Adults	Adolescents	Children
	Fatty food consumption score β (95% CI)		
Male and Female	2.3 (2.0;2.6)	2.9 (2.4;3.4)	0.5 (0.1;0.9)
Male	2.2 (1.7; 2.8)	2.4 (1.6; 3.2)	0.4 (−0.3; 1.0)
Female	2.2 (1.8; 2.5)	3.2 (2.6; 4.0)	0.7 (0.1; 1.2)
Under-/normal weight	2.6 (2.1; 3.0)	3.0 (2.3; 3.6)	0.7 (0.2; 1.1)
Overweight/Obese	1.9 (1.5; 2.3)	2.8 (1.8; 3.9)	0.4 (−0.5; 1.3)
Low/medium education level	2.3 (1.9; 2.8)	2.8 (2.0; 3.5)	0.4 (−0.2; 1.1)
High education level	2.1 (1.6; 2.5)	3.1 (2.4; 3.9)	0.5 (−0.0; 1.0)

4. Discussion

Self-reported sweet and fatty taste preferences were positively associated with self-reported sweet and fatty food propensity scores, respectively, in children, adolescents, and adults. This indicates that taste preferences are indeed associated with actual food choices; the higher the sweet or fatty preference score, the higher the sweet or fatty propensity score, respectively. Overall the consumption of sweet and fatty foods increased by 2% per unit increase of the sweet and fatty preference score category. For adolescents and parents, the increase was 3% and for children it was between 0.5% and 1% per unit increase. The strength of the association was strongest for adults and adolescents. The estimates for children 6 to 12 years old were weaker, suggesting that younger children consume food and drink offered at home by their parents. As parents act as gatekeepers with regard to the availability of food and drink [21] children might not be able to consume only what their taste preferences would imply but rather what their parents want them to consume. This might have attenuated the investigated association in the group of children. Our FBPQ may thus be considered as a useful instrument to provide valid data on self-reported sweet and fatty taste preferences for multi-country epidemiological field studies.

The questionnaire used in I.Family to assess taste preference in children, adolescents, and their parents was developed on the basis of two existing tools. As no questionnaire for taste preference assessment in children, adolescents, and adults across Europe existed, there was a need to develop and test the validity of the I.Family FBPQ. Two recent studies validated their food and beverage preference questionnaires to administer to adults across two cultures; English and Arab [22] and Australian and Thai [23]. Besides questionnaires for observation studies, food and beverage preference questionnaires were tested for reliability and validity both in a laboratory setting [24] and under free-living conditions [24–26]. Thus, validated preference questionnaires exist, but the present study contributes a validated questionnaire allowing for investigating associations between sensory taste preferences and health outcomes, even in a cross-cultural setting including a wide range of age-groups.

As we had no objective measurement to validate the FBPQ, we used the self-reported food consumption frequencies for the validation. The validity of the FFQ itself could be questioned due to social desirability or recall bias and misclassification may potentially attenuate the observed associations. While we acknowledge the possible attenuation, we nevertheless believe that our FFQ provides useful information since it has been previously validated [10,11] and tested for reproducibility [12].

In comparison to the FFQ, the FBPQ is a tool especially designed to assess taste preferences. It has been analyzed before and has been found to be applicable in children from the age of six years upward [8]. This instrument is easy to apply and faster to complete compared to an FFQ. Further, as it is not necessary to recall the diet of the previous month, it is likely to provide more robust information with regard to recall bias.

The confirmation of the relative validity of the FBPQ is important as the tool can be used in future studies to investigate additional aspects of taste preferences, such as the longitudinal development of taste preferences and possible associations with health outcomes in young European populations. With respect to so-called "upstream factors" of taste preference development, studies investigating regional or temporal changes of determinants will also be able to make use of the present results. Further, the associations observed in this study will contribute toward the development of successful interventions, health programs, and policies aiming at improving the dietary behavior of children as well as adolescents and adults.

Limitations and Strengths

Our study has some limitations that need to be addressed. The FFQ for children below the age of 12 years was proxy-reported by the parents, hence social desirability potentially affected our data. Parents might have responded to the questions of the FFQ in a way they thought to be more socially acceptable. This might have led to an attenuation of the studied association. In addition, as the number of children in the lowest sweet and fatty taste preference score category was very small, the calculation of sweet and fatty food propensity scores within those categories was based on very small numbers and was thus not representative for this age-group. Despite this potential limitation, we are nevertheless convinced that the results presented in the current paper provide important information for public health stakeholders, policy makers, and researchers.

In the present study, information on restrained eating could not be considered. This could possibly have led to an attenuation of our results regarding the association between taste preferences and food consumption frequency. We obtained information on current dieting only for adolescents. In a sensitivity analysis within the group of adolescents, we adjusted the regression analysis for currently being on a diet and could see that the associations under investigation remained positive and significant. Unfortunately, we could not adjust the whole analysis for restrained eating, but the results of the sensitivity analysis suggest that this does not alter the investigated association.

Another limitation concerns the unequal distribution of the sexes within the group of adults, whereby the majority was female. The stratified results, however, showed no differences between men and women.

The strengths of our study are the large multi-country study sample and the broad age-range of participants. Due to the large study sample and the assessment of a broad range of health-related information, we were able to adjust the analysis for several covariates, such as sex, weight status, country, and education level. Further, we were even able to also include data of underweight and overweight/obese participants. In order to account for the bidirectional association between weight status and food consumption/energy intake, we analyzed the data stratified by weight status. The studied associations were positive and significant for under-/normal weight as well as for overweight/obese participants. The finding, which was that the association for fatty taste was not significant in children, could be due to the fact that parents of overweight/obese children are more restrictive with regard to sweet and fatty foods. This would then lead to the observed attenuation of the association between taste preferences and food consumption frequencies.

5. Conclusions

Although food choices are influenced by various factors, we were able to show a positive association between sweet and fatty taste preferences, assessed via an FBPQ, and sweet and fatty food propensity scores, assessed via a validated FFQ, in a large multi-country epidemiological cohort

study in children, adolescents, and adults. We conclude that our FBPQ is a valid instrument for epidemiological field studies aiming to characterize taste phenotypes.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/7/1453/s1, Table S1: Estimates of fixed effect sizes with reference category in parentheses in relation to sweet and fatty food consumption frequencies in adults, adolescents and children.

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Review

A Biopsychosocial Model of Sex Differences in Children's Eating Behaviors

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Abstract: The prevalence of obesity and eating disorders varies by sex, but the extent to which sex influences eating behaviors, especially in childhood, has received less attention. The purpose of this paper is to critically discuss the literature on sex differences in eating behavior in children and present new findings supporting the role of sex in child appetitive traits and neural responses to food cues. In children, the literature shows sex differences in food acceptance, food intake, appetitive traits, eating-related compensation, and eating speed. New analyses demonstrate that sex interacts with child weight status to differentially influence appetitive traits. Further, results from neuroimaging suggest that obesity in female children is positively related to neural reactivity to higher-energy-dense food cues in regions involved with contextual processing and object recognition, while the opposite was found in males. In addition to differences in how the brain processes information about food, other factors that may contribute to sex differences include parental feeding practices, societal emphasis on dieting, and peer influences. Future studies are needed to confirm these findings, as they may have implications for the development of effective intervention programs to improve dietary behaviors and prevent obesity.

Keywords: sex differences; eating behavior; food intake; biopsychosocial; children; brain imaging

1. Introduction

Sex and gender are important characteristics that contribute to individual variability in the development of disordered eating and obesity, but the extent to which they impact eating behaviors in children is less clear. It has been assumed that sex differences in eating behavior arise in adolescence because of the physiological changes and sociocultural pressures experienced during this developmental period. Prior to adolescence, sex-based influences on eating behavior have been thought to be minimal. However, there are both biological (e.g., sexual dimorphic patterns of in utero neural development and genetics) and psychosocial (e.g., parental feeding practices and societal body ideals) factors that may affect the way children eat prior to puberty. Despite these potential influences, this period of development has received little attention in the literature. Because of the sex differences that occur in the prevalence of both disordered eating [1–3] and obesity [4,5], there is a need to understand the role of sex in the development of behaviors involved with the etiology of these diseases prior to puberty. To call attention to this gap, this paper reviews the extant literature and presents new data demonstrating that sex differences in eating behavior arise prior to puberty and have effects on children's appetitive traits and neural responses to food cues.

The National Academy of Sciences has outlined rationale for when sex differences should be studied [6]. Several of their criteria apply to eating and weight disorders and therefore are relevant to the current paper. The first criterion is if there are known sex differences in the prevalence or incidence of a disease. Eating disorders occur nearly eight times more frequently in females than males [1–3]. At the same time, data from the National Health and Nutrition Examination Survey (NHANES) show across all age groups a higher prevalence of obesity among male children compared to females [7]. These striking statistics provide support for studying the role of sex in eating behaviors because they are integral to the development of these conditions. Another criterion outlined in this report is if there are known sex differences in disease severity, progression, or outcome. In the case of obesity, there are well-described differences in body composition, with adult males carrying fat around the abdomen and chest (i.e., visceral adipose tissue), which is associated with higher metabolic risk, while some pre-menopausal adult females are metabolically protected by accumulating fat in the lower extremities [8,9]. In addition, males tend to have more fat free mass than females. These differences are present in infancy [10–12] and persist throughout development, becoming more robust at puberty [13]. Furthermore, symptomology associated with binge eating (i.e., frequency and level of distress) is more severe in females relative to males [14]. A final criterion suggested in the report is if sex influences the success or outcome of interventions [6]. In both children [15] and adults [16], males tend to be more responsive to weight loss interventions than females. With the potential promise of personalized medicine for treatment of complex diseases such as obesity, understanding how sex influences response to treatment could highlight novel therapies that could specifically be targeted to males or females.

Before reviewing the literature, it is worth noting that much of the research in this area has not distinguished between the constructs of "sex" and "gender". Sex refers to the biological classification of male or female according to chromosomes and reproductive organs. Gender, on the other hand, refers to one's self-representation, which is influenced by sociological and cultural factors [17]. Often one's biological sex matches with self-assigned gender, but this is not always the case. The multitude of factors influencing both sex and gender have made the study of individual differences between males and females complicated. Because we are applying a biopsychosocial framework to describing how sex influences eating behavior, we include discussion of biological factors more likely to influence sex and social and psychological factors more likely to influence gender. However, as most prior studies do not clarify whether they distinguished between the two constructs when collecting participant data, it is not possible to make clear distinctions about how the terminology is used when referring to these studies. To avoid switching between "sex" and "gender" throughout the paper, we use the term "sex" as a combined term that includes not only biological, but also social and psychological influences.

The goal of this paper is to present evidence that sex influences eating behaviors in childhood and to examine the source of these influences using a biopsychosocial framework. In the last section of the paper, we present new data analyses that have been informed by the biopsychosocial model. This paper is not intended to be a systematic review, but rather is a starting point for framing research questions that can systematically address the role of sex in childhood eating behavior. To support the argument that sex differences in childhood eating behavior are relevant to the development of eating and weight-disorders, we have selectively focused on some aspects of eating behavior (i.e., food acceptance, food intake, picky eating, appetitive traits, eating compensation, eating in the absence of hunger, and meal-specific microstructural patterns (e.g., bite rate and eating speed)). However, other important contributors to eating behavior that may not as directly impact risk for eating and weight disorders (e.g., oral sensory responses and olfactory sensations) have been omitted. Additionally, to avoid the inclusion of effects on eating behavior that could be influenced by the physiological and hormonal events related to puberty, to the extent possible, the literature review focuses on children age 11 years and younger, although it is recognized that this may not fully eliminate pubertal influences. However, due to the paucity of evidence in some sections, we have included a few studies that report on an age range beyond 11 years, although we recognize that the results may be influenced by pubertal

development. Within the age group of children discussed, infants are defined as <1 year, toddlers as 1–2 years, preschool children as 3–5 years, and middle childhood as 6–11 years.

2. Evidence for Sex Differences in Children's Eating Behavior

The first part of the paper provides an overview of the available literature to support the role of sex in childhood eating behaviors. This section is divided into studies that have examined sex differences in food acceptance, food intake, appetitive traits, eating-related compensation, eating in the absence of hunger, and meal-specific microstructure.

2.1. Sex Differences in Food Acceptance/Preference

The literature has consistently shown sex differences in children's food acceptance and preference patterns, particularly for foods that impact weight status and overall dietary quality (i.e., fruits, vegetables, proteins, etc.). For food acceptance patterns, Cooke and colleagues [18] found that females (ages 4–7 years) liked a greater number of foods than male children. With regards to specific foods or food groups, studies including children from various countries have shown that females rate liking of fruits [18–21] and vegetables [18–24] higher than males, while male children report higher liking for meat, fish, poultry, and high-fat foods compared to females [18–20,25]. Furthermore, male children in middle childhood have higher acceptance of fatty and sugary foods [18] and foods and beverages characterized as "unhealthy" (e.g., sweet snacks, savory snacks, and sugar sweetened beverages) compared to female children [20]. Additionally, females in middle childhood show increased liking for vegetables [22] while males have greater liking for meat products [18].

While the aforementioned studies demonstrate sex differences in food acceptance in middle childhood, studies in toddlers and preschool-aged children have shown no differences [26,27]. However, it is not clear if null findings are in part due to a lack of sensitivity in the methods available to measure liking in preschool children (i.e., hedonic facial scales and parental report). These results demonstrate that. in middle childhood, females typically like or prefer foods that are often regarded as lower in energy and nutrient dense, such as fruits and vegetables, whereas males tend to like meats, meat products, and foods high in fat and sugar. The sensory and/or nutritional characteristics of the foods that drive these sex-effects are not known.

2.2. Sex Differences in Dietary Patterns

As liking and preference are primary determinants of what children eat [28,29], it is likely that sex also influences children's dietary intake. This is especially apparent for fruits and vegetables [28]. In children as young as two years, intake of vegetables [30–33], fruits [24,31,32,34] and fruits and vegetables combined [31,35–37] is higher among females than males. Female children have similarly reported greater intake of foods classified as "healthy" and lower intake of "unhealthy" foods when compared to males [32]. Since these studies used self- and parentally-reported measures of food intake, there is potential for response bias as fruit and vegetable intake is a socially desirable behavior. However, studies using more objective assessment methods in schools have also observed that female students are more likely to consume from a salad bar than males [38,39]. The alignment with observational data strengthens the findings from questionnaires, suggesting that female children tend to consume more fruits and vegetables than males.

In addition to fruits and vegetables, self-reported intake of other foods and food groups also varies by sex. In cohorts of European children, males report consuming more sugar and sweets [36,40], breakfast cereals, full-fat milk, meats/meat products, and baked beans while females consumed more oily fish, eggs, and cheese [36]. In the United States, male children tend to have higher intake of most food groups, as well as higher overall energy intake [37,41], although overall variety of foods consumed tends to be higher in females [42]. This finding supports the previously discussed observations that found females also *liked* a greater number of foods than male children [18]. Although these studies provide support for the notion that sex differences in eating behavior arise in childhood, not all

studies agree [27,43]. Inconsistencies across studies could be due to variability in how dietary intake is measured (e.g., 24-h recall, food frequency, and direct observation), who is reporting dietary intake (e.g., parent vs. child), and the age and cognitive abilities of the child being studied [44]. There is a need to conduct more observational studies where food intake is directly measured to confirm sex-effects on reported intake in children.

2.3. Sex Differences in Questionnaire Measures of Appetitive Traits

The literature reviewed in the preceding sections on both food acceptance and intake supports the notion that female children like and consume more foods that are typically thought to be protective against excess weight, e.g. fruits and vegetables. However, these associations between sex and liking/preference do not provide insight into why females are at greater risk for eating and weight disorders. An additional possibility is that females differ from males in appetitive traits that might make them more susceptible to eating in response to external food cues or less susceptible to feedback from internal satiety cues. In the following section, evidence for sex differences in parentally reported measures of eating behaviors and appetitive traits are presented.

2.3.1. Picky Eating

Picky eating is commonly observed in young children [45] and is associated with lower consumption of fruits and vegetables [30,46]. In the literature, picky eating has been conceptualized by two related constructs: (1) food neophobia, which is the rejection of novel or unknown foods; and (2) food fussiness, which is the rejection of many known, familiar foods. Whether the prevalence of picky eating differs by sex is unclear. No sex differences were evident for picky eating, more generally, in a sample of Canadian preschool-aged children [47] or in a review of studies in toddlers ($\leq$30 months) [45]. For food neophobia, a study in French toddlers found males to have higher neophobia than females [48], however, other studies did not find sex differences [49,50]. In contrast, food fussiness, an eating trait assessed with the Children's Eating Behavior Questionnaire (CEBQ) [51], has shown more consistent sex differences, however, the pattern of results is inconsistent. Males have been reported as fussier eaters than females in a cohort of 2–7-year-olds from the United Kingdom [23] and in 6–7-year-olds from the Netherlands [52], while, in toddlers from China, females were reported to be fussier eaters [53]. In general, there appears to be greater evidence for picky eating in males than females, but the inconsistent findings emphasize the need to delineate the underlying constructs, examine potential confounding factors (e.g., parental characteristics, and child age and temperament), and have appropriately powered samples (i.e., not over or under powered).

2.3.2. Appetitive Traits

Other studies have investigated whether there are sex differences among other appetitive traits assessed by the CEBQ [51]. Using this instrument, some investigators have divided appetitive traits into those related to food avoidance (i.e., slowness in eating, satiety responsiveness, emotional undereating, and food fussiness) and those related to food approach (i.e., enjoyment of food, food responsiveness, desire to drink, and emotional overeating) [51]. Higher scores on food approach related subscales and lower scores on food avoidant related subscales have been positively associated with weight status in children [46,54–56]. While generally most studies have not shown systematic differences in appetitive traits between male and female children, a few studies have reported sex differences. For example, in a cohort study of middle childhood, males from Thailand had greater enjoyment of food than females [57], however the opposite was found in a cohort of 6–7-year-old Dutch children (i.e., females higher than males) [52]. When looking more broadly across appetitive traits, male children showed greater desire to drink [57], emotional overeating [52], and food responsiveness [53]. In contrast, females showed greater avoidance behaviors (e.g., slowness of eating and satiety responsiveness) [46,58].

Evidence of greater food approach behaviors among male compared to female children may be in part due to differential parent feeding strategies that reinforce these behaviors. Mothers of female children report greater concern about them putting on weight [59], and therefore they may encourage greater food avoidant strategies. On the other hand, male children receive greater encouragement to eat [60,61] and are served larger portion sizes from a virtual buffet than female children [62]. These domain-specific parenting strategies [63] may encourage the development of more avid appetites among males and more food avoidant strategies among females. Therefore, it is critical for future studies to take into account the role of parents in the development of eating behaviors in males and females.

2.4. Evidence of Sex-Effects on Laboratory Measures of Self-Regulatory Eating

The literature reviewed in the previous section indicates few systematic sex differences in parent-reported measures of children's appetite. While questionnaires are convenient for capturing an overview of child behaviors, responses may be affected by the biases parents have about feeding male versus female children. Objective measures are necessary to provide additional support for the role of sex in childhood eating behaviors. In the following section, results are reviewed from studies that have used laboratory methods to characterize "self-regulatory eating", broadly defined in this context as the ability to regulate energy consumption in response to internal or external signals.

2.4.1. Compensation Protocols

One of the most frequently used methods to assess self-regulatory eating is the compensation or preloading paradigm. Using a crossover design, children consume appetizers or "preloads" on two separate visits. Preloads are matched for taste, sensory characteristics, and often volume, but are covertly manipulated to vary in energy density (kcal per weight or volume of food or beverage) and/or macronutrient content. Participants are compelled to finish the preload and are served an ad libitum meal some time later (often 25–30 min with children) to measure consumption. Children who have "good" energy compensation can adjust their intake at the subsequent meal based on the energy content of the preload [64,65]. Poorer compensation ability has been associated with higher weight status in children [66–68], suggesting that performance on this measure may generalize to eating regulation more broadly. Several studies that have used this protocol in preschool children found that males have better energy compensation than females [66,67,69,70], which is consistent with some studies in adults [71,72]. Notably, other studies in preschool children do not report sex differences [73–77] and the individual variability in this measure is poorly understood. Of note, all the studies that have found that males compensate better than females have used beverages as a preload, raising the possibility that sex differences in energy compensation may be specific to the ability to regulate calories in liquid rather than solid form.

The notion that sex differences around eating self-regulation are specific to beverages is further supported by studies that have tested the effect of varying the energy density of a beverage served *within* a meal. Whereas the traditional preloading study measures "satiety" by testing the extent to which a preload or snack delays hunger at the following meal, serving a beverage within a meal captures "satiation" by determining the effect of varying energy content on total meal intake. Kling and colleagues [78] tested the effect of varying the energy density (ED) of milk on satiation by conducting a crossover study where either lower—(1% fat) or higher—(3.25% fat) ED milk was served to children with a typical preschool meal served in a childcare setting. When the higher-ED milk was served, males decreased their intake of the other meal items, whereas females did not. Thus, compared to males, females were less accurate at adjusting their intake to account for additional energy consumed from the higher-ED milk. These sex differences were independent of possible confounders, including the type of milk children consumed at home, child age and body size, milk liking and preference ratings, children's appetitive traits, and parent feeding practices. The pattern of sex differences observed in both satiety and satiation studies challenges the notion that compensatory responses are solely due to

the delay between the preload and subsequent meal that allows for the release of sensory and nutrient signals that influence fullness.

2.4.2. Eating in the Absence of Hunger

Eating in the absence of hunger (EAH) is a standard paradigm to assess hedonic eating [79–81]. It is thought to be stable through childhood [82], and is considered a phenotypic characteristic of childhood obesity [83]. Studies in preschoolers [75,83] and middle childhood [84] have found greater eating in the absence of hunger in males compared to females. However, in 5–18-year-old Hispanic children from the United States, sex differences did not persist after adjusting for energy needs [85]. Although individual differences in EAH may be partially driven by child energy needs, there is evidence that sex may moderate the relationship between EAH and outcomes such as child weight status [68,84,86,87], parental dieting characteristics, and feeding practices [80,86]. For example, some maternal behaviors such as dietary disinhibition [86] and restriction [88] are more predictive of EAH in females than in males. On the other hand, greater use of pressure to eat has been found to be a stronger predictor of EAH in males than in females [89,90]. These findings highlight the need to model the relationships between child level (i.e., sex and weight status) and parent level (i.e., feeding practices, eating styles, sex, and weight status) variables to elucidate the pathways leading to excess energy consumption in males and females.

2.4.3. Meal-Related Microstructure

Although not specifically related to the ability to regulate food intake, some investigators have referred to eating behaviors that make up meal microstructure (e.g., bite rate, eating rate, and bite size) as indicators of satiety responsiveness [91]. Of these characteristics, eating rate has been most consistently associated with weight status in adults [92] and children [93], and is therefore a target for interventions to treat obesity [94]. Observational coding of meal-time behaviors in the GUSTO cohort from Singapore showed that male children have faster eating rate (g/min), larger bite size (g/bite), and shorter oral exposure (min) than female children [95]. Similar findings have been reported in adolescents [96,97]. As masticatory development has been thought to be similar in males and females before puberty [98], it is unlikely that faster eating speed among male children can be attributed to stronger muscular force supporting the jaws. It has also been reported that eating rate has a genetic component, which may help to shed light on these differences [99]. While the research in this area is limited, the observation of sex differences in eating speed and oral processing time prior to puberty has implications for the development of personalized interventions to reduce overeating in males and females.

In addition to the aforementioned paradigms, other measures have been considered to assess self-regulatory eating in children, for example measuring children's intake in response to manipulations in food portion size [100,101], energy density [102], or self-serving conditions [103]. To the best of our knowledge, sex differences have not been observed in self-regulatory eating using these assessments, thus they are not discussed further in this paper.

While there is a lack of investigations that have included sex as a primary determinant of eating behaviors, the studies reviewed are suggestive of male–female differences in food liking and intake, appetitive traits, self-regulatory eating, and meal-related microstructure. In addition, there is evidence that child weight status may moderate the relationship between sex and eating in the absence of hunger. Since these differences could impact the success of dietary and behavioral interventions [15], additional research focused on clarifying the pathways by which eating behaviors develop in males and females is needed.

3. Biopsychosocial Contributions to Sex Differences

This section explores possible mechanisms for the observed sex differences in children's eating behaviors. The scope of the discussion has been limited to: (1) neural responses to food cues (a potential

biological influence); (2) body image and weight concerns (potential psychological influences); and (3) parental feeding attitudes and practices (potential social influences). Additional potential influences within these biological, psychological, and social constructs are presented in Figure 1, but are not explored at length in this paper. For additional insight on mechanistic pathways in the development of childhood eating behaviors, the reader is directed to recent reviews [104,105].

In addition to serving as a framework for presenting potential mechanisms that influence the development of eating behaviors in males and females, the biopsychosocial model can help guide the planning of new studies. The model can provide insight to help in the generation of new hypotheses that can be tested to further understanding of how eating behaviors develop in males and females. In addition, it can inform the types of questionnaires and measures that should be included when planning a study and can suggest potential interactions between variables to query during statistical analyses.

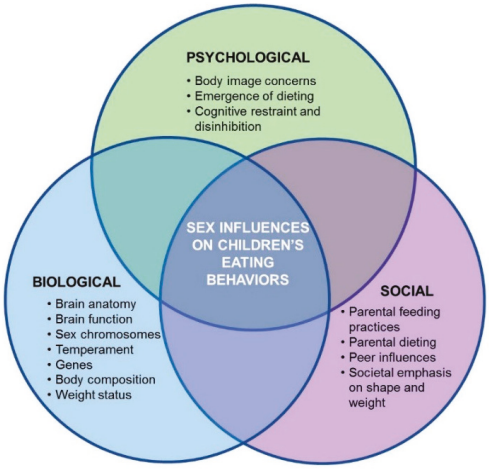

Figure 1. Biopsychosocial model of sex effects on children's eating behaviors. Potential biological influences could come from differences in brain anatomy or brain function that arise early in development, effects due to sex chromosomes, temperament, genes, or differences in body composition and/or weight status that can influence food intake regulation. Psychological influences include body image concerns, dieting, and cognitive restraint and disinhibition, typically observed more frequently in females than males. Social influences include differences in parental feeding practices directed at males and females, parental dieting, peer influences, and societal emphasis on "thinness" in females and "bigness" in males.

3.1. Neural Differences in the Response to Food Cues

One potential contribution to differences in eating behavior between male and female children is variation in neural processing of food cues. Food cues elicit responses in brain regions implicated in executive function, subjective valuation (e.g., orbitofrontal cortex), and visual processing (e.g., fusiform gyrus) [106] that are correlated with eating behaviors [107,108]. Several studies have observed sex differences in neural response to food cues. For example, in adult samples that have used functional magnetic resonance imaging (fMRI) to assess food cue reactivity, females show greater activation than males in a number of brain regions associated with executive function (i.e., dorsolateral and ventromedial prefrontal cortex) [109,110], visual processing [111] (e.g., fusiform gyrus), taste and interoceptive processing [111] (e.g., insula), and reward (e.g., caudate) [112]. To date, only one study has reported sex differences in children, although the findings contradict those from adults. Luo and colleagues [113] found that, compared to females, 7–11-year-old males had greater activation to food

relative to non-food images in the right posterior hippocampus and temporal occipital fusiform cortex, regions implicated in memory and visual processing. To date, the developmental trajectory of neural response to food cues remains unclear, making it difficult to interpret the inconsistent patterns of sex differences between adult and child samples.

3.2. Body Image and Weight Concerns

From a young age, individual differences in eating behaviors may in part be driven by sex differences in perceived ideal and preferred body size. Sex differences in dieting and body image concerns have been consistently documented in children as young as eight years [114]; however, differences in younger children are less consistent [114–116]. Compared to males, school-aged females report higher levels of weight-related behaviors and concerns, including desire to lose weight [117], dieting behavior [115], level of worry about weight and thoughts about which foods might promote weight gain [115,118,119], and feelings of guilt over eating too much [118]. Females also tend to be more dissatisfied with their bodies [116,117,120–122] and have lower self-esteems [121,123,124]. By eight years of age, females have greater body dissatisfaction than males [114,116–118,122,124,125] and this tends to increase during middle childhood [124]. Overall, greater emphasis on the maintenance of an ideal body weight in females than males may encourage sex differences in eating behaviors that are adopted to achieve "the perfect figure".

3.3. Parental Feeding Styles and Practices

The greater emphasis on "thinness" as a cultural ideal in females likely encourages sex differences in parental feeding practices and attitudes directed at children. In general, parents are more concerned about weight status in female children than they are in males [63,117]; thus, they are more likely to assume an active role in training, redirecting, and encouraging desired eating behaviors in female children [63,117]. Studies have also found that male children are encouraged to eat more than female children [60,61], while females are more likely to seek parental praise and approval for meal-time behaviors [60]. In response to maternal concerns, female children are more likely than male children to change eating behaviors [125,126]. These observations could partially explain sex differences in food acceptance and intake, whereby female children show more nutritious food intake patterns than males [32]. Greater need for external attentions, such as praise, among females could mean that they are less attentive to internal signals of hunger and fullness when compared to males, which may increase their risk for disordered eating behaviors.

The influence of controlling feeding practices, such as restriction and pressure-to-eat, have also been found to vary depending on the sex of the child. Observational coding of meals in Singapore revealed that mothers respond to faster eating in females by using more restriction and control-related prompts, but similar relationships were not found in males [127]. Greater laboratory [80,128] and parentally-reported restriction [67,129,130] have been associated with higher weight status in primarily Caucasian females, but not males. In addition, Arredondo and colleagues [131] found in Latino families that greater parental control over feeding is associated with increased reported intake of "unhealthy" foods (e.g., sodas, sugar sweetened beverages, chips, and sweetened cereals) in females, but not males. In general, mothers tend to use greater feeding control with female than male children [132]. Increased use of parental control, specifically within the domain of feeding, may weaken females' ability to eat in response to internal satiety signals, which may ultimately increase weight gain and risk for disordered eating. Notably, these patterns have not been consistently observed across studies. Studies in both preschool children [89] and a Dutch sample in middle childhood [90] found that controlling feeding practices were associated with greater eating in the absence of hunger [89] and external and emotional eating [90] in males, but not females. Overall, the influence of child age, ethnicity, and socioeconomic status, as well as parental factors including education, weight status, and general parenting style have not been clarified and require additional investigation.

3.4. Peer and Social Influences

In addition to parental influences, societal ideals related to expectations about what and how males and females should eat may also engender different eating behaviors in children. A feminine identity is characterized by eating smaller portions, consuming less meat, and preferring healthier options to maintain appearance, while a masculine eating identify is characterized by feeling full, with a focus on physical performance [133,134]. Within these ideals, female children are seen as more effective at modeling healthy behaviors than males [135,136]. Furthermore, females are also more likely to respond to modeled eating behaviors including vegetable acceptance [135] and fruit and vegetable intake [137]. The higher success of modeling and dietary interventions among females suggests a greater awareness of social expectations related to eating [138]. Moreover, greater self-control among females [139,140] may help facilitate greater uptake of these behaviors.

4. Applying the Biopsychosocial Model to Interpret Evidence of Sex Differences in Children's Eating Behaviors

In the prior two sections of the paper, we reviewed evidence from the literature of sex differences in children's eating behaviors and provided a biopsychosocial model as a framework for understanding how eating behaviors develop in males and females. A theme across the various studies reviewed is that the relationship between weight status and eating behaviors differs in males and females, and these differences may stem in part from parental feeding practices and societal pressures on ideal/acceptable body weights that differ by sex. Adding to this theme, the last section of the paper presents previously unpublished, secondary data analyses to determine the influence of sex and weight status on children's appetitive traits and neural response to food cues.

4.1. Case Study #1. Influence of Age, Sex, and Adiposity on Appetitive Traits

Although previous studies found higher food approach related behaviors among males than females [52,53,57], it is unclear how age and/or development might influence the relationship between appetitive traits and sex, as children might show higher food approach related behaviors during times rapid growth. For this reason, it is essential to understand whether the relationship between appetitive traits differs by child age and weight status. To shed light on this relationship, we examined CEBQ scores from 11 datasets collected from studies conducted at the Children's Eating Behavior Laboratory at The Pennsylvania State University during 2012–2018. A total of 263 (M = 133; 50.6%) 3–12-year-old children had complete parent-reported anthropomorphic and CEBQ data as well as measured child anthropometrics. Males and females did not differ by age ($t(260) = 0.553$, $p = 0.581$, $d = 0.07$), body mass index (BMI)-for-age percentile (BMI%; $t(260) = -0.859$, $p = 0.391$; $d = 0.11$), race (Fisher's $p = 0.276$), ethnicity (Fisher's $p = 0.999$), maternal education ($t(260) = 0.551$, $p = 0.58$, $d = 0.07$;), or CEBQ subscales (p values ranging 0.073–0.681; see Supplementary Materials, Table S1). Although maternal education did not differ by child sex, it was used as a proxy for socioeconomic status as maternal education has been shown to be more highly associated with adiposity than income [141]. Child weight status was assessed by measuring height and weight on a digital scale (Tanita, Arlington Heights, IL, USA) and stadiometer (SECA, Chino, CA, USA) and children were categorized as either having healthy weight (BMI-for-age < 85th percentile) or overweight/obesity (BMI-for-age $\geq$ 85th percentile) (Table 1).

Table 1. Demographic Characteristics of children enrolled in studies that assessed sex differences in appetitive traits[a] and neural responses to food cues[b].

	CEBQ [a]		Fmri [b]	
	Males (*n* = 133)	**Females (*n* = 130)**	**Males (*n* = 20)**	**Females (*n* = 25)**
Age (years)	7.40 (2.28)	7.56 (2.10)	8.75(0.99)	9.06(1.34)
BMI percentile	61.53 (29.06)	58.50 (28.20)	52.50(27.12)	53.57(30.93)
Maternal Ed. (years)	16.19 (2.63)	16.35 (2.71)	16.91(2.49)	16.88(1.90)
Weight Status (*n*)				
Obese/Overweight	43	27	3	6
Healthy Weight	90	103	19	19
Ethnicity (*n*)				
Not Hispanic/Latinx	94	84	20	25
Hispanic/Latinx	4	4	1	0
Not Reported	35	35	1	0
Race (*n*)				
Black/African American	6	2	2	0
White	119	112	19	25
Other	7	4	1	0
Not Reported	1	2	0	0
SES (*n*)				
>$100,000	16	19	7	5
$51,000–$100,000	30	29	11	15
≤$50,000	18	18	3	5
Not Reported	69	64	1	0

Means (SD) reported for Age, BMI percentile, and Maternal Education. Weight Status categories defined by BMI percentile: Obese/Overweight ≥ 85th percentile; Healthy Weight < 85th percentile. BMI, body-mass index; CEBQ, Child Eating Behaviors Questionnaire Sample; fMRI, functional Magnetic Resonance Imaging Sample. [a] Sample assessing appetitive traits in case study #1; [b] Sample assessing neural responses to food cues in Case Study #2.

Food approach and avoidance, as measured with CEBQ, were examined separately using the same hierarchical model steps: (1) child age and maternal education; (2) a quadratic age term; (3) child sex and adiposity; (4) a sex X age interaction; and (5) a sex X adiposity interaction (Table 2). The change in model fit, R^2, was tested at each step to determine whether the model explained significantly more variance with the added terms. Once the best model was identified, exploratory analyses examined the component subscales that contribute to the food avoidance and approach scores to determine whether the effect seen was consistent across subscales or driven by an individual subscale.

Individual differences in CEBQ avoidance and approach behaviors were best fit by different models. Child sex was not a significant predictor of avoidance for any of the models where it was included. In contrast, food approach was best modeled by including the interaction between child sex and weight status (Table 2). The interaction between sex and weight status was significant such that the association between having overweight or obesity and greater food approach was stronger for females than males. This suggests that weight status may be more predictive of food approach behaviors in females than in males. Exploratory analyses of approach subscales indicated that this finding was primarily driven by the food responsiveness subscale, which showed a suggested sex by weight status interaction (β(SE) = −0.36 (0.20), p = 0.073). The interactions between weight status and other CEBQ approach subscales were not significant (p values ranging from 0.155–0.255). Overall, these results suggest that, in female children, food responsiveness could be a better predictor of weight status than other CEBQ approach subscales, and therefore may be a target for intervention studies in this population.

Table 2. Hierarchical Regression for Approach and Avoidance Scales of the Child Eating Behavior Questionnaire.

Food Approach															
	1			**2**			**3**			**4**			**5**		
	B	**SE**	**β**	**B**	**SE**	**β**	**B**	**SE**	**β**	**B**	**SE**	**β**	**B**	**SE**	**β**
Maternal Education	−0.005	0.01	−0.027	−0.005	0.01	−0.028	−0.005	0.01	−0.025	−0.005	0.01	−0.025	−0.003	0.01	−0.015
Age	0.008	0.01	0.034	−0.003	0.09	−0.015	0.0003	0.01	−0.001	0.001	0.02	0.003	−0.002	0.01	−0.008
Age-squared				0.001	0.01	0.050	–	–	–	–	–	–	–	–	–
Weight Status							0.288	0.07	0.572 ***	0.288	0.07	0.288 ***	0.455	0.11	0.902 ***
Sex							−0.119	0.06	−0.002	−0.107	0.22	−0.237	−0.046	0.07	−0.091
Sex X Age										−0.002	0.03	−0.007	–	–	–
Sex X Weight Status													−0.286	0.14	−0.567 *
R^2			0.002			0.002			0.071			0.071			0.086
Δ R^2 F						[1] 0.017			[1] 9.59 ***			[3] 0.003			[3] 4.21 *
Food Avoidance															
Maternal Education	0.017	0.01	0.094	0.016	0.01	0.093	0.017	0.01	0.094	0.017	0.01	0.097	0.017	0.01	0.093
Age	−0.029	0.01	−0.137 *	−0.072	0.08	−0.339	−0.027	0.01	−0.126 *	−0.035	0.02	−0.165	−0.026	0.01	−0.125 *
Age-squared				0.003	0.01	0.205	-	-	-	-	-	-	-	-	-
Weight Status							−0.073	0.07	−0.156	−0.071	0.07	0.151	−0.087	0.10	−0.184
Sex							0.068	0.06	0.143	−0.049	0.21	0.143	-	-	-
Sex X Age										0.015	0.03	0.072	0.062	0.07	0.131
Sex X Weight Status													0.023	0.13	0.049
R^2			0.029			0.030			0.038			0.039			0.038
Δ R^2 F						[1] 0.303			[1] 1.137			[1] 0.0.87			[1] 0.765

Standardized coefficients (B) and standard errors are presented along with the unstandardized coefficients (β) for model Steps 1–5. For change in R-square, the number in the brackets indicate which model step it was tested against. * $p < 0.05$, *** $p < 0.001$. [1] Model was tested against model 1; [3] Model was tested against model 3

4.2. Case Study #2. Influence of Sex on Neural Food Cue Responsivity

In a separate dataset of 7–11-year-old children who had participated in a study on the neural determinants of food portion size and energy density [108,142], we followed up findings from Luo and colleagues [113] to investigate potential sex differences in children's food cue reactivity. As with the first case study, child weight status was treated as a key moderating factor. Males (N = 22) and females (N = 25) did not differ by age ($t(45) = 0.89$, $p = 0.378$, $d = 0.260$), BMI-for-age percentile ($t(45) = 0.125$, $p = 0.901$, $d = 0.036$), race (Fisher's $p = 0.095$), ethnicity (Fisher's $p = 0.456$), or maternal years of education ($t(45) = -0.045$, $p = 0.964$, d = 0.013) (Table 1).

On the day of the MRI, children arrived after a 2-h fast and were scanned during a usual meal-time. Before and after the scan, children rated fullness level on a validated, pictorial visual analog scale [143]. Children were imaged at 3T (MAGNETOM Trio) with a T1-weighted structural (MPRAGE) sequence and a T2*-sensitive gradient echo pulse sequence (see Supplementary Materials for image acquisition parameters). Food images were presented using MATLAB Version 8 [144] and viewed through a mirror mounted on the head coil using a magnet-compatible projector. The protocol for task design and image development has been reported elsewhere [108,142]. In brief, children viewed a total of 180 images (120 food, 30 furniture, and 30 scrambled images) presented in block design. The food cues differed in portion size (large or small) and energy density (high-ED or low-ED). High-ED foods were >1.5 kcal/gram and included French fries, chicken nuggets, cookies, and pizza. Low-ED foods were <1.5 kcal/gram and included grilled chicken, carrots, broccoli, and apples. Data were preprocessed and analyzed using Analysis of Functional NeuroImages (AFNI) [145] using standard preprocessing steps (see Supplementary Materials for details). Four participants (3 male and 1 female) were excluded due to excessive motion (defined as fewer than 4/6 usable runs; see Supplementary Materials for motion and outlier criteria). For each subject, a general linear model was constructed including 6 parameters of interest (i.e., one for each image condition) and 12 parameters of no-interest to control for motion (see Supplementary Materials). Group analyses were then conducted using energy density contrasts (high-ED – low-ED) derived from parameter estimates for each portion size condition separately, as well as a composite (i.e., across both portion sizes). Multiple comparisons were controlled by using Monte-Carlo simulations ([146] $p < 0.001$; $k = 29$) using AFNI's 3dClustSim to achieve a final $p < 0.05$.

As there was no main effect of portion size, or a portion size × sex interaction on neural response to high- or low-ED cues (see Supplementary Materials), the remaining group analyses focused on the ED contrast collapsed across portion size. An analysis of covariance (ANCOVA; 3dMVM [147]) showed a significant sex × BMI z-score interaction in right superior temporal gyrus, extending to both parahippocampal and fusiform gyri F(1,39) = 29.21; peak: x = −37.5, y = 37.5, z = 7.5; k-173; Figure 2A). Post-hoc correlations confirmed a significant positive association between BMI z-score and neural response to higher than lower ED food images in females ($r = 0.598$; $p = 0.002$), while in males this relationship was negative ($r = -0.667$; $p = 0.002$) (Figure 2B). There was no evidence for a main effect of BMI z-score or sex. Although pre- and post-scan fullness differed in males and females, the same pattern of results was seen when controlling for fullness ratings and when analyses included ED contrasts for each portion size (see Supplementary Materials Figure S1) (Figure 2A,B).

Although preliminary, these results suggest that increased weight status in female children is positively related to neural engagement to high- relative to low-ED food cues in regions typically associated with contextual processing (i.e., parahippocampal gyrus) and visual object recognition (i.e., fusiform gyrus), while in male children the opposite pattern was observed. While additional studies are needed to confirm these findings, they suggest that weight status in female children may be more associated with differential patterns of food-cue related brain activation than weight status in male children. Questions for additional investigation include determining whether brain alterations precede or follow the development of excess weight and understanding the behavioral implications for these neural responses.

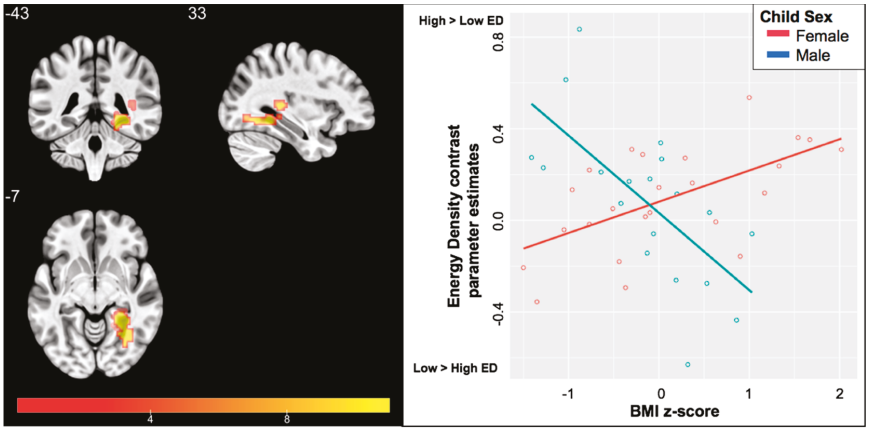

Figure 2. (**A**) Statistical parametric map (F-statistic) of the interaction between BMIz and child sex on neural responses to high-ED compared to Low-ED food cues. Cluster extends from the right superior temporal gyrus into the parahippocampal and fusiform gyri. (**B**) Extracted energy density contrast (high-ED–low-ED) parameter estimates, illustrating increased activation to high-ED compared to low-ED food cues for girls with BMIz greater than the 50th percentile and increased activation to high-ED compared to low-ED food cues for boys with BMIz greater below the 50th percentile.

5. Summary and Conclusions

In this paper, we review evidence of sex differences in children's eating behaviors and present new data showing that sex and weight status interact to differentially influence appetitive traits and neural response to food images in males and females. In the reviewed literature, we identified sex differences in food acceptance, food intake, appetitive traits, and laboratory measures of self-regulatory eating. In addition, new analyses showed that child weight status interacts with sex to influence appetitive traits such that food approach behaviors (i.e., food responsiveness) are stronger predictors of increased weight status in females than in males. Similarly, in a separate cohort of 7–11-year-olds, we found that sex and weight status interact to influence children's neural responses to food images that vary in energy density. In females, greater activation to higher energy food cues in brain regions implicated in contextual processing, memory, and object recognition was positively related to weight status, while the opposite pattern was observed in males. Although we cannot fully discount the possibility that some of the observed differences are driven by physiological changes that occur with puberty, the focus on children under 11 years of age likely reduces these influences. The evidence presented underscores the need to study the etiology and implications of sex differences in children's eating behaviors.

Despite inconsistencies across the literature, a few consistent themes are apparent. First, sex differences in children's eating behaviors were more often found in school-aged children. Few consistent differences in eating behaviors were identified among infants and toddlers. It is possible that differences are present in younger children but are unable to be measured due to methodological limitations. Perhaps more likely, however, is that these patterns arise during childhood due to differential parenting practices and social influences directed at males and females. Second, female children tend to report liking and eating more foods that are lower in energy density and higher in critical nutrients (i.e., fruits and vegetables) than males. Due to the lack of clear biological differences in taste anatomy [148], these differences are also likely to be influenced by parent, peer, and societal factors. Importantly, the self-report nature of most of this literature highlights the need to confirm these findings with more objective measures of eating behavior. Third, sex differences in appetitive traits,

EAH, and parental feeding attitudes are influenced by complex interactions with child weight status. In general, parents are more concerned about excess weight in female compared to male children. As a result, they likely feed children differently depending not only on the sex of the child, but also their perception that the child is at risk for developing overweight. It is likely that parental characteristics, such as dieting history, cognitive restraint, socioeconomic status, and weight status, influence the relationship between child sex and eating behaviors, highlighting the need to conduct larger studies that are sufficiently powered to query three-way interactions (e.g., child sex × child weight status × parent weight history).

6. Recommendations for Future Research

When planning and reporting on future studies, it is important that researchers clearly define the constructs of sex and gender, in terms of how they are measured and reported. In addition, in studies that statistically control for sex as a covariate, it would be helpful for researchers to report applicable estimates, coefficients, and *p*-values for covariates, either in the manuscript or in Supplementary Data. This would facilitate the ability to conduct systematic reviews on this topic. Moreover, research in children, especially infants and preschool children, should utilize objective and observational measures of children's eating behaviors and intake when possible to limit the influence of parental beliefs and perceptions along with probable response bias for questionnaires. Lastly, in regards to intervention efforts for obesity, sex or gender should be considered when determining target behaviors as well as evaluating the impact of the intervention on primary and secondary outcomes. Together, these recommendations will help advance our understanding of the role that sex and gender play in the development of weight and eating disorders.

Caution is recommended when interpreting the findings discussed, both from the literature and the new analyses presented. First, the majority of studies that have reported sex differences were not designed to detect sex as a primary determinant of outcomes; thus, it is not possible to rule out chance findings. Second, among the studies that did not report differences, sex was often controlled for as a covariate, but results for main outcomes were not stratified and reported by sex. This makes it difficult to determine whether primary eating behavior outcomes differed in males and females and limits the ability to conduct meta-analyses across studies. Third, determining the underlying mechanisms for sex differences in eating behavior is complicated by the lack of clarity in how sex and gender are defined in the literature. A concern moving forward is that researchers will overgeneralize findings by developing separate intervention approaches for males and females without considering that sex and gender are non-binary, multidimensional constructs. To avoid this type of overgeneralization, we caution against using sex or gender as the basis to group participants prior to assigning treatments. Instead, sex and gender should be measured and considered such as other individual subject characteristics and used to provide information to help phenotype risk groups.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/3/682/s1, Table S1: Parent Report for Child Eating Behaviors Questionnaire, Figure S1: Overlap of significant clusters from the four analyses: (1) ANCOVA with overall ED contrast (red), (2) Linear mixed effects model with ED contrasts for each portion size (yellow), (3) ANCOVA with overall ED contrast and pre-MRI fullness covariate (cyan), (4) ANCOVA with overall ED contrast and post-MRI fullness covariate (blue).

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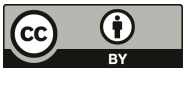

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Influences of Psychological Traits and PROP Taster Status on Familiarity with and Choice of Phenol-Rich Foods and Beverages

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Abstract: Plant phenolics are powerful antioxidants and free radical scavengers that can contribute to the healthy functional properties of plant-based food and beverages. Thus, dietary behaviours rich in plant-based food and beverages are encouraged. However, it is well-known that the bitter taste and other low-appealing sensory properties that characterize vegetables and some other plant-based foods act as an innate barrier for their acceptance. The aim of this study was to investigate the influence of psychological traits and PROP status (the responsiveness to bitter taste of 6-n- propylthiouracil) on the choice of and familiarity with phenol-rich vegetables and beverages varying in recalled level of bitterness and astringency. Study 1 aimed at assessing the variations of the sensory properties of vegetable and coffee/tea items with two check-all-that-apply (CATA) questionnaires (n = 201 and n = 188 individuals, respectively). Study 2 aimed at investigating how sensitivity to punishment, to reward, and to disgust, food neophobia, private body consciousness, alexithymia, and PROP responsiveness affect choice and familiarity with phenol-rich foods (n = 1200 individuals). A Choice Index was calculated for vegetables (CV) and coffee/tea (CC) as a mean of the choices of the more bitter/astringent option of the pairs and four Familiarity Indices were computed for vegetables (FV) and coffee/tea (FC), higher (+) or lower (-) in bitterness and astringency. Subjects higher in food neophobia, sensitivity to punishment or sensitivity to disgust reported significantly lower choice indices than individuals lower in these traits, meaning that they systematically opted for the least bitter/astringent option within the pairs. Familiarity with vegetables was lower in individuals high in sensitivity to punishment, in food neophobia and in alexithymia, irrespective of their sensory properties. The Familiarity Index with coffee/tea characterized by higher bitterness and astringency was lower in individuals high in food neophobia, sensitivity to disgust, and alexithymia. No significant effect of PROP was found on any indices. The proposed approach based on product grouping according to differences in bitterness and astringency allowed the investigation of the role

of individual differences in chemosensory perception and of psychological traits as modulators of phenol-rich foods preference and consumption.

Keywords: choice; familiarity; PROP; food neophobia; sensitivity to disgust; sensitivity to punishment; vegetables; caffeinated beverages; bitterness; astringency

1. Introduction

Diets rich in plant-based food and beverages are encouraged, given general agreement on their positive health outcomes. Meta-analyses of the effects of such foods indicate that a reduced risk of coronary heart disease, stroke, and diabetes are associated with a regular intake of non-starchy vegetables and moderate consumption of tea and coffee [1].

Plant phenolics are powerful antioxidants and free radical scavengers that can contribute to the healthy functional properties of plant-based food and beverages [2]. However, phenol compounds from vegetable sources are characterized by bitterness, astringency, and pungency [3–5], sensations that may limit food acceptability [6,7]. Human beings, long sensitized to the bitter taste of plant toxins, consider excessive bitterness the principal reason for food rejection [8]. The tactile sensation of astringency discourages animals from ingesting foods too high in tannins, thus protecting them from the tannin's potential harmful anti-nutritional effects [9]. A high intensity of perceived astringency negatively impacts the acceptance for high phenol containing foods [3]. The high phenol binding proteins from parotid glands exert a protective role against dietary phenols, and astringency arises from phenol interactions with the adsorbed glycoprotein layer, with the consequent oral cavity delubrication [10,11].

Sensory properties drive liking for vegetables [12], and it is well-known that bitterness and other unpalatable sensory properties may act as a barrier for vegetable acceptance [8,9,13,14]. Moreover, while bitterness and astringency are important qualities in tea and coffee, and may contribute to consumer appreciation of these products [15,16], in actual consumption conditions, masking ingredients (sweeteners, milk) are often used to modify these sensations to levels compatible with individual preferences [17].

Healthy individuals substantially differ in chemosensory perception, and such variability has been extensively studied in recent years. Most notably, the inherited capacity to perceive the bitterness of propylthiouracil (PROP) is considered a reliable broad marker for individual differences in taste responsiveness that may influence food preferences and eating behaviour [18]. The effect of the PROP phenotype (PROP bitterness ratings on the generalized Labeled Magnitude *Scale* (gLMS): ≤17, non-taster (NT); 18–52, medium taster (MT); and ≥53, supertaster (ST), according to Hayes et al. and Fischer et al. [19,20]) on the intake and preference of bitter foods and beverages has been examined in several studies, with mixed results, mainly because demographics, genetics, and other environmental factors may influence both phenotypic responses to oral stimulation and affective response to food [21,22]. Those who are insensitive to PROP bitterness (non-tasters) were found to consume more vegetables and more bitter vegetables than the other taster phenotypes, PROP medium-tasters and super-tasters [23,24]. The super-taster PROP phenotype was associated with a lower preference for bitter vegetables [25]. On the other hand, no differences between PROP phenotypes were found in preferences for plant-based bitter foods [26] or for actual vegetable intake in children [27–29]. PROP supertasters gave higher bitterness, sourness, and astringency ratings for coffee, but these did not significantly affect liking [17] or consumption [30]. In general, these results are inconsistent and the causal models envisaging straight associations of variations in taste abilities with food perception and choice show a weak predictive power.

Recent studies have shown that personality has a hugely important role in preferences and choices and, in some cases, in determining sensory responses to foods. One such key personality variable is the trait of food neophobia (FN), originally defined as the reluctance to try or eat unfamiliar foods.

High levels of food neophobia have been associated with reduced preference and intake for many food products belonging to different categories, including fruits and vegetables, in adults [31,32] and children [33]. In particular, food neophobia was found to affect the liking of foods and beverages characterized by high intensities of bitterness, astringency, sourness, and pungency. Those high in food neophobia (neophobics) reported liking such vegetables, beverages, fruits, and spicy foods less than those low in food neophobia (neophilics). Conversely, few differences between food neophobia groups were found for the liking of bland vegetables and beverages, or for sweets and desserts [32,34]. Neophobics perceive pungency and astringency in food products as more intense, and like the most pungent and astringent samples less than neophilics [34,35].

Other personality traits have been found to be associated with lower preferences for pungent foods. Individuals highly sensitive to visceral disgust (disgust related to rotten food, vermin, and body fluids) [36,37] find pungent foods more intense and like and choose them less [35]. Two other personality traits, sensitivity to punishment and sensitivity to reward, describe individual differences in reactivity and responsivity to the behavioural inhibition and activation systems, respectively [38]. Sensitivity to punishment was found to be negatively associated with liking of spicy foods [39] and pungent food choice in females [35]. Sensitivity to reward was found to be positively associated with chili intake, liking of spicy foods, and choice of pungent foods [35,39,40]. Recent studies have also highlighted an association between sensitivity to reward and unhealthier food behaviours, such as a preference for sweet and fatty foods, higher fat intake, higher alcohol consumption, and smoking frequency [41–43]. Alexithymia, defined as the inability of individuals to identify and name their emotional states [44], was found to be associated with food preferences, with high alexithymia associated with a liking for alcohol, sweets, and fats/meats, and lower alexithymia with a liking for vegetables, condiments, and strong cheeses [45].

The complexity of these factors and the sometimes mixed reports on their effects indicate that the interplay of several dimensions, such as gender, age, personality traits, and taste responsiveness, influence choice and intake of foods and beverages. In addition, food products are selected based on culture, which means that some products are far more contextually appropriate and/or familiar than others. While a positive relationship between familiarity and choice can be expected, the strength of this relationship is unclear. Many contextual situational factors may play a role in choice, while familiarity covers both features of frequency of consumption (occasional and regular) and levels of knowledge (from product name to product taste) that are less affected by contextual factors (see, for example, the scale developed by Tuorila and colleagues [46]). In addition, it is not known if, or in what way, the relationship between choice and familiarity is affected by personality traits or taste responsiveness. Although some studies have investigated how taste responsiveness affects food familiarity or food choice, the literature on the role of psychological traits is quite limited, and the relationships between these variables remain little explored [35]. Exploring the factors that influence choice of and familiarity with phenol-rich foods and beverages is of interest to better understand food behaviour and to shed light on the role of personality traits and taste responsiveness as barriers to heathy eating.

The grouping of food and beverages based on their overall sensory characteristics has already been used to explore individual differences in preferences and consumption. PROP status only marginally affects the preference expressed for specific foods selected to represent sensations generally disliked by PROP supertasters, such as bitterness and pungency [26]. Food neophobia level significantly influenced preference for and familiarity with food and beverages categorized as “mild” and “strong” flavors [34]. Grouping vegetables as having low and high appeal was used to investigate demographic and attitudinal variables affecting vegetable consumption in European adolescents [14]. Existing data from sensory evaluations of trained and untrained assessors, as well as the chemical composition, were the criteria generally used for grouping the foods [12,14,47–51].

In the present study, an original approach to phenol-rich product grouping based on differences in bitterness and astringency is proposed. This approach was used to investigate the influence of individual variation in psychological traits and PROP status on choice of and familiarity with phenol-rich

vegetables and beverages, varying in recalled levels of bitterness and astringency. Furthermore, the relationship between familiarity with and choice of phenol-rich vegetables and beverages with a high recalled level of bitterness and astringency as a function of personality traits and PROP status was investigated.

2. Materials and Methods

The experimental plan consisted of two independent studies: one preliminary study and one main study, conducted with two different subject groups. The preliminary study was conducted in order to validate the differences in expected level of bitterness and astringency within each pair included in the vegetable choice questionnaire (V-IT-FCQ) and coffee/tea choice questionnaire (C-IT-FCQ) used in the main study. The main study aimed at investigating how PROP responsiveness and psychological traits affect familiarity with, and choice of, vegetables and coffee/tea, presented in pairs with two options with different levels of bitterness and astringency. The studies were conducted in agreement with the Italian ethical requirements on research activities and personal data protection (D.L. 30.6.03 n. 196) and the respondents gave their written informed consent at the beginning of the study. The protocol of the studies was approved by the Ethics Committee of Trieste University. The respondents gave their written informed consent at the beginning of the test, according to the principles of the Declaration of Helsinki.

2.1. Participants

Participants were recruited on a national basis by means of announcements published on social networks (Facebook), articles published in national newspapers, and in magazines. Furthermore, each research unit recruited subjects locally by means of social networks, mailing lists, pamphlet distribution, and word of mouth. The exclusion criteria were pregnancy and not having lived in Italy for at least 20 years.

2.1.1. Preliminary Study—Validation of the Differences in Bitterness and Astringency within Pairs of the Choice Questionnaires used in the Main Study

Subjects completed an online questionnaire aimed at measuring the sensory response (bitterness and astringency) to vegetables (201 subjects: 77.7% females; age range 18–70; mean age 40.3 ± SD 14.1) and coffee/tea (188 subjects: 75.4% females; age range 19–68; mean age 40.1 ± SD 14.3) products (presented with names) selected for the questionnaires used in the main study (§ 2.1.2).

2.1.2. Large Scale Data Collection

Data were collected on 1200 Italian subjects (58% females; age range 18–60 years; male mean age 35.9 years ± SD 12.8; female mean age: 35.2 years ± SD 12.9) on a national basis. In order to explore possible age-related differences, subjects were divided into three age groups: 18–30 years (45.6%), 31–45 years (28.0%), 46–60 years (26.4%).

2.2. Procedure

2.2.1. Preliminary Study—Validation of the Differences in Bitterness and Astringency within Pairs of the Choice Questionnaires

Two check-all-that-apply (CATA) questionnaires [52] with forced choice (yes/no) were developed to describe the sensory properties of items to be included in the vegetable food choice questionnaire (V-IT-FCQ) and coffee/tea choice questionnaire (C-IT-FCQ) used in the main study. The vegetable CATA questionnaire included fourteen items: "pumpkin risotto", "risotto with radicchio", "lettuce and valerian salad" (*Valerianella locusta,* also known as corn salad or mâche), "radicchio and rocket salad", "green salad", "bean sprout salad", "chard", "chicory", "zucchini", "asparagus", "carrots", "cauliflowers", "cucumber", and "radish". The coffee/tea CATA questionnaire included coffee and tea

items with/without ingredients (milk and sugar) masking the perception of bitterness and astringency. The coffee/tea CATA questionnaire included six items: "coffee with sugar"; "coffee without sugar"; "tea with sugar"; "tea without sugar", "macchiato", and "cappuccino". The list of sensory properties included 19 and 13 descriptors in the vegetable and coffee/tea questionnaires, respectively, but in the present paper only bitterness and astringency were considered. Both the products and the sensory properties were presented using words in a randomized order. The participants filled in the questionnaire online. The online platform SurveyGizmo (surveygizmo.eu) was used for data collection.

2.2.2. Large Scale Data Collection

Participants were asked to fill in an online questionnaire, and they then attended a session at the laboratory. Socio-demographic (gender, age, education) information and familiarity with foods were collected through online questionnaires before the test sessions. In the lab session, participants were asked to fill in a set of questionnaires to measure personality and psychological traits and to complete the choice questionnaires. PROP responsiveness was also measured. The study included sensory tests, questionnaires, and the collection of other data (see Monteleone et al., [53] for a complete overview of data collection), but only a selection of variables are presented here.

Psychological Traits

Sensitivity to punishment (SP) and sensitivity to reward (SR), related to responsiveness of behavioural inhibition and activation systems, were quantified using the sensitivity to punishment and sensitivity to reward questionnaire (SPSRQ) questionnaire developed by Torrubia, Ávila, Moltó, and Caseras [54]. Items 4, 8, 16, 25, 32, 34, and 36 were discarded based on the validation of the questionnaire in Italian (see Spinelli et al 2018 [36]). The sensitivity to punishment and sensitivity to reward scales were scored with a yes/no format. For each subject, sensitivity to punishment and sensitivity to reward scores were computed by summing up the yes answers (SP score range 0–23; SR score range 0–18), so that a higher score indicated a higher sensitivity to punishment and to reward.

Food neophobia (FN), defined as the reluctance to try and eat unfamiliar foods, was quantified using the 10-statement scale developed by Pliner and Hobden [55] and validated in Italian by Laureati and colleagues [34]. Individual food neophobia scores were computed as the sum of ratings given to the 10 statements, after reversing the neophilic items (using a seven point Likert scale: disagree strongly/agree strongly). The scores ranged from 10 to 70, with higher scores corresponding to higher food neophobia.

Sensitivity to disgust (DS), defined as the responsivity to core-visceral disgust (rotten food, vermin, body fluids), was quantified using the eight-item short form of the disgust sensitivity scale developed by Inbar, Pizarro, and Bloom [56] and validated in Italian by Spinelli and colleagues [35]. The scale includes two subscales, each presented with a specific scale ranging from 1 = strongly disagree (very untrue about me) to 5 = strongly agree (very true about me) (subscale 1) and from 1 = not at all disgusting to 5 = extremely disgusting (subscale 2). The individual scores ranged from 5 to 40, with higher scores reflecting a higher sensitivity to disgust.

Private body consciousness (PBC), defined as the disposition to focus on internal bodily sensations (awareness of internal sensations), was quantified using the five-item instrument developed by Miller, Murphy, and Buss [57]. The individual score was computed as the sum of the ratings given for the five statements (using a five-point scale: extremely uncharacteristic/extremely characteristic). The scores ranged from 5 to 25, with higher scores reflecting higher private body consciousness levels.

Alexithymia (TAS), defined as a specific disturbance in psychic functioning, characterized by difficulties in the capacity to verbalize affect and to elaborate fantasies, was quantified using the Toronto Alexithymia Scale (TAS) developed by Parker, Bagby, Taylor, Endler, and Schmitz [58] and validated in Italian by Bressi and colleagues [59]. The individual alexithymia total score was computed as the sum of ratings given to the 20 statements (using a five-point Likert scale: disagree strongly/agree

strongly). The alexithymia total scores ranged from 20 to 100, with a higher score indicating a greater level of alexithymia.

PROP Phenotyping

PROP taster status was assessed using a 3.2 mM PROP solution, prepared by dissolving 0.545 g/L of 6-n-propyl-2-thiouracil (European Pharmacopoeia Reference Standard, Sigma Aldrich, Milano, Italy) in deionized water [60]. Subjects were presented with two identical 10 mL samples, each coded with a three-digit code. Subjects were instructed to hold each sample in their mouth for 10 s, then to expectorate, wait 20 s, and evaluate the intensity of bitterness using the general label magnitude scale (gLMS; 0 = no sensation–100 = the strongest imaginable sensation of any kind) [61]. Verbal instructions were given that the top of the scale represented the most intense sensation that subjects could ever imagine experiencing. To ensure appropriate use of this scale, practise using a variety of remembered sensations from different modalities, including loudness, oral pain/irritation, and tastes, was provided. Subjects had a 90 s break to control for carry-over effects after the first sample evaluation. During the break, subjects adopted a washing procedure to rinse their mouth with distilled water for 30 s, ate some plain crackers for 30 s, and finally rinsed with water for a further 30 s before they evaluated the second PROP sample [5]. PROP taster status was based on the average rating of the two replicates and groupings were based on previously published cut-offs [19,20]: PROP non-tasters (NT) ≤17 (n = 274); PROP medium tasters (MT), 18–52 (n = 505); and PROP supertasters (ST) ≥53 (n = 421) on the gLMS.

Choice of and Familiarity with Vegetable and Coffee/Tea items

The choice of phenol-rich vegetables and coffee/tea between pairs of two food items characterized by different levels of bitterness and astringency was assessed with the V-IT-FCQ and C-IT- FCQ (Table 1). Vegetable and coffee/tea pairs in the choice questionnaires were selected so that the options in each pair significantly differed for bitterness and astringency, based on the results of the preliminary CATA study. V-IT FCQ consisted of seven pairs of vegetables, selected to represent possible options for the same main dish (risotto with different condiments: pumpkin or zucchini) and for similar side dishes consisting of raw (leafy/green salads: lettuce and valerian or radicchio and rockets; green salad or bean sprouts; salad ingredients: cucumbers or radishes) or cooked (leafy green: chard or chicory; others: zucchini or asparagus; carrot or cauliflower) vegetables. Similarly, coffee and tea options were selected to represent possible alternatives of the same hot beverage, including or excluding ingredients masking the perception of bitterness and astringency (i.e., milk and sweeteners).

Table 1. Pairs of food items included in the vegetable choice questionnaire (V-IT-FCQ) and coffee/tea choice questionnaire (C-IT-FCQ).

Vegetable Choice Questionnaire (V-IT-FCQ).	
0: Options lower in bitterness and astringency	**1: Options higher in bitterness and astringency**
Pumpkin risotto	Risotto with radicchio
Lettuce and valerian salad	Radicchio and rocket salad
Green salad	Bean sprout salad
Chard	Chicory
Zucchini	Asparagus
Carrots	Cauliflower
Cucumber	Radish
Coffee/Tea Choice Questionnaire (C-IT-FCQ)	
Macchiato	Coffee
Coffee with sugar	Coffee without sugar
Cappuccino	Coffee
Tea with sugar	Tea without sugar

For each pair, participants were asked to indicate which food they would ideally choose, pointing out that the answer would describe not what they usually choose but rather what they would like to choose in a situation of absence of restrictions (e.g., due to health or weight concerns). The choice for vegetables was asked in the context of a main meal and the choice for coffee/tea was asked in the context of breakfast. Options within the pairs were coded as "0" for the lowest level of bitterness and astringency and "1" for the highest level of bitterness and astringency. Here, for each subject, a choice index was calculated for vegetables (CV) and coffee/tea (CC) as a mean of the choices of the more bitter/astringent option (range from 0 to 1). Transformation in continuous variables of the binary data has been proposed in order to simplify analysis and use standard statistical methods frequently used for sensory data [62,63]. The approach for the calculation of a choice index as a sum of the options 1 (within the pairs) was already used in Spinelli et al. [35].

Familiarity with vegetables and coffee/tea items was assessed by a five-point labelled scale (1 = I do not recognize it; 2 = I recognize it, but I have never tasted it; 3 = I have tasted it, but I don't eat it; 4 = I occasionally eat it; 5 = I regularly eat it) developed by Tuorila and colleagues [46]. Two indices of familiarity with vegetables and coffee/tea higher in bitterness and astringency (+) were obtained by the sum of ratings of familiarity with the items that, within each pair, were higher in these sensations, based on the results of the preliminary study: FV+: risotto with radicchio, radicchio and rocket salad, bean sprout salad, chicory, asparagus, cauliflower, radish; ranging from 7 to 35; FC+: coffee and tea without sugar; ranging from 2 to 10. Two indices of familiarity with vegetables and coffee/tea lower in bitterness and astringency, respectively, were obtained by the sum of ratings of familiarity with the items that, within each pair, showed a lower level of bitterness and astringency (-), based on the results of the preliminary study: FV-: pumpkin risotto, lettuce and valerian salad, chard, zucchini, carrots, cucumber; ranging from 6 to 30; FC-: coffee and tea with sugar; ranging from 2 to 10.

The presentation order of the food items in the familiarity and choice questionnaires was randomized across participants.

2.3. Data Analysis

2.3.1. Preliminary study—Validation of the Differences in Bitterness and Astringency within Pairs of the Choice Questionnaires

Cochran Q-tests were performed to assess the differences between the frequency of selection of bitterness and astringency within the pairs of the V-IT-FCQ and C-IT-FCQ. Post-hoc pairwise comparisons were calculated using the McNemar procedure and the level of significance was set at 5% [43,52].

2.3.2. Large Scale Study

Cronbach's α was computed to check for the internal reliability of each psychological trait questionnaire. Two-way ANOVA models were used to determine the main effects of gender (males; females) and age class (18–30; 31–45; 46–60) and their interactions on psychological trait scores and on PROP bitterness intensity. Three-way ANOVA models were used to test the effects of gender, age, and psychological trait level (low, medium, and high) and PROP status (NT, MT, and ST) and their interactions on choice (CV and CC) and familiarity (FV+, FV-, FC+, FC-) indices.

The robustness of the ANOVA models was verified; the residuals of each ANOVA model were inspected for normality by histograms and Q–Q plots and for heteroscedasticity using Levene's test. A p-value of 0.05 was considered the threshold for statistical significance and post-hoc using the Bonferroni test adjusted for multiple comparisons were used. Pearson's correlation coefficients were computed to explore the association between familiarity and choice (FV+ and CV; and FC+ and CC, respectively) in subject groups with different levels of expression of psychological traits (L, M, and H) and PROP status (NT, MT, and ST). A p-value of 0.05 was considered the threshold for

statistical significance. Fisher's r to z transformation was used on the correlation coefficient to assess the significance of the differences (p-value of 0.05).

The XLSTAT statistical software package version 19.02 (Addinsoft) was used for data analysis.

3. Results

3.1. Preliminary Study—Validation of the Differences in Bitterness and Astringency within Pairs of the Choice Questionnaires

Significant differences were found between the items of each pair belonging to the vegetable choice questionnaire (V-IT-FCQ) and to the coffee/tea choice questionnaire (C-IT-FCQ) in both bitterness and astringency frequency of selection, with the exception of green salad/bean sprout salad in bitterness ($p = 0.262$) and carrots and cauliflower in astringency ($p = 0.827$) (Table 2).

Table 2. Percentage of participants who selected the terms "bitterness" and "astringency" in the check-all-that-apply (CATA) experiment. Cochran's Q test was used to determine significant differences between samples.

Vegetable Choice Questionnaire (V-IT-FCQ)							
Option 0 (lower in bitterness and astringency)	**Option 1 (higher in bitterness and astringency)**	**Bitterness (%)**			**Astringency (%)**		
		p	**option 0**	**option 1**	***p***	**option 0**	**option 1**
Pumpkin risotto	Risotto with radicchio	**	1.6	69.9	**	7.1	21.9
Lettuce and valerian salad	Radicchio and rocket salad	**	18.9	82.1	**	6.5	27.9
Green salad	Bean sprout salad		16.4	12.9	*	6.0	13.4
Chard	Chicory	**	27.4	81.6	**	13.4	30.3
Zucchini	Asparagus	**	11.9	34.8	**	5.0	13.4
Carrots	Cauliflower	**	3.0	16.9		7.5	7.0
Cucumber	Radish	**	31.3	46.3	*	19.4	29.9
Coffee/Tea Choice Questionnaire (C-IT-FCQ)							
Option 0 (lower in bitterness and astringency)	**Option 1 (higher in bitterness and astringency)**	**Bitterness (%)**			**Astringency (%)**		
		p	**option 0**	**option 1**	***p***	**option 0**	**option 1**
Macchiato	Coffee	*	50.5	97.9	*	13.3	41.0
Coffee with sugar	Coffee without sugar	*	19.7	97.9	*	20.2	41.0
Cappuccino	Coffee	*	21.8	97.9	*	6.4	41.0
Tea with sugar	Tea without sugar	*	4.3	67.0	*	30.3	44.1

* $p \leq 0.01$, ** $p \leq 0.001$.

3.2. Large Study on Familiarity with and Choice of Phenol-Rich Foods and Beverages

3.2.1. Personality Trait Questionnaires

The internal reliability of the questionnaires measuring psychological traits was satisfactory, with Cronbach's alpha ranging from 0.86 to 0.70 (Table 3). Based on the percentile limits, the population was grouped into Low-L (1° quartile), Medium-M (interquartile), and High-H (3° quartile) levels of expression of each trait (Table 3).

Table 3. Psychological traits: internal reliability (Cronbach's α–α), limits of the first (1st Q) and the third (3rd Q) quartiles, number of observations for each group (Low, Medium, High).

Trait	α	1st Q	3rd Q	*n* Low	*n* Medium	*n* High
Sensitivity to Punishment	0.85	5	13	310	537	353
Sensitivity to Reward	0.77	3	9	329	540	331
Food Neophobia	0.86	18	36	334	558	308
Sensitivity to Disgust	0.70	25	33	303	533	364
Private Body Consciousness	0.71	16	21	368	490	334
Alexithymia	0.82	38	55	314	567	312

Both gender and age affected individual variation in personality traits (Table 4). A significant gender effect was found for private body consciousness, sensitivity to punishment, sensitivity to reward, and sensitivity to disgust. Females were significantly higher in private body consciousness, sensitivity to punishment, and sensitivity to disgust than males, while males were more sensitive to reward. A significant effect of age was found for sensitivity to punishment, sensitivity to reward, sensitivity to disgust, alexithymia, and food neophobia. Sensitivity to punishment, sensitivity to reward, and alexithymia decreased with age, while food neophobia and sensitivity to disgust increased with age. The effect was further characterized by an interaction in the case of gender with private body consciousness: a decrease in private body consciousness with age was found in males, but not in females.

Table 4. Two-way ANOVA: gender, age and their interaction effect on psychological traits and on propylthiouracil (PROP) bitterness scores. F, *p*, and mean values.

Trait	Gender				Age					Gender × Age	
	F	*p*-Value	Mean Values		F	*p*-Value	Mean Values			F	*p*-value
			Females	Males			18–30	31–45	46–60		
Sensitivity to Punishment	37.1	<0.0001	9.9	8.0	32.4	<0.0001	10.5 (a)	8.2 (b)	8.2 (b)	1.6	0.2058
Sensitivity to Reward	72.7	<0.0001	5.1	6.8	85.8	<0.0001	7.6 (a)	5.6 (b)	4.7 (c)	0.8	0.4343
Food Neophobia	0.5	0.4701	27.2	27.7	10.0	<0.0001	26.1 (b)	26.6 (b)	29.7 (a)	0.2	0.8198
Sensitivity to Disgust	90.1	<0.0001	30.6	27.6	14.6	<0.0001	28.0 (b)	29.2 (a)	30.1 (a)	3.0	0.0513
Private Body Consciousness	25.3	<0.0001	18.7	17.4	1.1	0.3410	18.2	18.1	17.7	7.2	0.0008
Alexithymia	0.1	0.7899	46.0	46.2	37.9	<0.0001	49.8 (a)	43.4 (b)	45.0 (b)	0.4	0.6821
PROP	22.8	<0.0001	44.6	36.9	12.6	<0.0001	45.2 (a)	41.3 (a)	35.6 (b)	3.0	0.0495

Different letters indicate significantly different values ($p \leq 0.05$).

3.2.2. PROP Responsiveness

Effects of both gender and age were found on responsiveness to PROP (Table 4). The effects were further characterized by an interaction with gender, in that females were more responsive to PROP. PROP responsiveness decreased from the age class 18–30 to 31–45 and then remained stable in females, while a decrease in PROP responsiveness in males was reported in the age class 46–60.

3.2.3. Vegetable Choice Index (CV) and Coffee/Tea Choice Index (CC)

The effects of individual variation in psychological traits and PROP status, gender, age, and their interactions on choice indices are reported in Table 5.

Table 5. Three-way ANOVA. Psychological trait level (high, medium, and low), PROP Status (NT, MT, ST), gender, age, and relevant two-way interaction effects on the choice index for vegetables (CV), choice index for coffee/tea (CC), indices for familiarity with vegetables with high (FV+) and low (FV-) bitterness and astringency and indices for familiarity with coffee/tea with high (FC+) and low (FC-) bitterness and astringency. F and *p* values. Significant differences ($p \leq 0.05$) are emboldened.

	Choice Index for Vegetables		**Choice Index for Coffee/Tea**		**Familiarity with Vegetables Higher in Bitterness and Astringency**		**Familiarity with Vegetables Lower in Bitterness and Astringency**		**Familiarity with Coffee/Tea Higher in Bitterness and Astringency**		**Familiarity with Coffee/Tea Lower in Bitterness and Astringency**	
	F	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***	**F**	***p***
Sensitivity to Punishment	6.4	**0.0017**	3.4	**0.0323**	11.5	**<0.0001**	4.4	**0.0122**	2.1	0.1259	1.6	0.2055
Gender	21.2	**<0.0001**	0.6	0.4306	9.3	**0.0024**	64.7	**<0.0001**	0.3	0.5740	0.0	0.9520
Age	33.0	**<0.0001**	2.2	0.1085	31.4	**<0.0001**	10.8	**<0.0001**	0.0	0.9862	0.6	0.5285
Gender × SP	0.0	0.9683	2.0	0.1414	0.3	0.7644	0.5	0.6182	2.8	0.0628	0.8	0.4683
Age × SP	1.8	0.1286	0.8	0.5416	1.0	0.4138	1.7	0.1581	0.5	0.7620	0.5	0.7682
Sensitivity to Reward	0.8	0.4392	1.3	0.2696	0.1	0.9507	0.1	0.9164	0.1	0.9186	0.1	0.9351
Gender	25.4	**<0.0001**	0.4	0.5273	4.4	**0.0369**	56.3	**<0.0001**	0.8	0.3789	0.0	0.8636
Age	36.2	**<0.0001**	1.8	0.1607	37.8	**<0.0001**	12.6	**<0.0001**	0.2	0.8098	0.1	0.9339
Gender × SR	1.7	0.1766	0.6	0.5717	0.5	0.6215	0.3	0.7440	1.5	0.2328	0.3	0.7591
Age × SR	0.2	0.9501	0.6	0.6883	1.0	0.4203	0.2	0.9312	1.4	0.2288	0.4	0.7848
Food Neophobia	11.7	**<0.0001**	6.8	**0.0012**	34.1	**<0.0001**	14.9	**<0.0001**	16.1	**<0.0001**	5.4	**0.0048**
Gender	32.0	**<0.0001**	0.2	0.6378	3.6	0.0595	58.5	**<0.0001**	0.1	0.7986	0.0	0.8339
Age	40.0	**<0.0001**	4.2	**0.0159**	47.9	**<0.0001**	18.3	**<0.0001**	0.7	0.5207	0.3	0.7172
Gender × FN	1.5	0.2130	0.4	0.6563	0.8	0.4484	1.3	0.2825	1.1	0.3275	0.2	0.8262
Age × FN	0.2	0.9313	2.0	0.0967	1.0	0.4138	0.8	0.5048	0.9	0.4711	0.7	0.5971
Sensitivity to Disgust	13.0	**<0.0001**	4.2	**0.0154**	10.1	**<0.0001**	2.9	0.0545	3.8	**0.0233**	2.2	0.1071
Gender	14.4	**0.0002**	0.8	0.3572	9.6	**0.0019**	58.8	**<0.0001**	2.9	0.0894	0.1	0.7851
Age	45.7	**<0.0001**	3.9	**0.0201**	49.6	**<0.0001**	16.9	**<0.0001**	0.6	0.5310	0.5	0.6163
Gender × DS	0.2	0.7832	0.3	0.7663	0.2	0.8558	0.7	0.4758	1.3	0.2706	0.9	0.4071
Age × DS	0.7	0.6250	1.4	0.2469	1.8	0.1198	1.0	0.3823	1.4	0.2297	1.1	0.3743
Private Body Consc.	0.9	0.4203	0.0	0.9670	4.4	**0.0123**	1.7	0.1773	2.0	0.1346	1.2	0.2918
Gender	24.4	**<0.0001**	0.4	0.5240	3.9	**0.0489**	49.3	**<0.0001**	0.3	0.5837	0.0	0.8951
Age	40.1	**<0.0001**	2.4	0.0889	42.9	**<0.0001**	15.5	**<0.0001**	0.7	0.4892	0.4	0.6871
Gender × PBC	3.6	**0.0267**	0.2	0.8372	2.2	0.1113	0.4	0.7034	0.2	0.8094	1.7	0.1922
Age × PBC	2.0	0.0905	1.5	0.1919	2.3	0.0603	0.8	0.5297	1.3	0.2852	0.7	0.6041
Alexithymia	2.1	0.1184	2.9	0.0547	7.7	**0.0005**	5.4	**0.0046**	3.5	**0.0292**	1.5	0.2127
Gender	20.8	**<0.0001**	1.2	0.2750	5.5	**0.0195**	56.3	**<0.0001**	0.7	0.4148	0.2	0.6722
Age	30.5	**<0.0001**	2.0	0.1400	32.2	**<0.0001**	10.0	**<0.0001**	0.0	0.9958	0.4	0.6632
Gender × TAS	0.8	0.4407	3.0	**0.0504**	0.4	0.6933	0.0	0.9542	2.0	0.1423	1.4	0.2358
Age × TAS	1.3	0.2528	1.2	0.3312	0.1	0.9693	0.3	0.8856	0.6	0.6304	0.3	0.8903
PROP	0.5	0.5969	0.6	0.5439	0.1	0.8819	0.0	0.9585	0.3	0.7432	1.5	0.2324
Gender	25.7	**<0.0001**	0.8	0.3615	7.4	**0.0067**	67.0	**<0.0001**	1.1	0.2856	0.0	0.9142
Age	33.2	**<0.0001**	2.5	0.0848	39.2	**<0.0001**	14.1	**<0.0001**	0.4	0.6744	0.4	0.6583
Gender × PROP	1.2	0.2968	0.2	0.8411	3.0	0.0526	5.5	**0.0042**	1.8	0.1711	0.0	0.9752
Age × PROP	0.9	0.4888	0.5	0.7087	0.7	0.6255	0.2	0.9591	0.3	0.8853	1.7	0.1386

SP: Sensitivity to punishment; SR: Sensitivity to reward; FN: Food neophobia; DS: Sensitivity to disgust; PBC: Private Body consciousness; TAS: Alexithymia; PROP: PROP taster status.

A significant effect of both gender and age was found for the vegetable choice index in each ANOVA model. The coffee/tea choice index was significantly affected by age only in the food neophobia and sensitivity to disgust models, while no effect of gender on the coffee/tea choice index was reported. These effects were not further characterized by an interaction between gender and age. The vegetable choice index was higher in males and increased with age. When the effect was found to be significant, the coffee/tea choice index increased with age.

The effect of food neophobia, sensitivity to punishment, and sensitivity to disgust was significant for both the vegetable choice index and coffee/tea choice index. These effects were not further characterized by interactions with age and gender. Individuals who scored higher in food neophobia, sensitivity to punishment, or sensitivity to disgust reported significantly lower choice indices than individuals low in these traits, meaning that they systematically opted for the least bitter/astringent option within the pairs (Figure 1a–b).

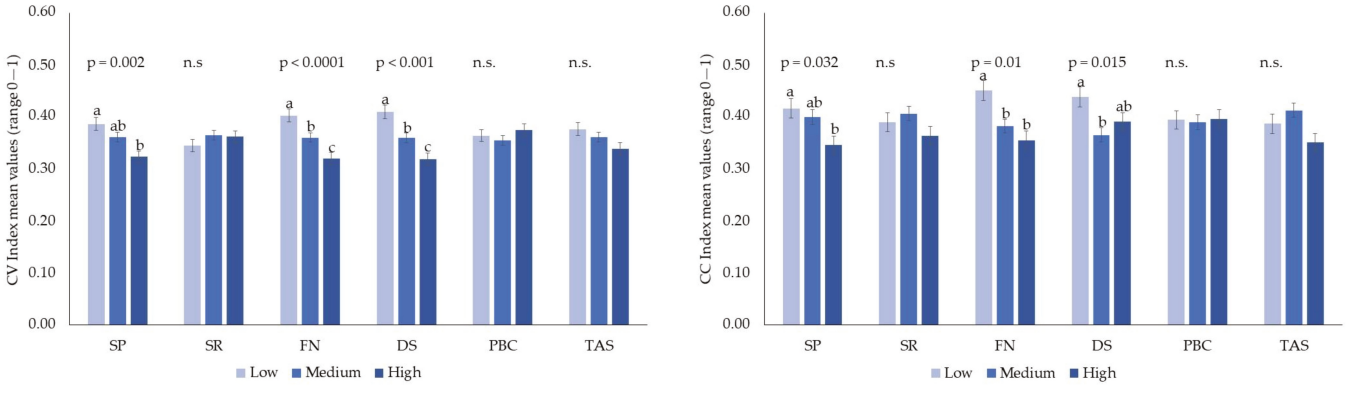

Figure 1. (**a**) Effects of psychological traits (sensitivity to punishment, SP; sensitivity to reward, SR; food neophobia, FN; sensitivity to disgust, DS; private body consciousness, PBC; and alexithymia, TAS) on the choice index for vegetables (CV Index). (**b**) Effects of psychological traits (sensitivity to punishment, SP; sensitivity to reward, SR; food neophobia, FN; sensitivity to disgust, DS; private body consciousness, PBC; and alexithymia, TAS) on the choice index for coffee/tea (CC). Different letters represent significantly different values ($p \leq 0.05$). n.s.= non-significant ($p > 0.05$).

A significant interaction was found for alexithymia (TAS) and gender (coffee/tea choice index), but no significant difference was found in a Bonferroni pairwise comparison. A significant interaction was found for private body consciousness (PBC) and gender (vegetable choice index), with males medium and high in private body consciousness reporting a higher choice index than females medium and high in private body consciousness.

PROP responsiveness. No effect of PROP responsiveness was found on either choice index.

3.2.4. Familiarity with Vegetables (FV+ and FV-)

Individual variation in psychological traits significantly affected familiarity with vegetables in the case of sensitivity to punishment (F = 9.6; $p < 0.0001$), food neophobia (F = 30.1; $p < 0.0001$), disgust sensitivity (F = 7.8 $p = 0.0004$), and alexithymia (F = 8; $p = 0.0003$). Higher levels in these traits corresponded to a lower familiarity with vegetables. This was further investigated, considering the vegetable groups varying in bitter and astringency. Table 5 reports the effects of individual variation in psychological traits and PROP status, gender, age, and their interactions on familiarity indices with vegetables high (+) and low (-) in bitterness and astringency.

A significant effect for both age and gender was found on the familiarity index for vegetables higher in bitterness and astringency and the familiarity index for vegetables lower in bitterness and astringency in each ANOVA model, with the only exception being gender in the model with food neophobia. These effects were not further characterized by an interaction (gender and age). Females were more familiar with vegetables irrespective to their bitterness and astringency level. Both vegetable familiarity indices increased with age.

A significant effect for food neophobia, alexithymia, and sensitivity to punishment was found on both indices, while a significant effect for private body consciousness and sensitivity to disgust was found only on the familiarity index with vegetables higher in bitterness and astringency. These effects were not further characterized by an interaction with age or gender. Both familiarity indices were lower in neophobics, in individuals higher in sensitivity to punishment and higher in alexithymia. The familiarity index with vegetables characterized by high unappealing sensations was lower in individuals higher in sensitivity to disgust. For private body consciousness, the post hoc test did not show significant differences between individuals high and low in this trait. The effect of individual variation in psychological traits on the familiarity index for vegetables high in bitterness and astringency is reported in Figure 2.

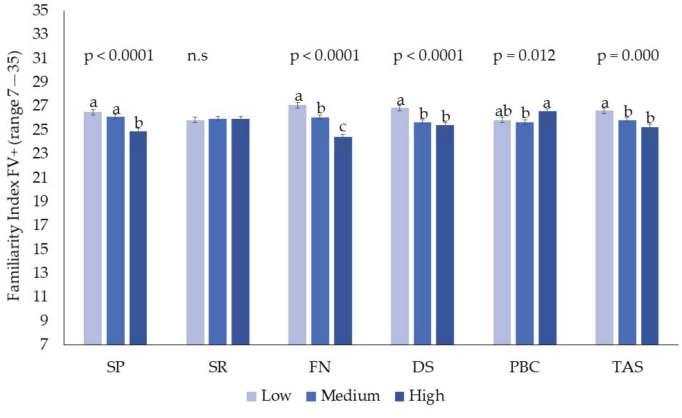

Figure 2. Effect of psychological traits (sensitivity to punishment, SP; sensitivity to reward, SR; food neophobia, FN; sensitivity to disgust, DS; private body consciousness, PBC; and alexithymia, TAS) on the familiarity index with vegetables higher in bitter and astringency (FV+). Different letters represent significant different values ($p \leq 0.05$). n.s.= non-significant ($p > 0.05$).

No effect of PROP responsiveness was found on either index, while a significant interaction between PROP and gender was observed on the familiarity index with vegetables lower in bitterness and astringency, confirming that females were more familiar than males with vegetables lower in bitterness and astringency, irrespective of PROP status.

3.2.5. Familiarity with Coffee/Tea (FC+ and FC-)

No effect of age, gender, or their interaction was found on the familiarity index with coffee/tea characterized by high or low bitterness and astringency in any model.

A significant effect of food neophobia was found on both indices. Neophobic subjects were less familiar with coffee/tea without sugar and more familiar with their version with sugar. Neophilic subjects showed a median familiarity score for this beverage group of eight; this means that, at least occasionally, they consumed both unsweetened coffee and tea or that they regularly consumed only one of these beverages. Neophobic subjects showed a median familiarity value of seven, indicating that they do not consume one of the items and only occasionally consume the other. Individual variations in sensitivity to disgust and alexithymia significantly affected the familiarity index, with coffee/tea characterized by highly unappealing sensations. Subjects with high sensitivity to disgust and high alexithymia were found to be less familiar with the without sugar coffee/tea group of products. The effect of individual variation in psychological traits on the familiarity index for coffee/tea high in bitterness and astringency level is reported in Figure 3.

No significant effect of PROP was found on either index of familiarity.

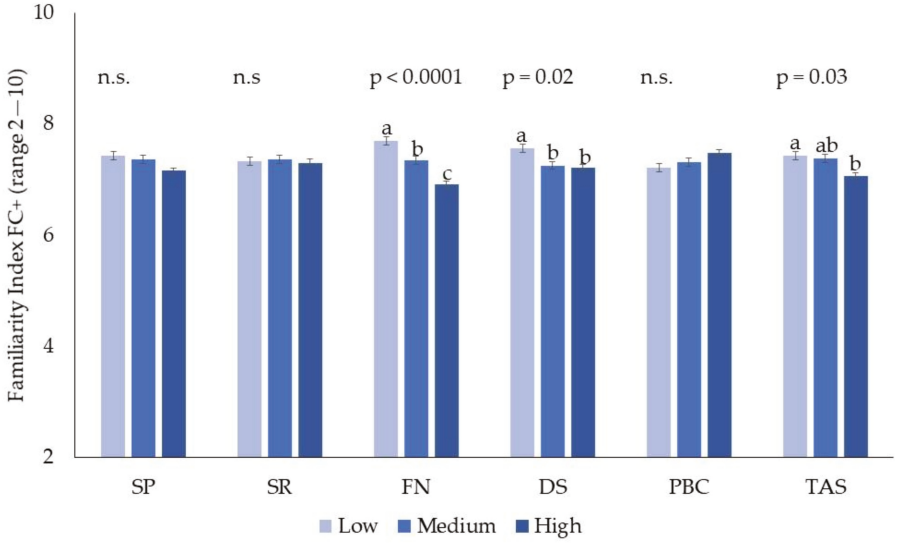

Figure 3. Effect of psychological traits (sensitivity to punishment, SP; sensitivity to reward, SR; food neophobia, FN; sensitivity to disgust, DS; private body consciousness, PBC; and alexithymia, TAS) on the familiarity index with coffee/tea higher in bitterness and astringency (FC+). Different letters represent significant different values ($p \leq 0.05$).

3.2.6. Correlation between Choice of and Familiarity with Bitter/Astringent Option

Significant positive correlations between the vegetable choice index and familiarity index with vegetables higher in bitterness and astringency, and between the coffee/tea choice index and familiarity index with coffee/tea higher in bitterness and astringency, were found in each subgroup of individuals (low, medium, and high) for each personality trait and in each PROP status class (NT, MT, and ST). The

correlation coefficient ranged from 0.25 to 0.41 in the case of vegetables and from 0.42 to 0.57 in the case of beverages (Table 6).

Table 6. Pearson correlation coefficients between the vegetable choice index (CV) and familiarity index with vegetables higher in bitterness and astringency (FV+) and the Pearson correlation coefficients between the coffee/tea choice index (CC) and familiarity index with coffee/tea higher in bitterness and astringency (FC+) within the three levels (low, medium, high) of each psychological trait and PROP status (NT, MT, ST).

Vegetable Choice Index/Familiarity Index with Vegetables Higher in Bitterness and Astringency (CV/FV+)				
Trait	**Low**	**Medium**	**High**	**Diff. among groups**
Sensitivity to Punishment	0.25	0.38	0.38	*
Sensitivity to Reward	0.28	0.40	0.37	*
Food Neophobia	0.25	0.37	0.41	*
Sensitivity to Disgust	0.34	0.39	0.32	n.s.
Private Body Consciousness	0.34	0.41	0.32	n.s.
Alexithymia	0.33	0.36	0.37	n.s.
PROP status	**NT**	**MT**	**ST**	
PROP	0.30	0.38	0.37	n.s.
Coffee/tea choice index/familiarity index with coffee/tea higher in bitterness and astringency (CC/FC+)				
Trait	**Low**	**Medium**	**High**	**Diff. among groups**
Sensitivity to Punishment	0.49	0.50	0.56	n.s.
Sensitivity to Reward	0.56	0.51	0.49	n.s.
Food Neophobia	0.57	0.53	0.42	*
Sensitivity to Disgust	0.55	0.51	0.50	n.s.
Private Body Consciousness	0.54	0.49	0.54	n.s.
Alexithymia	0.55	0.52	0.48	n.s.
PROP status	**NT**	**MT**	**ST**	
PROP	0.49	0.49	0.57	*

All correlations are significant ($p \leq 0.05$). * significant pairwise differences. Vegetables—Sensitivity to Punishment: Low–Medium ($p = 0.02$), Low–High ($p = 0.03$); Sensitivity to Reward: Low–Medium ($p = 0.03$); Food Neophobia: Low–Medium ($p = 0.03$), Low–High ($p = 0.01$). Coffee/tea—Food Neophobia Low–High ($p = 0.01$), Medium–High ($p = 0.02$), PROP status: Medium–High ($p = 0.05$). n.s. = non-significant ($p > 0.05$).

Individuals lower in food neophobia, sensitivity to punishment, and sensitivity to reward reported significantly lower correlations between the vegetable choice index and familiarity index with vegetables higher in bitterness and astringency compared to individuals higher in these traits. Individuals lower in food neophobia reported a significantly higher correlation coefficient between the coffee/tea choice index/familiarity index with coffee/tea higher in bitterness and astringency compared to individuals higher in food neophobia. The correlation coefficients for the coffee/tea choice index/familiarity index with coffee/tea higher in bitterness and astringency increased in ST compared to NT and MT.

4. Discussion

The selection of food and beverages to be included in the CATA questionnaire was performed based on pre-existing sensory data from consumers and trained panels. The vegetable CATA questionnaire included vegetables described by potentially unpleasant sensory properties due to their chemical composition, such as a bitter taste, astringent sensations, objectionable flavours, and a dark, unattractive colour (radicchio, rocket, chicory, asparagus, and radish) [64–68] and vegetables characterized by a sweet taste, delicate flavour, and a bright, appealing colour (pumpkin, lettuce, valerian, green salad, chard, and zucchini) [69–72]. The range of differences between the two options in each pair was relatively high, with the exception of two pairs (carrot versus cauliflower, and lettuce versus bean sprout), for which these sensory properties were checked by less than 20% of the respondents

and a significant difference was found for only one of the two sensory properties. These pairs were included in Study 2 based on the fact that a subtle but significant difference was found for at least one of these sensations (carrot versus cauliflower for bitterness and lettuce versus bean sprout for astringency). The coffee/tea CATA questionnaires included versions of the of the same hot beverage varying in bitter and astringency due to the inclusion or exclusion of ingredients masking the perception of bitterness and astringency (i.e., milk and sweeteners). Findings from the CATA questionnaires confirmed that vegetable and coffee/tea items included in the choice and familiarity indices significantly varied in bitterness and astringency. This substantiates the screening of items based on the hypothesis that they should represent phenol-rich dishes/beverages varying in the level of bitterness and astringency sensations.

Based on the results from the two CATA questionnaires, it was possible to divide questionnaire items into two groups, each representing the lower and higher bitterness/astringency option for vegetable-based dishes or for coffee/tea beverages, according to consumer expectations. Two main features characterized the approach for food grouping proposed in the present paper: (1) sensory differences between selected vegetable/beverages items were defined according to the response of the target population rather than derived from existing data on other consumer groups (e.g., other food cultures or trained panels); (2) the individual propensity to prefer more or less bitter/astringent options of the phenol-rich foods and beverages was investigated by means of indices computed on choice of and familiarity responses with vegetable and coffee/tea groups rather than considering the response to specific single food/beverage items. These features allowed the highlighting of the importance of individual differences in psychological traits and chemosensory ability in affecting familiarity with, and choice for, phenol-rich foods. The approach based on CATAs to group foods differing in bitter and astringency limits bias due to misinterpretation of the consumer expectation for sensory differences between foods. Furthermore, the computation of indices minimized the impact of individual preferences for specific food/beverages items (for example, a specific bitter vegetable might be very popular and well accepted in some regions and not in others).

The characteristics of the population participating in the study confirmed existing data on gender and age effects on psychological traits and PROP status. We found no effect of gender on neophobia, in line with previous findings that reported no [73] or small [53] effects, and we confirmed an increase in neophobia with age [46,74,75]. The gender effect for the other traits was also consistent with previous results, with females more sensitive to punishment than males, and males more sensitive to reward than females [54,76], females more sensitive to disgust [36], and no gender effect on alexithymia [59]. For age, with some exceptions, comparisons with previous studies are more limited, considering that much of the extant literature involved younger individuals or a specific age class. In our sample, we found a decrease in alexithymia with age, in contrast to findings in an adult population in Finland [77].

Results from this study confirmed previous findings on the age and gender effect on PROP responsiveness, with aging negatively associated with PROP responsiveness [21,53,78–80]. Females rated PROP bitterness higher than males, confirming other results showing that females are more sensitive to PROP than males, and more likely to be tasters [53,80,81]. While females were more familiar with vegetables, independent of their bitterness and astringency, the choice of the most bitter and astringent vegetable option was higher in males than females and increased with aging, irrespective of their psychological traits. A higher preference for sweetness in females is well documented [82] and this may explain our results in the choice test.

The comparison of choice and familiarity indices for vegetables indicated that bitterness and astringency did not represent a barrier to vegetable consumption in females. At the same time, the choice for bitter/astringent food did not appear a reliable predictor of vegetable consumption in males. A greater appreciation of health-related food aspects, greater nutritional and culinary knowledge, and an increased interest in preparing home-cooked meals are all positively associated with vegetable consumption [83] and were likely to be responsible for the higher familiarity for vegetables in females than in males in the current study.

The positive association of aging with the choice of vegetables higher in bitterness and astringency can be explained by the repeated exposure—an effect that may allow initial avoidance to be overcome, at least partly through "learned safety" [84]. Thus, a food that is initially disliked could become familiar and potentially preferred [85,86]. Furthermore, the increased attention to the health-related aspects of eating associated with aging [53,87] might further help in promoting choices for healthier vegetable options, even if they are less palatable initially.

Neither choice of nor familiarity with vegetables was affected by PROP status, consistent with the results of previous study showing a lack of association of bitter vegetable preference with responsiveness to PROP bitterness [24,26,34]. Evidence from recent studies highlighted that a complex network of both genetic and environmental factors appears to influence responsiveness to PROP [18,21]. However, this phenotype is still widely used, with the purpose of exploring the associations of chemosensory ability and vegetable preferences [24,26,88]. Among the several alternative methods for evaluating PROP and determining group assignment, in this study we opted for the one solution test [60,89] and the a priori cut-offs for non-tasters (from 0 to 17), medium tasters (MT from 18 to 52), and supertasters (from 53 to 100) [19,20], widely documented in the literature. Alternative chemosensory indices taking into account broader differences in taste systems might offer a new perspective in looking at the association of dietary style and taste responsiveness phenotypes [90,91]. However, based on the results from the present study, and in line with the newer multidimensional models of food preference and choice, environmental factors might mitigate the impact of biology in determining food preferences, such that phenotype differences in responsiveness to bitterness may not be enough to influence food choice and intake [92].

In general, data on choice of and familiarity with vegetables indicated the relevant roles of food neophobia, sensitivity to punishment, and sensitivity to disgust as determinants of vegetable eating. These psychological traits were negatively associated with both the choice of vegetables with higher bitterness and astringency and the familiarity with vegetables in general, irrespective of their sensory properties. This is in line with previous findings, which show that food neophobia in adults is associated with a reduced dietary variety, which is most evident in a lower acceptability and intake, particularly of vegetables, fruits, and protein foods [31,93]. Our findings align also with the hypothesis that higher punishment sensitivity is associated with more unhealthy behaviours, as it was found previously to be associated with a higher sugar intake [43]. Individuals with higher alexithymia declared a lower familiarity with vegetables independently of their bitterness and astringency, while no effect on choice was reported. Similarly, Robino and colleagues [45] reported a negative relationship between alexithymia and stated liking for vegetables. The fact that we did not find an effect of this trait on choice may suggest that this trait modulates vegetable consumption independently from the sensory characteristics of vegetables and thus affects the whole product category.

The correlation between choice and familiarity indices significantly varied according to the level of food neophobia and sensitivity to punishment, thus indicating potential differences between what individuals would like to choose and what they declare they consume normally. The correlation value decreased with neophobia and sensitivity to punishment, indicating that low food neophobia and sensitivity to punishment individuals were likely to have a wider vegetable repertoire. In older adults, a positive association between the willingness to try new foods and a wider variety of consumed vegetables has already been observed [94]. On the other hand, the high level of food neophobia and sensitivity to punishment traits were associated with an increased correlation between choice and familiarity. Neophobic individuals tended to be more consistent with what they preferred and what they declared to consume, and this possibly indicates a restricted spectrum of vegetables included in their daily diet.

These findings, taken together, confirm the hypothesis that personality variables—specifically food neophobia, sensitivity to punishment, and sensitivity to disgust—may act to facilitate or inhibit the preference and intake of vegetables characterized by unpleasant sensations, consistent with what

has been previously found for pungency [35] and, in the case of food neophobia, for bitterness and astringency [34].

Aging was positively associated with the choice of the more bitter/astringent coffee/tea options, suggesting the effects over time of learned positive flavour–flavour and/or flavour consequence conditioning via the stimulatory impact of caffeine, leading to the bitter taste of coffee/tea becoming acceptable [95,96]. Taste motives are among the main reasons for caffeinated beverages consumption [97] and a bitter taste contributes to the appreciation for caffeinated beverages drinkers [15].

PROP status did not affect choice and familiarity with coffee/tea items, thus adding to the negative findings in data on causal relationships between PROP bitterness perception and coffee/tea preference and consumption [17,98]. Several factors other than sensory properties, such as functional motives, health beliefs, tradition, and culture, shape the personal preferences for caffeinated beverages [97]. Recent findings on genetic of bitterness perception indicate an opposite causal relationship between PROP responsiveness and coffee and tea consumption [98]. This possibly further accounts for the lack of significant effect of PROP status on choice and familiarity indices, since they are based on responses to both tea and coffee. However, differences in correlations between choice and familiarity indices indicated that ST, more than MT and NT subjects, tended to consume the most preferred option. This may imply that these subjects, more sensitive than the rest of the population to unappealing sensations, tended to adopt more strictly the consumption conditions that better adapt to their personal preference.

Food neophobia, sensitivity to punishment, and sensitivity to disgust appeared to act as barriers to the choice of the more bitter/astringent coffee/tea options. High food neophobia and sensitivity to disgust levels were associated with a lower familiarity with the unsweetened version of coffee/tea items and to a higher familiarity with the least bitter/astringent option for neophobic subjects only. A lower preference for coffee has been already reported for individuals higher in neophobia [93].

Food neophobia significantly affected the strength of the correlation between the choice and familiarity indices of the most astringent/bitter coffee/tea options. The correlation value was significantly higher in subjects with lower than with higher food neophobia. Habit, defined as a ritual or a daily routine, was one of the main motivational factors for caffeinated beverages consumption [15], but neophobic subjects were less familiar with coffee/tea and were only occasional consumers of unsweetened coffee/tea beverages, and this could account for the weaker correlation between choice and familiarity for unsweetened coffee/tea indices. It has been shown that a variety of motivations play a role in the consumption of coffee beverages [99] and that sensory properties are more relevant for individuals who consume more coffee daily and with a faster caffeine metabolism index [100]. We may hypothesise, therefore, that while for individuals lower in neophobia the sensory properties are of importance, thus explaining their preference for the unsweetened options, for those higher in neophobia, coffee preference may be more explained by situational and social factors (e.g., social rituals).

While this study benefits from a large sample and the study of the impact of psychological traits on choice, some aspects have remained underexplored. Thus, the foods and beverages considered in the study might differ for properties other than bitterness and astringency, such as texture or energy content. Differences in these aspects might have a role in choice and familiarity that has not been taken into account in the present paper, thus possibly limiting the interpretation of the results. Further studies are encouraged, taking into account a larger variety of dimensions.

5. Conclusions

The approach proposed in this study for product grouping based on sensory properties was effective and allowed the investigation of the role of individual differences in chemosensory perception and psychological traits as modulators of phenol-rich foods preference and consumption. Individual differences in psychological traits (food neophobia, sensitivity to punishment, and sensitivity to disgust), rather than responsiveness to PROP, influenced both the preference and consumption of phenol-rich foods. Furthermore, psychological traits significantly affected the degree of coherence

between what individuals preferred and what they consumed in their daily life, thus, in the ultimate analysis, determining their diet variety.

A positive correlation between familiarity and choice was confirmed, but the two measures were found to provide different information. While in vegetables the traits food neophobia, sensitivity to punishment, and sensitivity to disgust were found to be associated with a lower familiarity with vegetables independent of their sensory properties, in coffee/tea, food neophobia, sensitivity to disgust, and alexithymia were associated with a lower familiarity with the unsweetened options. To build on these interpretations of food preference and consumption behaviour, the systematic explorations of individual differences in psychological traits should also take place in applied settings.

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Abbreviations

DS: Sensitivity to Core Disgust; FN: Food Neophobia; PBC: Private Body Consciousness; SP: Sensitivity to Punishment; SR: Sensitivity to Reward; TAS: Alexithymia; V-IT-FCQ: Vegetable Choice Questionnaire; C-IT-FCQ: Coffee/Tea Choice Questionnaire; CV: Vegetable Choice Index; CC: Coffee/Tea Choice Index; FV+/FV-: Indices of Familiarity with vegetables high (+) or low (-) in bitterness and astringency sensations; FC+/FC-: Indices of Familiarity with coffee/tea high (+) or low (-) in bitterness and astringency sensations

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Communication

A Brief Review of Genetic Approaches to the Study of Food Preferences: Current Knowledge and Future Directions

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Abstract: Genetic variation plays a crucial role in individual differences in food preferences which ultimately influence food selection and health. Our current understanding of this pathway has been informed through twin studies (to assess the heritability of food preferences), candidate gene studies, and genome-wide association studies (GWAS). However, most of this literature is mainly focused on genes previously identified as having taste or smell functions. New data suggests that genes not associated with taste or smell perception may be involved in food preferences and contribute to health outcomes. This review highlights these emerging findings and suggests a polygenic risk assessment approach to explore new relationships between food preferences and health risks.

Keywords: genetics; food preferences; heritability; candidate gene; GWAS; adiposity; polygenic risk score

1. Introduction

Food preferences are shaped by a high number of environmental, cultural, and nutritional factors, including genetic ones. The first evidence for genetic influences on food preferences came from family and twin studies [1–12]. However, over the last few decades, rapid advances in molecular genetics have revolutionized the understanding of individual differences in many aspects of human behavior. These advances give researchers the tools to conduct genetic association studies on a large scale to better understand the role of specific gene loci in sensory perceptions, food liking/disliking, preference, and intake, as well as on food-related habits [13–18].

To date, the vast majority of studies on food liking and preference have focused on identifying specific genes and traits associated with sensory perceptions (mainly taste and smell perception). The effects of taste and smell genes on food habits [19–36] and health status [30,31,37–48] have also been extensively investigated. However, gaps in understanding still exist, and emerging evidence suggests that novel genes (not necessarily related to taste or smell perception) may play a critical role in these relationships [13–16].

Thus, a potential new area in nutrition research is the investigation of the genetic bases of food preferences, broadly defined to include both taste/smell-related and non-related genes.

Obtaining a comprehensive picture of genetic effects on food preferences and habits and their consequences for food-related diseases, such as being overweight or obesity, is of considerable public health importance and interest to the food industry.

This review focused on current knowledge, linking genetic variability to food preferences. Specifically, we reviewed studies on food preferences (defined as the selection of one food rather than another) and food liking (meaning the degree of liking or disliking towards a food).

2. Genetic Dissection of Food Preferences

The genetic background of a trait can be investigated through several methods. Firstly, heritability analysis allows one to estimate the proportion of variation of a phenotype, which is due to genetic differences between individuals. However, heritability studies do not provide any information on specific genes and polymorphisms related to a given trait. Specific information can be identified through genetic association analysis such as candidate gene and genome-wide approaches. A candidate gene study investigates variations within specific genes of interest selected on the basis of existing knowledge or hypotheses. In contrast, a Genome-Wide Association Study (GWAS) is conducted without suppositions or previous knowledge and the whole genome is scanned so that new genetic variants may be discovered [49–51].

Here, we report different approaches through which the genetics of food preferences can be dissected. Firstly, we review studies that provide evidence for a genetic basis of food preferences (heritability studies) and then studies that identified underlying genes (candidate genes and genome-wide association). Finally, we describe the possible relationships between genes linked to food preferences and health status, and we present an example of the predictive power of polymorphisms associated with vegetable liking on adiposity measures.

2.1. Heritability Studies

Heritability is the proportion of the phenotypic variation in a population explained by genetic effects; it is a measure of the inheritance of a trait. Usually, heritability estimation requires data where familial relationships are known (twins or family studies) and does not provide information about which genes are responsible for the trait. Heritability has been widely estimated in twin studies, where monozygotic twins (identical twins with almost no differences in their DNA) are compared to dizygotic twins (fraternal twins who share, on average, half of their DNA). This comparison allows one to evaluate the proportion of variation of a trait ascribable to genetic factors, while the remaining variance is assumed to derive from environmental factors. Heritability estimation ranges from 0 to 1: a high value indicates that genetics plays a major role, while low values indicate that most of the variation is due to environmental factors. High heritability does not necessarily imply that a single gene is the cause of trait variation. It is possible that multiple genes, each of them having a small effect, contribute to this variation [52].

Evidence on the heritability of food preferences has been reported in both adult and children twin studies. For example, studies of 3–5 year-old children provide evidence for high or moderate heritability for liking of vegetables (from 0.37 and 0.54), fruits (from 0.51 to 0.53), and proteins (from 0.48 to 0.78) [4,5]. Moderate heritability for specific food preferences such as vegetables (0.54), fruits (0.49), meat or fish (0.49), and dairy (0.44) has also been observed in adolescents (18–19 years of age) [6]. Similar findings have been reported in adults. In a cohort of ~600 adult female twins in the UK, Keskitalo and colleagues reported that 0.49, 0.54, and 0.53 of the variation in liking for a sweet solution, liking and use-frequency of different sweet foods (sweet desserts, sweets, sweet pastry, ice cream, hard candy, and chocolate), respectively, was explained by genetic factors [8,9]. Similarly, a study in young adult Finnish twins showed that genetic effects account for 0.18–0.58 of the variation in the pleasantness of oral pungency, spicy foods, and pungent sensations [10]. In the same cohort, genetic influences on sour foods were studied, and 0.14 and 0.31 of the variation in pleasantness and intensity of orange juice spiked with citric acid was reported [11]. Moreover, these same authors also found that genetic effects accounted for 0.34–0.50 of the variation in pleasantness and use-frequency of sour foods categorized into three groups as follows: sour fruits and berries (red currant, red currant juice, cranberry, lingonberry, lemon, and rhubarb), sour dairy products (natural cultured milk, natural yogurt, and sour milk), and less-sour berries and fruits (strawberry, orange, blueberry, peach, and banana) [11].

Differences in heritability results across studies can be explained by the small sample size of most studies and by the minimal number of foods analyzed (i.e., different from study to study and mainly focused on the taste perception of foods). Moreover, differences in the data collection and analysis (i.e.,

age differences of participants, use of different questionnaires and measurements, analysis of single foods or a set of clustered foods) could also be responsible for this variability.

More recently, a large study of more than 2000 UK twins analyzed the heritability of different liking patterns using data from an online food liking–disliking questionnaire including 87 different foods and beverages. This study revealed four food-liking patterns by principal component analysis (PCA): fruit and vegetables; sweet and high carbohydrates; meat; distinctive tastes (including chili pepper, garlic, or other foods with strong taste). Moderate heritabilities were obtained for all of them (fruit and vegetables: 0.36; sweet and high carbohydrates: 0.52; meat: 0.44; distinctive tastes: 0.58), corroborating past works on genetic influences of food liking–disliking [12]. However, similar heritability estimates reached by studies with both large and small sample size suggest that environmental factors also play a crucial role.

Overall, these studies are useful in providing a quantitative estimate of the heritability of food preferences and in supporting the idea that genetic determinants play a role. However, as already mentioned, they do not give information concerning specific genes accounting for food preferences.

2.2. Candidate Gene Studies

A candidate gene study requires an "a priori" hypothesis based on a potential role of a given gene on a given trait of interest [53]. Regarding food preferences, this approach has been used to examine the possible role of polymorphisms in genes already known to be involved in taste or smell perceptions. These two senses allow us to recognize and to discriminate foods and are among the most important determinants of food liking/disliking [54–56]. For these reasons, DNA polymorphisms in taste and smell genes have played an important role in individual variability on food choices.

2.2.1. Taste Receptor Genes

It is well known that genetic factors influence taste perception. Genes encoding taste receptors have been identified and genetic variability of sweet, umami, and bitter perceptions have been intensely investigated, although knowledge gaps exist for sour and salty perception [19–37]. As stated above, comprehensive reviews have already been published on the relationship between variations in taste receptors and food preferences [38–44], thus in the present review, we only present a few examples.

A very well-known example is that of the *TAS2R38* bitter receptor, a major contributor to individual differences in bitter taste perception of PROP (6-n-propylthiouracil) or PTC (phenylthiocarbamide). About 30%–40% of the European population is taste-blind to these compounds or perceive them as weakly bitter (so-called non tasters), while the remaining 70%–60% can perceive them as moderately or intensely bitter (so-called tasters). Three SNPs (Single Nucleotide Polymorphisms) in the *TAS2R38* gene (rs1726866, rs10246939, rs713598) result in three amino acid substitutions defining two main haplotypes, namely AVI and PAV, that confer differences in the ability to taste PTC/PROP. Indeed, individuals homozygous for the AVI haplotype are mainly non tasters, while homozygous for the PAV haplotype and heterozygous individuals are likely to be tasters [19,20,57,58].

Although controversial results have emerged in the literature, the variation in the ability to perceive PROP has been widely related to preferences for different foods such as brassica vegetables, other bitter foods, sweets, added fat, spicy foods, and alcoholic beverages [37,38,59–62]. For example, Mennella and collaborators showed that in children, but not in adults, *TAS2R38* variations partially explained individual preferences for sucrose or beverages and cereals with a high sugar content [63]. A study in Malaysian adults showed mixed results. Specifically, they reported that aversions to individual foods such as green tea, mayonnaise, and whipped cream were associated with *TAS2R38* genotypes, while no associations were observed for vegetables and sweet/fatty foods [64]. More recently, a study by Shen et al. showed that AVI/AVI subjects liked brassica vegetables more than PAV/AVI and PAV/PAV individuals [65]. In another recent work, Perna and collaborators reported that one specific polymorphism in the *TAS2R38* gene was associated with preferences for beer, butter, and cured meat [66]. However, a link between *TAS2R38* genetic variants and food liking has not been

observed in other studies and several reasons could be responsible for the inconsistent findings such as food assessment methods, sample size, cultural habits, or other environmental factors that may influence the association.

Evidence for a relationship between other bitter taste receptor genes and liking of common foods and beverages have also been reported. For example, variation in the *TAS2R19* bitter-taste gene showed associations with grapefruit juice bitterness and liking [37], while another bitter-taste gene, *TAS2R43*, has been related to coffee liking [67]. Data also suggested a possible influence of genetic variation in the *TAS1R3* sweet receptor gene on sweet preferences in children [68], as well as a link between variations in the *CD36* gene (responsible for fat taste perception) and fat preferences [31].

The studies reviewed above have limited implications for general food preferences because they only analyze one or few genes (or SNPs) and they examine liking for just one or few foods. To address this shortcoming, our group examined the relationship between a broad spectrum of food preferences and DNA variants in several taste and olfaction genes in a large cohort of >400 individuals. Statistically significant associations were identified for genes involved in chemosensory functions (i.e., *TRPV1* and *TAS1R2*) or in signal transduction (i.e., *PLCβ2* and *ITPR3*). One of the most interesting associations was found between the *TAS1R2* gene (coding for a sweet taste receptor) and liking of alcoholic beverages, according to data reporting a link between ethanol preference and liking for sweet taste. Specifically, the lower frequent allele for two different SNPs (rs3935570 and rs4920566) in the *TAS1R2* gene were positively associated with the liking of vodka and white wine. Another noteworthy association was detected for tea and the *PLCβ2* gene, a marker for type II taste bud cells, which is involved in the caffeine response and is also expressed in the sensory cells of the olfactory epithelium. In this case, the rarest allele of rs2290550 SNP was negatively correlated with tea liking [15].

2.2.2. Olfactory Receptor Genes

Humans vary in their capacity to perceive several odors, and their variation in olfactory receptor (OR) genes may be responsible for these differences [69,70]. Despite more than 400 genes/receptors being involved in smell perception, little is known about the link between these genes and specific odorants as well as their possible influence on food preferences. One of the most recognized examples is the role of the olfactory receptor gene *OR7D4*, which is partially responsible for individual differences in the ability to smell androsterone [69]. Androsterone is undetectable for some people, others define it as foul smelling or urine and sweat smelling, while others describe it as sweet or floral smelling. Two SNPs in the *OR7D4* gene are responsible for two amino acid substitutions that impair the ability to perceive androstenone [70]. Androstenone is present in the meat of male pigs. A recent study confirmed that *OR7D4* variants were associated with the sensory perception of pork meat containing androstenone as well as lower liking for the flavor and odor of pork meat by androstenone-sensitive individuals [71].

Another example is the *OR2J3* gene, which is associated with individual differences in detecting *Cis*-3-hexen-1-ol (C3HEX), an odorant with a green/grassy smell and is present in several fruits and vegetables. Polymorphisms in this gene are responsible for amino acid substitutions impairing the ability to smell C3HEX. Subjects can be classified as C3HEX-sensitive or C3HEX-insensitive [72,73]. Moreover, foods spiked with C3HEX were less acceptable than the unspiked foods; however, the reductions in acceptability were more marked in C3HEX-sensitive individuals if compared to C3HEX-insensitive individuals [74].

Finally, studies examined variation in the *OR5A1* gene, related to β-ionone odor sensitivity. β-ionone aroma is a fruity/floral aroma that is present in several foods and beverages [75–78]. A series of studies by Jaeger and co-workers showed that a DNA variation (rs6591536 SNP) in the *OR5A1* gene is the causal variant for β-ionone odor sensitivity, explaining 96.3% of the phenotypic variation. They also reported that β-ionone sensitive individuals can easily differentiate between foods (such as milk chocolate or apple juice) with and without added β-ionone, and they can also recognize β-ionone

in foods when compared to less-sensitive individuals. Moreover, sensitive individuals prefer foods without β-ionone rather than with β-ionone [79].

2.3. GWA Studies

Over the past decade, the GWAS approach has become one of the most common tools for the identification of genes associated with complex traits and diseases. In these studies, a large number of participants are genotyped for a large number of genetic markers (usually SNPs) covering the whole genome and their relationships with the trait of interest are examined, allowing for the identification of novel gene variants and genomic loci [80].

To date, very few GWAS have been conducted on food preferences, which are summarized in Table 1. Although a genome-wide scan typically analyzes thousands or even millions of SNPs, Table 1 reports only GWAS significant SNPs with p-value $< 5 \times 10^{-8}$. This p-value is equivalent to the Bonferroni-corrected threshold ($\alpha = 0.05$) for 1 million independent variants (approximately the number of independent SNPs analyzed in a GWAS).

The first GWAS was carried out on cilantro (or coriander) liking in a large cohort of unrelated European subjects belonging to the 23andMe cohort [81], who responded to an online questionnaire asking whether they taste cilantro as soapy and whether they like it. An association among the rs72921001 SNP, soapy taste, and disliking of cilantro was found. This SNP falls within a cluster of eight olfactory receptor genes on chromosome 11. Among them, the authors suggested that a good candidate for cilantro preferences could be the *OR6A2* gene coding for a receptor that can be activated by several aldehydes responsible for the characteristic odor of cilantro [18].

More recently, we conducted the first GWAS on red and white wine preference assessed by survey-reported food liking in 3885 adults coming from different geographic areas (Italy, the Netherlands, and Central Asia). In this work, we detected a significant association between white wine liking and rs9276975 SNP in the *HLA-DOA* gene, encoding for a non-canonical MHC (major histocompatibility complex) II molecule. Although the mechanism of how MHC could be linked to wine preferences is unknown, the possible involvement of the olfactory system was hypothesized [16]. Moreover, another GWAS on the liking of 20 different foods was carried out on a large cohort of 4611 individuals, which identified 15 novel significant variants associated with 12 different foods. Some of these variants are located within genes that might represent good candidates for food choices. Interestingly, none of them belong to taste or olfactory receptor gene families, but are likely to be involved in reward response to food (i.e., *BPNT1*, *IRX4*, *CNTN5*, and *CSMD1* genes). For example, an association was detected between the liking of bacon and rs140738262 SNP in the *CNTN5* gene. This polymorphism also showed marginal association with the liking of other fatty foods such as lamb, pork chops, and goat cheese. This gene is expressed in the brain and has previously been associated with anorexia nervosa, suggesting a possible link with preferences for palatable food and the responsivity of the brain reward system to these foods. For vegetables, an association between chicory liking and rs138369603 SNP in the *CSMD1* gene has emerged. We hypothesized a possible role of this gene in the regulation of the food reward response since its variants were linked to differential activation of the cuneus, an area possibly involved in central reward processing [17].

Overall, these results represent a step in understanding the biological bases of food liking and suggest that the GWAS approach may be useful in identifying novel candidate genes for food preferences. Nowadays, thanks to the reduction of SNP genotyping costs as well as to the existence of large population biobanks, GWA studies could contribute to identifying many more loci, which will enhance insight into the genetic architecture of food preferences. Thus, further studies should be conducted to confirm previous findings, to extend the range of examined foods, and to also analyze other food groups.

Table 1. GWA studies of food liking.

Reference	Subjects (*n*)	Population	Food Liking Assessment	Associated Trait	SNP	Locus
Eriksson et al., 2012 [18]	26,455	Unrelated (European)	Responses to an online survey asking the following questions: - Does fresh cilantro taste like soap to you?" (Yes/No/I'm not sure) - Do you like the taste of fresh (not dried) cilantro?" (Yes/No/I'm not sure)	Cilantro	rs72921001	*OR6A2*
Pirastu et al., 2015 [16]	3885	Isolated population (European and Central Asia)	Survey-reported food liking (5-point scale or 9-point scale)	White wine	rs9276975	*HLA-DOA*
Pirastu et al., 2016 [17]	4611	Isolated population (European and Central Asia)	Survey-reported food liking (5-point scale or 9-point scale) (5-point scale or 9-point scale)	Artichokes Artichokes Artichokes Broccoli Broccoli Broccoli Bacon Oil or Butter on Bread Blue Cheese Ice Cream Liver Coffee	rs28849980 rs28849980 rs8034691 rs2530184 rs9832668 rs138369603 rs140738262 rs6661761 rs12994253 rs2035613 rs34088951 rs145671205	CCRN4L ADAMTS19-CHSY3 LOC100128714 NA RYBP CSMD1 CNTN5 BPNT1 KCMF1-TCF7L1 IRX4 RNU6-66 FIBIN

Associated trait refers to the associated food liking; SNP column shows the name polymorphism; Locus column refers to the gene closest to the most significant SNP; GWA refers to Genome-Wide Association.

3. From genetic Variations in Food Preference Genes to Health

There is a well-developed body of research examining the relationships between taste receptor genes and their downstream effects on food preferences and intake, which may in turn affect nutritional and health status [31,43–48]. These studies are reviewed elsewhere [82], however a few salient examples are discussed here. For instance, SNPs in the *TAS1R2* and *TAS1R3* genes, which codify for sweet taste receptors and are related to a higher preference and intake of sweet foods, have also been associated with increased dental caries [46,83,84]. Another example is the relationship between variations in the *TAS2R38* bitter taste gene and eating behavior as well as anthropometric and adiposity measures. Increased disinhibition has been described in women carrying the PROP-insensitive allele for the rs1726866 SNP [85]; while another finding reported higher BMI and waist circumference among PROP non-taster women with low dietary restraint [86]. In another study, differences in body fat percentage were associated with the three *TAS2R38* genetic variants, while no significant relationships with BMI and eating behavior were found [87]. Other studies did not support a relationship between *TAS2R38* variants and adiposity measures [64,86–88]. These inconsistent results could be ascribed to the presence of several confounding factors (i.e., sex, age, ethnicity, etc.) that may modulate the relationship among taste receptors and health status parameters.

Differences in bitter taste perception have also been associated with bitter taste receptor mRNA levels in taste cells [89,90], suggesting that gene expression is another factor to consider when the relationship with health measures is studied. Moreover, recent findings showed that the gene expression profile of fungiform taste papillae differs between lean and obese subjects [91]. Together, these findings highlight the need to conduct future studies to clarify their association.

Recent evidence also raises the possibility that taste and smell receptors residing in different bodily tissues may have multiple functions in health and disease. For example, taste receptors are also expressed in extra-oral tissues, such as the gastrointestinal tract, where they seem to be involved in digestive functions or homeostasis and energy metabolism [92–103].

It is also well known that the sense of smell is impaired in neurodegenerative diseases [104,105] and associations between olfactory genes (expressed in olfactory and non-olfactory tissues) and diet-related diseases such as obesity have also been demonstrated [104,106,107]. Notably, the *OR7D4* gene, recently related to preference for pork meat containing androstenone (described in [71]), was previously associated with adiposity, cognitive dietary restraint, and susceptibility to hunger in another study [108].

Despite these positive findings, very large GWAS on BMI or other health-related parameters have not found associations with SNPs in chemosensory genes [109–111], suggesting that their effects are likely to be very small and limited in predictive power.

Combining Several Genetic Variants: The Polygenic Risk Score

The evidence presented above suggests that a new paradigm may be needed to accelerate progress in understanding the relationships between food preferences and nutrition and health. Our findings [17] using the GWAS approach identified novel genes associated with food preferences with no known effects on chemosensory function. Thus, looking beyond the involvement of traditional chemosensory genes in food preferences may be important for gaining new insights.

Although GWA studies have led to progress in identifying common variations associated with many complex traits, the modest effect sizes have prevented risk prediction based on single genetic variants. More recently, polygenic risk score analyses that combine the effects of several genetic variants have shown some predictive ability for a wide range of complex traits [112]. In polygenic score (PGS) analysis, a set of SNPs identified in a GWAS is used to construct a polygenic score that is used for association testing or risk prediction.

To the best of our knowledge, polygenic risk score analyses for food preferences have not yet been conducted. Although the link between vegetable intake and adiposity measures was widely investigated [113–115], few studies focusing on the relationship between hedonic measures and

adiposity have been conducted. These studies have found no or weak association [116–118], suggesting that this complex relationship could be modulated by several factors, including genetic ones. Therefore, here, we report the data obtained from a PGS analysis to evaluate the predictive power of SNPs associated with food liking on adiposity measures (BMI and fat mass). Data was collected from 1140 individuals belonging to two Italian cohorts (Friuli Venezia Giulia and Val Borbera). Further details on data collection, sample characteristics, and polygenic score analysis are reported in supplementary materials (File S1).

We constructed a PGS for vegetables (PGS-vegetables) based on 6 SNPs significantly associated with preferences for different vegetables in our previous work: rs28849980, rs10050951, rs8034691 for artichokes, rs2530184, rs9832668 for broccoli, and rs138369603 for chicory (see Table 2 in [17]).

For each individual, PGS-vegetables represents vegetable preference predicted by the combination of the above mentioned 6 SNPs. In the first step, the allele count for each SNP was weighted by its per-allele association with food preferences. Specifically, for each identified SNP, an individual's genotypic score (0, 1, or 2 for genotyped SNPs, or any value between 0–2 for imputed SNPs) was multiplied by the effect size. SNPs were weighted such that a higher weight was associated with a higher preference for the associated vegetable. The final score (PGS-vegetables) was calculated for each individual by summing the values obtained in the first step across all six SNPs. Linear regression analysis was conducted to test the associations between adiposity measures (BMI and fat mass as dependent variables) and PGS-vegetables as the predictor variable, in models adjusted for sex, age, education level (as number of years of completed schooling), and physical activity (never/light/moderate/intense). Information on sample collection, genotyping, imputation, and phenotypes were reported in our previous works [16,17]. Table 2 shows that PGS-vegetables was a significant negative predictor of BMI and fat mass (p-value < 0.05), in addition to sex, age, education, and physical activity. Specifically, higher PGS-vegetables (corresponding to higher preferences for vegetable foods) was predictive of lower BMI and fat mass.

Table 2. Results of polygenic risk score analysis.

Predictor Variables	BMI, Kg/m^2	Fat Mass, Kg
Sex, male	**2.85 (<0.0001)**	−0.67 (0.2)
Age, years	**0.04 (<0.0001)**	**0.09 (<0.0001)**
Education level, years	**−0.14 (<0.001)**	**−0.26 (0.002)**
Physical activity	**−1.19 (<0.0001)**	**−2.56 (<0.0001)**
Vegetables PGS	**−0.98 (0.028)**	**−2.08 (0.023)**

Beta and p-value in brackets are shown. In bold: significant results (p-value < 0.05). BMI = Body Mass Index; PGS = Polygenic Score.

Although the PGS-vegetables variable accounted for only 0.28% of the variation in BMI and 0.33% of the variation in fat mass, the low number of SNPs included in the study could explain this finding.

These results on PGS represent a starting point in studying the polygenic effects of food preferences on health status. As the number of GWAS of food preferences increase, further studies considering more SNPs and other food categories should be conducted. Adopting the PGS approach would allow the development of more powerful genetic profiles to better predict the risk of disease.

4. Conclusions

In conclusion, the data reviewed here highlight the role of genetic variations in food preferences and their important contributions to nutrition and health.

There is a need to identify and investigate other genes involved in food preferences, besides those already implicated in olfactory and taste perception. These novel genes can be discovered through GWAS or other genomic approaches.

The use of polygenic risk analysis to assess associations between food preferences and disease outcomes could lead to important new insights in nutrition research.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/8/1735/s1, File S1: Data collection and polygenic score analysis.

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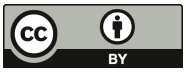

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Human Tongue Electrophysiological Response to Oleic Acid and Its Associations with PROP Taster Status and the *CD36* Polymorphism (*rs1761667*)

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Abstract: The perception of fat varies among individuals and has also been associated with *CD36 rs1761667* polymorphism and genetic ability to perceive oral marker 6-n-propylthiouracil (PROP). Nevertheless, data in the literature are controversial. We present direct measures for the activation of the peripheral taste system in response to oleic acid by electrophysiological recordings from the tongue of 35 volunteers classified for PROP taster status and genotyped for *CD36*. The waveform of biopotentials was analyzed and values of amplitude and rate of potential variation were measured. Oleic acid stimulations evoked positive monophasic potentials, which represent the summated voltage change consequent to the response of the stimulated taste cells. Bio-electrical measurements were fully consistent with the perceived intensity during stimulation, which was verbally reported by the volunteers. ANOVA revealed that the amplitude of signals was directly associated, mostly in the last part of the response, with the *CD36* genotypes and PROP taster status (which was directly associated with the density of papillae). The rate of potential variation was associated only with *CD36*, primarily in the first part of the response. In conclusion, our results provide direct evidence of the relationship between fat perception and *rs1761667* polymorphism of the *CD36* gene and PROP phenotype.

Keywords: electrophysiological recording from human tongue; fat perception; *CD36*; PROP tasting

1. Introduction

Over the last decade, multiple effects of dietary fatty acids as regulators of lipid and energy metabolism in human health and disease outcomes have been pointed out [1]. Therefore, the capability to distinguish them in a diet can have important nutritional implications for the health of volunteers, and studies aiming to analyze the fat perception and discrimination are important to understand the mechanisms involved in the choice of fat-rich foods [2].

Dietary fats were traditionally thought to have no 'taste' of their own, but rather to be sensed through their textural and odorant properties [3]. Emerging findings have since disputed this understanding by demonstrating an important involvement of taste in fat detection, which has been proposed as a sixth primary taste quality [2–6]. Although dietary lipids are mostly triglycerides, free long-chain fatty acids released from dietary lipids during oral processing seem to be accountable

for fat taste perception [7,8]. In fact, the cleavage of triglycerides into free fatty acids by a lingual lipase has been shown both in rodents [9] and in humans [5]. Various classes of fatty acid receptors have been proposed for the taste transduction of lipids [10,11], including the multifunctional CD36 scavenger receptor [11–16], which is primarily responsible for the detection of long chain fatty acids on the tongue [5,17,18]. CD36 is a membrane glycosylated protein whose expression is controlled by the *CD36* gene and regulated by its allelic diversity [19]. The exchange of A for G in the *rs1761667* single nucleotide polymorphism (SNP) has been shown to decrease protein expression [19], and is associated with a reduced oral ability to perceive fatty acids [5,20,21]. Ethnic-specific effects were also observed in one experiment where East Asians, but not Caucasians, with the AA genotype showed a reduced ability to perceive fatty acids [22]. The substitution of A for G in this SNP has also been shown to influence fat preference [23]. In addition, recent results suggest that this SNP is differentially related with body composition and endocannabinoid levels in lean and obese volunteers [24].

Some studies have also shown that changes in taste sensitivity to fats could be related to differences in general taste sensitivity as indicated by variations in the salivary protein expression of gustin (carbonic anhydrase VI), which has been associated with both taste perception [25], density of papillae and function [26,27]. In addition, it is known that sensitivity to and preference for fat has also been associated with the genetic ability to perceive the oral marker stimulus, 6-n-propylthiouracil (PROP) [21,28–33]. Specifically, volunteers who are very sensitive to PROP (super-tasters) and have a higher fungiform papillae density on their tongues [26,34–38], seem to have a higher sensitivity and a lower preference for high-fat foods [28,30,31,39–43], compared to those who taste PROP only at high concentrations or not at all (non-tasters) and show a higher preference for fat-rich foods. These considerations support the hypothesis that PROP sensitivity is negatively related to calorie consumption and body weight, as several studies have reported [30,41,44–48]. Besides, PROP taster status can affect lipid metabolism in normal weight [49] and obese [50] volunteers. However, the role of the PROP phenotype in fat perception is debatable [51–56]. Divergent results may be due to the fact that psychophysical methods which are based on self-reports can produce subjective evaluations.

Based on these statements and given the nutritional value of dietary fats, it is of great importance to characterize factors that may contribute to individual differences in fat taste perception with the aim of better understanding the mechanisms involved in the choice of fat-rich foods. We analyzed the relationships between oleic acid taste perception and *rs1761667* SNP in the *CD36* gene and PROP phenotype by direct evaluation of the degree of activation of the peripheral taste system in response to the oleic acid taste stimulation. These measures were carried out by means of electrophysiological recordings from the human tongue (Electrotastegrams, ETG), which yield data that are not influenced by the individual's subjective biases [57,58].

2. Materials and Methods

2.1. Participants

Thirty-five Caucasian and non-smoker volunteers (15 males, 20 females, age 28.6 $\pm$ 0.86 years) were recruited according to standard procedures at Cagliari University. All volunteers were originally from Sardinia island, Italy. No statistical analyses were performed to pre-determine the size of the sample. However, several guiding criteria were used. First, our sample size is comparable to those typically employed in electrophysiological recording experiments since they provide a direct measure of the degree of activation of the receptors or neurons under study [57]. Due to the high frequency of AG heterozygotes at the *rs1761667* SNP in the *CD36* gene among American Caucasian [22] and European populations reported in 1000 Genomes (dbSNP Short Genetic Variations, 2017), it was not possible to construct equal sample sizes within each of the genotype/phenotype subgroups. Therefore, volunteers were recruited to form three roughly equal-sized PROP-taster groups that were matched for age and gender. Volunteers had a normal body mass index (BMI) of 20.2 to 25.2 kg/m^2. None were dieting, taking medications that might interfere with oral sensory perception or had food allergies.

Their gustatory function was screened for four basic tastes by a taste strip test (Burghart Messtechnik, Wedel, Germany) to rule out any gustatory impairment. All volunteers were informed about the aim and protocol of the study and signed an informed consent form. The present study was conducted in accordance with the latest revision of the Declaration of Helsinki, and the procedures have been approved by the Ethical Committee of the University Hospital Company (AOU) of Cagliari, Italy. This trial was registered at ClinicalTrials.gov (identifier number: UNICADBSITB-1).

2.2. Experimental Protocol

Volunteers were tested in two sessions on two successive days. On the first day, each subject was classified for his/her PROP taster status, while on the second day he/she was tested for the electrophysiological response to oleic acid taste stimulation on a small area of the tongue tip and for the fungiform papillae density on the same area of the tongue surface. Volunteers were always requested to abstain from drinking (except water), eating, using chewing gum or oral care products for at least 2 h prior to testing. All had to be in the test room 15 min before the beginning of the session (9:00 AM) in order to acclimate to the constant environmental conditions (23–24 °C; 40–50% relative humidity). Women were always tested around the sixth day of the menstrual cycle to avoid taste sensitivity changes due to the estrogen phase [59]. All solutions (in spring water), which were used for the measures at room temperature, were prepared and stored in a refrigerator until 1 h before testing. Stimuli were presented.

At the end of the first visit, samples of whole saliva (2 mL) were collected from each volunteer into an acid-washed polypropylene test tube by means of a soft plastic aspirator. The samples were stored at −80 °C until being processed by the molecular analyses described below.

The study design is shown in Figure 1.

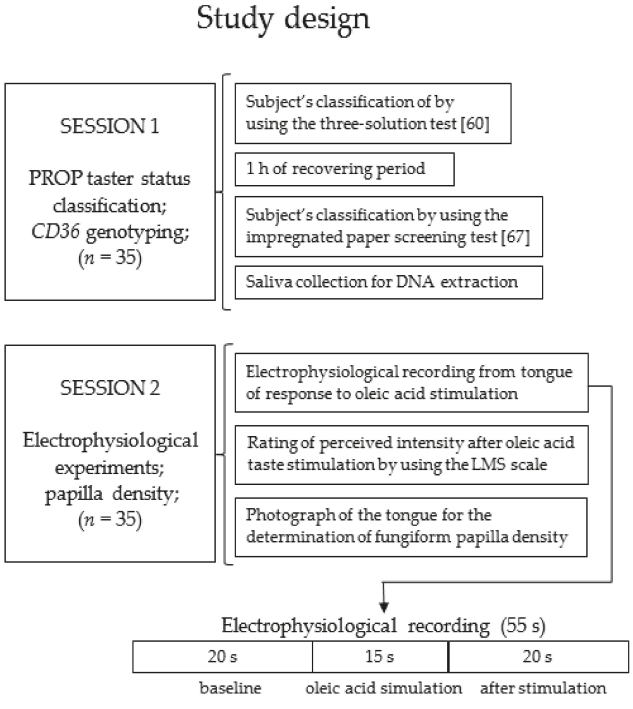

Figure 1. A graphic diagram representing the study design.

2.3. PROP Taster Status Classification

Volunteers were classified for their PROP taster status by two scaling measurements. All were first assessed using the three-solution test [60], which has been validated in numerous studies [61–65]. The perceived taste intensity ratings to PROP (0.032, 0.32, and 3.2 mmol/L) (Sigma-Aldrich, Milan, Italy) and sodium chloride (NaCl; 0.01, 0.1, 1.0 mol/L) (Sigma-Aldrich, Milan, Italy) solutions were collected by using the Labeled Magnitude Scale (LMS) [66]. The use of this scale gave the volunteers the freedom to evaluate the PROP bitterness intensity relative to the "strongest imaginable" oral stimulus ever perceived in their life. LMS is a semi-logarithmic 100-mm scale in which seven label verbal descriptors are arranged, in semilog intervals, along the length of the scale. The verbal labels and their positions on the LMS are: barely detectable, 1.4; weak, 6.1; moderate, 17.2; strong, 35.4; very strong, 53.3; and strongest imaginable, 100.

NaCl was used as a control because taste intensity to NaCl does not change with PROP taster status in this procedure [60]. Concentrations (10 mL samples) were presented in a random order. Volunteers who gave lower intensity ratings to PROP than to NaCl were classified as PROP non-tasters, those who gave overlapping ratings to both PROP and NaCl were classified as medium tasters, and those who gave higher ratings to PROP than to NaCl were classified as super-tasters. The classification of each subject as belonging to a PROP taster group (super-taster, medium-taster, or non-taster) was confirmed using the impregnated paper screening test [27,67] after a 1-h period. With this method, the two stimuli were presented sequentially to each subject by placing the paper disks on the tip of the tongue for 30 s; the first one was impregnated with PROP solution (50 mmol/L) and the second with NaCl (1.0 mol/L). The ratings of the perceived intensity on each paper disk were obtained by using the LMS scale (described above). Volunteers who rated the PROP disk lower than 15 mm on the LMS were categorized as non-tasters; those who rated the PROP disk higher than 67 on the LMS were categorized as super-tasters; all others were classified as medium tasters [67]. Volunteers who were classified differently by two methods were excluded from other tests. Based on the classification, which was documented by three-way ANOVA, 12 volunteers (5 males, 7 females, age 26.5 $\pm$ 2.6 years) were classified as non-taster (34.29 %), 13 (6 males, 7 females, age 29.2 $\pm$ 1.8 years) were medium taster (37.14 %) and 10 (4 males, 6 females, age 25.2 $\pm$ 2.6 years) were super-taster (28.57 %) (Table S1).

2.4. Molecular Analysis

Volunteers were genotyped for the *rs1761667* (G/A) single nucleotide polymorphism (SNP) of *CD36*, located at the —31118 promoter region of exon 1A. Briefly, analyses were performed by PCR followed by analysis with restriction enzyme (*Hha*I) of the fragments obtained according to Banerjee et al. 2010 [68]. This method has been validated by numerous studies [21,24,33]. The products of digestion were separated by electrophoresis on a 2% agarose gel and the bands of DNA were visualized by ethidium bromide staining and ultraviolet light to score the deletion. PCR 50 bp Low Ladder DNA was used as a molecular mass marker (Gene Ruler™-Thermo Scientific, Waltham, MA, USA).

Volunteers were also genotyped for the three SNPs of *TAS2R38* (gene which expresses the specific receptor of PROP [69]), that give rise to two major haplotypes, the taster variant (PAV) and the non-taster variant (AVI), and three rare ones (PVI, AAI, and AAV). Molecular analyses of *TAS2R38* locus were performed by Taqman® SNP Genotyping Assays (Applied Biosystems by Life-Technologies Italia, Europe BV) [57].

2.5. Electrophysiological Recordings

Differential electrophysiological recordings from the tongues of volunteers were performed according to Sollai et al. 2017 [57]. Briefly, two silver electrodes were used, one in contact with the tongue ventral surface and the other in perfect adhesion with the dorsal surface. The first electrode was a silver wire (0.50 mm) with the distal terminal rolled up to form a ball (about 5 mm of diameter) to obtain a good electrical contact and make the electrode safe for the sublingual mucosa. The second

one (patent WO 2017/212377) was made by depositing a silver film (100 nm thick) on a very thin (13 μm) polyimide layer (Kapton ©, DuPont, Wilmington, DE, USA) by means of evaporation in high vacuum. A film of insulating and biocompatible material (Parylene C, 2 μM thick) covered both sides of the electrode except for the area which must be in electrical contact with the tongue. The extreme suppleness of this electrode allows its perfect adhesion with the dorsal surface of the tongue. The distal part of this electrode had a circular hole which leaves a small area (6 mm of diameter) uncovered on the left side of the tongue surface tip when it is positioned on the tongue surface. This is the area of the tongue where oleic acid stimulation was delivered during the electrophysiological recordings, and the fungiform papillae density is calculated as described below. A third disposable adhesive electrode used as the ground terminal of the measuring instrument, was placed in an electrically neutral position (CDES003545, SpesMedica, Italy). After positioning the electrodes and verifying the electrical contact, the bio-potential recording started when a stable baseline was observed. Signals detected by the electrodes were recorded by a high input impedance polygraph for human use (Porti7 portable physiological measurement system; TMS International B.V., The Netherlands), which is an isolated certified Class IIa medical device. Signals were digitized, recorded and visualized in real time on a PC by PolyBench software (TMSI, Oldenzaal, The Netherlands). For each subject, the recording lasted 55 s (20 s baseline, 15 s during oleic acid simulation and 20 s after stimulation). Afterwards, the waveform of bio-potentials was analyzed (Clampfit 10.0 software, Berkeley, CA, USA) and the measures of voltage changes with respect to baseline in response to oleic acid (amplitude values) were determined at 2.5, 5, 10, and 15 s from stimulation onset. The rate of potential variation (mV/s) was also calculated at the same time intervals.

2.6. Oleic acid Taste Stimulations

The oleic acid taste stimulation was delivered by placing for 15 s a paper disk (6 mm dia) impregnated with 30 μL of oleic on the circular area of the tongue surface that was left free by the hole of the second electrode. Each volunteer was instructed to rate the perceived intensity by using the LMS scale [66]. Dry paper disks were also used as control.

2.7. Density Measurements of Fungiform Papillae

Fungiform papillae density was measured in the small circular area of the left side, close to the midline of the anterior surface of the tip of the tongue where oleic acid stimulation was delivered during the electrophysiological recordings according to our previous work [57]. Briefly, volunteers sat on a chair supporting their head with their hands in order to minimize movements. The area of the tongue was first dried and then stained by placing a circle of filter paper (6 mm in diameter) impregnated with a blue food dye (E133, Modecor Italiana, Italy) on the specified area. Photographs of the stained area of the tongue surface were taken for each volunteer using a Canon EOS D400 (10 megapixels) camera with lens EFS 55–250 mm. The digital images were analyzed using a "zoom" option in the Adobe Photoshop 7.0 program. The fungiform papillae were separately identified and counted by three trained operators who were uninformed of the PROP taster status and *CD36* genotype of volunteers [26,37,57]. The density/cm^2 was calculated.

2.8. Statistical Analysis

Simple linear correlation analysis was used to investigate the relationship between the density of fungiform papillae and perceived intensity in response to oleic acid taste stimulation. Linear correlation analysis was also used to elucidate the relationships between signal amplitude (mV) and biopotential variation rate (mV/s) with density of fungiform papillae, or perceived taste intensity of oleic acid. Fisher's method (Genopop software version 4.0) [70] was used to test *CD36* genotype distribution and allele frequencies according to PROP taster status. One-way ANOVA was used across PROP taster groups, *TAS2R38* genotype groups, *CD36* genotype groups or gender, to compare mean values ± SEM of the perceived taste intensity, density of fungiform papillae, signal amplitude

(mV), and biopotential variation rate (mV/s). Repeated measures ANOVA was used across PROP taster groups or *CD36* genotype groups, to evaluate the differences of mean values ± SEM of the signal amplitude (mV) and biopotential variation rate (mV/s), at 2.5, 5, 10, and 15 s after the application of oleic acid stimulation. Data were verified for the assumptions of homogeneity of variance, normality and sphericity (when applicable). To determine if the sphericity assumption was violated, a Greenhouse–Geisser correction or Huynh–Feldt correction was applied. Post-hoc comparisons were conducted with the Fisher LDS test, unless the assumption of homogeneity of variance was violated, in which case the Duncan's test was used. Statistical analyses were conducted using STATISTICA for WINDOWS (version 7; StatSoft Inc, Tulsa, OK, USA). p values ≤ 0.05 were considered significant.

3. Results

3.1. CD36 Genotyping and Phenotyping

Linear correlation analysis showed that the density of fungiform papillae in the small circular area of the tongue where oleic acid stimulation was delivered during the electrophysiological recordings is linearly correlated with the perceived taste intensity by volunteers ($r = 0.477$; $p = 0.005$).

Molecular analysis at the CD36 (SNP: *rs1761667*) gene identified 6 AA homozygous (3 males, 3 females, age 29.2 ± 2.9 years), 20 heterozygous (9 males, 11 females, age 26.1 ± 1.5 years), and 9 GG homozygous volunteers (3 males, 6 females, age 29.7 ± 3.4 years). PROP taster groups did not differ statistically based on genotype distribution and haplotype frequency of the *CD36* gene ($\chi^2 > 0.665$; $p < 0.71$; Fisher's test).

Mean values ± SEM of perceived intensity after taste stimulation with oleic acid in volunteers classified by their PROP taster status and genotyped for the *rs1761667*SNP of *CD36* gene are shown in Figure 2. The perceived intensity ratings to oleic acid stimulation were associated with PROP taster status. Specifically, the perceived intensity was higher in the super-taster volunteers than in non-taster or medium-taster ones ($p \leq 0.046$; Duncan's test subsequent to one-way ANOVA; $F_{2,32} = 3.138$; $p = 0.054$). No differences in intensity ratings between the medium-taster and non-taster volunteers were found ($p > 0.05$). In addition, volunteers with the GG genotype gave intensity ratings higher than volunteers with the AA genotype ($p = 0.047$ Fisher LDS test). No differences between the heterozygous and homozygous volunteers were found ($p > 0.05$).

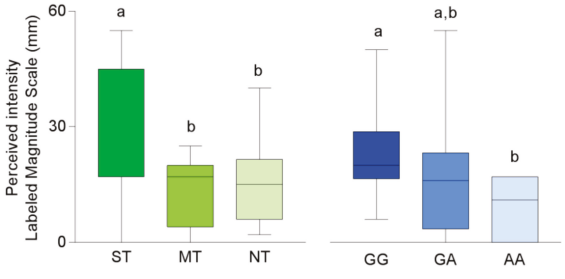

Figure 2. Box-and-whisker plots showing the minimum, first quartile, median, third quartile, and maximum of each set of perceived intensity data evoked by taste stimulation with oleic acid (30 μL) in super-tasters (ST; $n = 10$), medium-tasters (MT; n=13) and non-tasters (NT; $n = 12$) and in volunteers with genotypes GG ($n = 9$), GA ($n = 20$) and AA ($n = 6$) of *CD36*. Different letters indicate a significant difference ($p \leq 0.046$; Duncan's test and $p = 0.047$ Fisher LDS test, subsequent one-way ANOVA).

Fungiform papillae density varied with PROP taster status ($F_{2,32} = 18.712$; $p < 0.001$) (Figure 3). Super-tasters showed a higher density than medium-tasters ($p < 0.001$; Duncan's test), whose values were higher than those of non-tasters ($p = 0.032$; Duncan's test). No differences in fungiform papillae density related to the *CD36* polymorphism were found ($p < 0.05$).

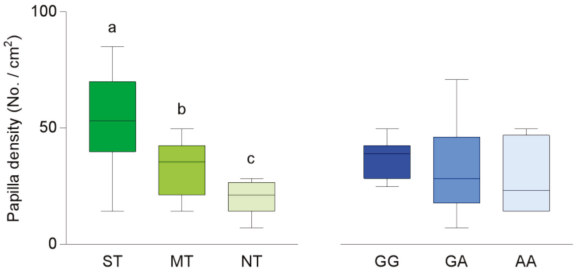

Figure 3. Box-and-whisker plots showing the minimum, first quartile, median, third quartile, and maximum of each set of density of fungiform papillae data in super-tasters (ST; n = 10), medium-tasters (MT; n = 13) and non-tasters (NT; n=12) and in volunteers with genotypes GG (n = 9), GA (n = 20) and AA (n = 6) of *CD36*. Different letters indicate a significant difference ($p \leq 0.032$; Duncan's test subsequent one-way ANOVA).

3.2. Electrophysiolgical Responses to Taste Stimulation with Oleic Acid

The electrophysiological recording from the human tongue allowed us to determine bioelectrical potential changes in response to oleic acid taste stimulation. The analysis of the waveform of bioelectrical potentials showed that the oleic acid stimulation evoked positive monophasic potentials characterized by a faster initial rise followed by a slower phase, which continued for the whole duration of stimulation. The variation in voltage with respect to baseline (i.e., the amplitude of these signals, as well as the hyperpolarization rate) was highly variable among volunteers. The values of amplitude varied from a minimum of 0.64 mM (measured in a non-taster volunteer with the AA genotype in the *CD36* polymorphism) to a maximum of 91.99 mV (determined in a super-taster volunteer with the GG genotype in the *CD36* polymorphism). Examples of this variability are shown in Figure 4.

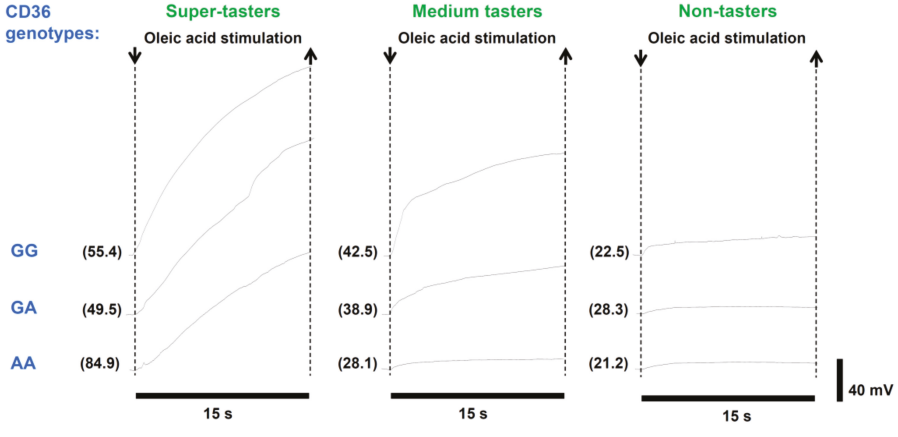

Figure 4. Examples of electrophysiological recordings in response to oleic acid (30 µL) taste stimulation in representative super-tasters, medium-tasters and non-tasters with different genotypes of the *CD36* gene. The very first data point on the left side of each electrophysiological recording represents the baseline. Numbers within parentheses on the left of each trace indicate the density of fungiform papillae (No./cm^2) of each subject calculated in the small circular area of the tongue where oleic acid stimulation was applied.

Linear correlation analysis showed that signal amplitude, as well as hyperpolarization rate, were linearly correlated to the density of fungiform papillae (r = 0.394; p = 0.028 and r = 0.410; p = 0.019,

respectively). No such correlation was found between the two electrophysiological parameters and perceived taste intensity ($r < 0.251$; $p > 0.06$).

Mean values ± SEM of the amplitude and hyperpolarization rate of signals recorded in response to oleic acid taste stimulation in volunteers categorized for their PROP taster status and genotyped for the *rs1761667* SNP of the *CD36* gene are shown in Figure 5. Values determined in super-taster volunteers were significantly higher than those measured in non-tasters, although they are at the limits of statistical significance ($p = 0.052$; Duncan's test subsequent one-way ANOVA), while medium-tasters showed biopotential changes which were not different from those of the other two taster groups ($p > 0.05$). In addition, the values determined in homozygous GG volunteers were higher than those of volunteers with the AA genotype ($p = 0.043$; Fisher LDS test subsequent one-way ANOVA). No differences between heterozygous and homozygous volunteers were found ($p > 0.05$). Volunteers with the GG genotype also showed higher values of hyperpolarization rate than volunteers with the AA genotype ($p = 0.028$; Fisher LDS test subsequent one-way ANOVA), while heterozygous volunteers did not show different hyperpolarization rate values from the other groups ($p > 0.05$). No differences in the rate values related to PROP taster status were found ($p > 0.05$). One-way ANOVA also showed that no differences in the amplitude and hyperpolarization rate of signals were found in relation to the *TAS2R38* genotype (Figure S1) or gender ($p > 0.05$).

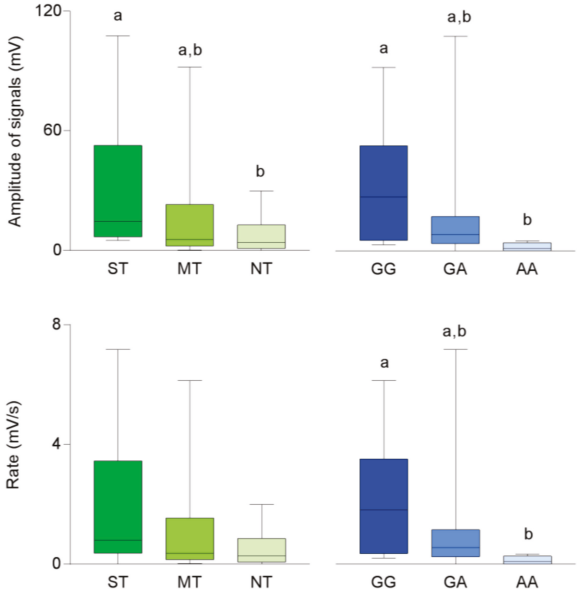

Figure 5. Box-and-whisker plots showing the minimum, first quartile, median, third quartile, and maximum of each data set of amplitude and rate of signals evoked in super-tasters (ST; $n = 10$), medium tasters (MT; $n = 13$) and non-tasters (NT; $n = 12$); and in volunteers with genotypes GG ($n = 9$), GA ($n = 20$) and AA ($n = 6$) of *CD36* by oleic acid (30 µL) taste stimulation. Different letters indicate a significant difference ($p \leq 0.05$; Fisher LDS or Duncan's test subsequent one-way ANOVA).

The same data from Figures 2, 3 and 5 are also reported as distributions of the original points and mean values ± SEM for each PROP taster and *CD36* genotype group as shown in Figures S2–S4.

Mean values ± SEM of signal amplitude and hyperpolarization rate, determined after 2.5, 5, 10, and 15 s after the application of oleic acid taste stimulation according to PROP taster status and CD36 polymorphisms, are shown in Figure 6. The time course of the hyperpolarization amplitude and variation rate were different in volunteers with different PROP phenotypes or *CD36* genotypes.

In particular, the amplitude of signals increased during the stimulation up to 15 s in super-taster and medium-taster volunteers ($p < 0.001$ and $p = 0.004$; Fisher LDS or Duncan's test subsequent repeated measured ANOVA), but they did not change in non-tasters ($p > 0.05$). The hyperpolarization values increased to the end of stimulation in volunteers with the GG genotype in the CD36 gene ($p \leq 0.001$; Fisher LDS or Duncan's test subsequent repeated measured ANOVA), but only for a duration of 10 s in heterozygous volunteers ($p = 0.025$; Fisher LDS test subsequent repeated measured ANOVA). These values did not change with time in volunteers with the AA genotype ($p > 0.05$). The hyperpolarization rate decreased (at 10 s and 15 s) in super-tasters ($p \leq 0.037$; Fisher LDS or Duncan's test subsequent repeated measured ANOVA) and more rapidly in medium-tasters ($p \leq 0.019$; Fisher LDS or Duncan's test subsequent repeated measured ANOVA), while it decreased only at 5 s in non-tasters ($p = 0.049$; Fisher LDS test subsequent repeated measured ANOVA). The hyperpolarization rate rapidly decreased across the whole-time course of recordings in volunteers having the GG genotype in the *CD36* gene ($p \leq 0.037$; Fisher LDS or Duncan's test subsequent repeated measured ANOVA). The hyperpolarization rate only decreased at 10 s in heterozygous volunteers ($p = 0.005$; Fisher LDS test subsequent repeated measured ANOVA) and did so more slowly (at 15 s) in volunteers with the AA genotype ($p > 0.05$).

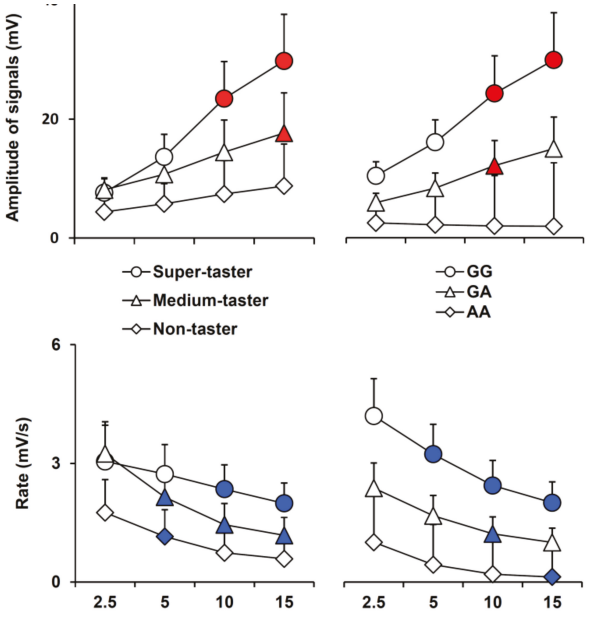

Figure 6. Time course of amplitude (mV) or hyperpolarization rate (mV/s) of the signal across PROP taster status or *CD36* polymorphism groups during stimulation time. Data (mean values ± SEM) are determined after 2.5, 5, 10, and 15 s from the application of oleic acid (30 µL). $n = 10$ super-tasters, $n = 13$ medium tasters and $n = 12$ non-tasters; $n = 9$ volunteers with genotypes GG in CD36, $n = 20$ GA genotypes and $n = 6$ AA genotypes. Solid symbols (red for amplitude of signals and blue for rate) indicate a significant difference with respect to the previous value of the corresponding group ($p \leq 0.05$; Fisher LDS or Duncan's test, subsequent to repeated measures ANOVA across PROP taster groups or *CD36* genotype of volunteers).

Dry paper disks which were used as controls evoked no potential variations.

4. Discussion

A great deal of conflicting data has been collected over the last decade on individual differences in fat perception, preference and consumption related to genetic variation in PROP taste sensitivity [23,51–56,71]. Emerging evidence suggests that variation in other genes such as polymorphisms in the gene of the CD36 scavenger protein, may also be involved in fat perception [5,20,21]. The present study provides the first direct demonstration of the roles of PROP phenotype and a *CD36* polymorphism in individual variability in fat perception. In fact, the highly reliable ETG method used here, allowed us to obtain direct and quantitative data that are free from individual subjective responses.

We found that oleic acid taste stimulation evoked positive monophasic potentials, which lasted for the entire duration of the stimulation and, in most recordings, even longer. On the other hand, the control stimulations were ineffective in evoking this response. The extended activation that we found in response to oleic acid could reflect the persistence of stimulation over time (a limitation of our method is that the stimulus cannot to be removed), and its slow increase of amplitude could depend on the high surface tension of lipid molecules. The biopotential variations recorded in response to oleic acid possibly represent a measure of the summated voltage change resulting from the response of stimulated taste cells, as already shown in response to other stimuli in our previous study [57]. These variations are also similar to those recorded from the olfactory epithelium [72,73], where the electrical activity has been reported as the summated generated potential by the population of stimulated olfactory neurons [74]. The fact that the recorded signals effectively correspond to the summated response of stimulated taste cells seems to be confirmed by the changes of the amplitude of signals among the PROP taster groups, which vary in density of papillae. This interpretation is further supported by the direct and linear correlation found between the amplitude and rate of signals and density of fungiform papillae. Density of papillae at the tongue tip, which is highly correlated with their total number on the tongue [37], was also positively correlated with the perceived taste intensity by the volunteers.

In addition, our results show a direct relationship between the amplitude of biopotentials recorded and PROP phenotype. Specifically, we recorded the largest amplitude values in PROP super-tasters who had the highest density of fungiform papillae in the same area of the tongue where oleic acid stimulation was delivered during the recordings. Likewise, the smallest amplitude values were recorded in non-tasters who had the lowest density of papillae, and intermediate amplitudes were recorded in medium-tasters with an intermediate density of papillae. These results strongly support previous psychophysical experiments showing a direct relationship between fat perception and PROP taster status, [21,29–31,48,75] that can be linked to differences in the density of papillae across the three PROP taster categories [26,34–38]. In fact, no differences in electrophysiological responses to oleic acid were found in relation to *TAS2R38* polymorphisms. This suggests that the phenotypic expression of the trait, which is strongly associated with papillae density, is a critical determinant of electrophysiological responses to fatty acids on the tongue rather than strictly the presence or absence of specific genetic variants in *TAS2R38*. This is consistent with data from another study, showing that the PROP phenotype is a better predictor of adiposity in women than the *TAS2R38* genotype, which is unrelated to adiposity [46]. Importantly, the signal amplitudes that we recorded in the PROP taster groups, as well as in the *CD36* genotype groups, agree with the perceived intensities reported by the volunteers during oleic acid stimulation, thus indicating that our bioelectrical measurements are fully consistent with common human psychophysical observations. The lack of a direct linear correlation between the electrophysiological and psychophysical measurements may simply reflect the presence of background 'noise' in the electrophysiological measures, and further refinement of the recording procedure will presumably reduce this noise.

Another important physiological feature we observed was a relationship between the values of the hyperpolarization amplitude and rate, and the *rs1761667* polymorphism of the *CD36* gene. In agreement with evidence showing that the presence of the homozygous AA genotype at this location of the *CD36* gene is characterized by reduced protein expression [19] and low taste sensitivity to

fats [5,20–22], we measured the lowest amplitude values and biopotential variation rates in volunteers carrying two A alleles who verbally reported the lowest values of perceived intensity. Likewise, we measured the highest bioelectrical values in volunteers with the GG genotype who perceived the highest intensity of oleic acid and intermediate values in heterozygous volunteers who reported intermediate values of perceived intensity. As expected, no variations in the density of papillae related to *CD36* genotypes were found.

The analysis of the time course of the responses showed that the amplitude of the signal increased during stimulation, mostly in the last portion of the response, and this effect was most prominent in PROP tasters who had a higher number of papillae, and in volunteers having at least one G allele in *CD36*, which is known to be associated with an increase of receptor expression [19]. On the contrary, signal amplitude did not change in PROP non-tasters who had a low number of papillae and in volunteers homozygous for the non-tasting (AA) form of this polymorphism in *CD36*, which is associated with reduced protein expression [19]. In addition, the hyperpolarization rate rapidly decreased across the whole time course of recordings in volunteers with two tasting (GG) alleles, who showed at 2.5 s after stimulus onset, values about twice as high as those of the volunteers with only a single G allele. In turn, the hyperpolarization rate slowly decreased in both heterozygous (GA) volunteers and in volunteers with two non-taster variants in *CD36*. All these results suggest that the presence of the tasting variant in the specific receptor is the most important condition to elicit a prompt response and, in addition, turns out to be the most important condition to evoke an intense perception when the volunteers have a high number of fungiform papillae in their tongue.

Future studies should confirm the results in a larger population (with a higher number of subjects in each study groups). In fact, a limitation of this work is the small size of the examined sample mostly regarding the group of subjects with the homozygous AA genotype at this *CD36* locus.

5. Conclusions

In conclusion, the present work builds on our previous psychophysical studies documenting a role for *rs1761667* polymorphism in *CD36* and polymorphisms in the *TAS2R38* gene (indexed by PROP phenotype in the current study) in the perception of oleic acid [21]. Here, we used a novel physiological recording technique [57] to directly measure the degree of activation of the peripheral gustatory system in response to taste stimulation with oleic acid on a localized area of the tongue. We found that both genes contributed to variations in the perception of oleic acid, but they had somewhat different effects on specific features of the electrophysiological response. Rate variation seemed to be influenced mostly by the *CD36* gene, early in the time course, whereas signal amplitude was more influenced by PROP status during the latter part of the time course. The influence of PROP status on signal amplitude may reflect a summation effect associated with higher density of papillae, a well-known anatomical characteristic of PROP-tasting volunteers [26,34–38], although other authors did not find links between the density of papillae and PROP status [76]. Our findings support the notion that several overlapping mechanisms are involved in fat perception, one related to PROP status and papillae density, and another related to *CD36* genotypes. Undoubtedly, other gene effects play a role as well. Further investigation of these mechanisms using both electrophysiological and psychophysical methods could shed important light on how dietary fats are perceived and their contribution to food choice and nutritional status.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/2/315/s1, Table S1: Ratings of perceived taste intensity in response to three concentrations of PROP and NaCl in the taster groups. Figure S1: Box-and-whisker plots showing the minimum, first quartile, median, third quartile, and maximum of each set data of amplitude and rate of signals evoked by oleic acid (30 μL) taste stimulation in individuals with genotypes PAV/PAV (n = 7), PAV/AVI (n = 16), AVI/AVI (n = 10) and rare genotypes (n = 2) of *TAS2R38*. One-way ANOVA showed no difference of amplitude and hyperpolarization rate related to *TAS2R38* genotype ($p > 0.05$). Figure S2: Distribution of data of the perceived intensity after taste stimulation with oleic acid (30 μL) in super-tasters (ST; n = 10), medium-tasters (MT; n = 13) and non-tasters (NT; n = 12) and in volunteers with genotypes GG (n = 9), GA (n = 20) and AA (n = 6) of *CD36*. Mean values ± SEM are also shown. Different letters indicate a significant difference ($p \leq 0.046$; Duncan's test and $p = 0.047$ Fisher LDS test, subsequent

one-way ANOVA). Figure S3: Distribution of data of the density of fungiform papillae in super-tasters (ST; $n = 10$), medium-tasters (MT; $n = 13$) and non-tasters (NT; $n = 12$) and in volunteers with genotypes GG ($n = 9$), GA ($n = 20$) and AA ($n = 6$) of *CD36*. Mean values ± SEM are also shown. Different letters indicate a significant difference ($p \leq 0.032$; Duncan's test subsequent one-way ANOVA). Figure S4: Distribution of data of amplitude and rate of signals evoked in super-tasters (ST; $n = 10$), medium-tasters (MT; $n = 13$) and non-tasters (NT; $n = 12$) and in volunteers with genotypes GG ($n = 9$), GA ($n = 20$) and AA ($n = 6$) of *CD36*. Mean values ± SEM are also shown. Different letters indicate a significant difference ($p \leq 0.05$; Fisher LDS or Duncan's test subsequent one-way ANOVA).

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Fatty Acid Lingual Application Activates Gustatory and Reward Brain Circuits in the Mouse

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Abstract: The origin of spontaneous preference for dietary lipids in humans and rodents is debated, though recent compelling evidence has shown the existence of fat taste that might be considered a sixth taste quality. We investigated the implication of gustatory and reward brain circuits, triggered by linoleic acid (LA), a long-chain fatty acid. The LA was applied onto the circumvallate papillae for 30 min in conscious C57BL/6J mice, and neuronal activation was assessed using c-Fos immunohistochemistry. By using real-time reverse transcription polymerase chain reaction (RT-qPCR), we also studied the expression of mRNA encoding brain-derived neurotrophic factor (BDNF), Zif-268, and Glut-1 in some brain areas of these animals. LA induced a significant increase in c-Fos expression in the nucleus of solitary tract (NST), parabrachial nucleus (PBN), and ventroposterior medialis parvocellularis (VPMPC) of the thalamus, which are the regions known to be activated by gustatory signals. LA also triggered c-Fos expression in the central amygdala and ventral tegmental area (VTA), involved in food reward, in conjunction with emotional traits. Interestingly, we noticed a high expression of BDNF, Zif-268, and Glut-1 mRNA in the arcuate nucleus (Arc) and hippocampus (Hipp), where neuronal activation leads to memory formation. Our study demonstrates that oral lipid taste perception might trigger the activation of canonical gustatory and reward pathways.

Keywords: linoleic acid; gustation; hedonic; BDNF; fat taste; c-Fos; Zif-268; Glut-1

1. Introduction

Taste modality serves as an important factor for food choice and for appreciating its hedonic value [1]. There are five basic taste qualities known hitherto in rodents and humans: sweet, sour, bitter, salty, and umami [2]. The specific receptors and cells for each of the five basic taste modalities have been identified and characterized [3]. The series of events that occur before and after the ingestion of food, leading to taste perception and preference, are a topic of wide interest. Recently, convincing evidence has started to accumulate in favor of fat as the sixth fat taste quality in rodents and humans [4]. The two principal receptors of fat taste, CD36 and GPR120, have finally been identified in human taste bud cells, and their sensitivity to fatty acid stimuli has been shown to be altered in obesity [5]. The cellular and molecular mechanisms of fat taste perception have recently been elucidated [4,6].

There are a few studies that have shed light on fat-eating behavior and brain activity. Our laboratory has demonstrated that the addition of a fatty acid on mouse tongues induced the expression of c-Fos immunoreactivity in the nucleus of the solitary tract (NST), the first gustatory relay in the brain [7]. We did not address the question of whether other parts of the brain are also activated during this experimental approach, though several investigators, by employing different methods, have concluded that the primary taste cortex, orbitofrontal cortex, and amygdala are activated by the perception of dietary lipids [8–11]. Tzieropoulos et al. (2013) reported that dietary fat was able to induce sustained reward response in human brains. Eldeghaidy et al. [12] used functional magnetic resonance imaging (fMRI) in human subjects, and suggested that taste, appetite, and reward-related brain areas were responsive to nutritional status and received sensory and interoceptive signals of motivation and hedonic value in response to a fat-rich diet. Other fMRI studies in humans have also demonstrated that administration of dietary lipids activates cerebral taste, texture, and reward areas [13–16].

The abovementioned studies show that different brain areas might be activated by dietary fat; however, there is a dearth of information on the identification of sequential activation of cerebral areas/pathways that are activated in response to taste bud stimulation by dietary fat prior to ingestion of the bolus. Information on this subject would be crucial not only to better understand the fundamental mechanisms of fat intake and its related addiction, but also to modulate fat-eating behavior that is altered in obese subjects [17]. Keeping this argument in view, we designed the present study wherein we added linoleic acid (LA), a long-chain fatty acid, on the circumvallate papillae, which are rich in fat taste receptors, of conscious mice and assessed the neural activity using immunocytochemical localization of c-Fos protein in different brain areas. We also analyzed the mRNA expression of brain-derived neurotrophic factor (BDNF), involved in synaptic plasticity and memory processes, Zif-268, an immediate-early gene, and Glut-1, another marker of neuronal activation during enhanced glucose demand in three brain areas of these animals [18].

2. Materials and Methods

2.1. Animals and Experimental Set-Up

Experiments were carried out on 6–10-week-old C57BL/6J male mice (Janvier, Le Genest-St-Isle, Mayenne, France). Animals were group-housed under standard laboratory conditions (12 h:12 h, light/dark cycle; 22 ± 2 °C, 50–60% humidity) and fed with standard pelleted food (Scientific Animal Food & Engineering, Augy, France) and water ad libitum. All experiments were designed to minimize animal suffering and the number of animals. The protocols were approved by the regional ethical committee of Burgundy University in compliance with European guidelines for the use and care of laboratory animals.

In the first set of experiments, the neuronal activation along the canonical gustatory pathway was systematically assessed after oral lipid stimulation using c-Fos immunohistochemistry. In order to avoid any stressful situation, the mice were accustomed to gentle handling for 5 days in order to apply the fatty acid. On the 6th day, they were gently handled similarly and linoleic acid (LA, 18:2 n-6) (Sigma-Aldrich, St. Louis, MO, USA) at 50 µM in 70 µl (*w*/*v*) was slowly placed with the help of a spatula for 30 min on the circumvallate papillae. Xanthan gum (0.3% *w*/*v*, Sigma-Aldrich USA), which mimics lipid texture, was similarly applied onto the circumvallate papillae of control animals. At the end of oral stimulation, the animals were injected, intraperitoneally, with sodium pentobarbital (40 mg/kg), the thoracic cavity was opened, and mice were perfused intracardially with 50 mL of ice-cold saline (NaCl, 0.9%), followed by 50 ml of ice-cold 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. The entire brain was removed and post-fixed by incubation in 4% paraformaldehyde for 2 h. Samples were cryoprotected by overnight incubation in 30% sucrose in 0.1 M phosphate buffer. Brain samples were embedded in a Tissue-Tek®OCT compound (Sakura FineTek, Torrance, CA, USA), frozen on dry ice, and stored at −20 °C before c-Fos immunohistochemical processing.

In the second set of experiments, using real-time reverse transcription polymerase chain reaction (RT-qPCR), we investigated the downstream molecular pathways/candidates in NTS, arcuate nucleus, and hippocampus at mRNA level. The total RNA from different brain areas was isolated by Trizol Reagent (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The quality of isolated RNA was determined using denaturing agarose gel electrophoresis. Then, the RNA was quantified by determining its UV absorbance at 260 nm. Five hundred nanograms of total RNA was reverse-transcribed with an iScript cDNA synthesis kit according to the manufacturer's instructions (Bio-Rad, Berkeley, CA, USA). RT-PCR was performed on the iCycler iQ real-time detection system, and amplification was undertaken using SYBR Green detection. Primers against the genes of interest were as follow: BDNF (forward 5′-TTGGATGCCGCAAACATGTC-3′; reverse, 5′-CTGCCGCTGTGACCCACTC-3′), Zif-268 (forward, 5′-GCGAACAACCCTATGAGC-3′; reverse, 5′-GGTCGGAGGATTGGTC-3′), Glut-1 (forward, 5′-GCTGTGCTTATGGGCTTCTC-3′; reverse, 5′-CACATACATGGGCACAAAGC-3′). The cycling conditions used were: 95 °C for 10 min; and 40 cycles of 95 °C for 15 s, 60 °C for 1 min. The amplification was carried out in a total volume of 20 µL, which contained 10.0 µL SYBR Green Supermix buffer (50 mMKCl; 20 mMTris-HCl (pH 8.4); 3 mM $MgCl_2$; 0.2 mM of each dNTP, 0.63 U iTaq DNA polymerase, and SYBR Green 1.0 nM fluorescein), 0.75 µL (0.3 mM) of each primer, and 1.5 µL diluted cDNA. Results were evaluated using iCycleriQ software, and a relative quantification of mRNA in different groups was determined. The relative amounts of RNA were normalized to the amount of the endogenous control 18S using StepOne software version 2.2-2010 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) and the Δ Ct method.

2.2. Immunohistochemistry

Fos Immunostaining

Fifty µm coronal sections were cut with a cryostat at −20 °C through the NST (nucleus of the solitary tract, bregma −0.48 to −7.08 mm), PBN (parabrachial nucleus, bregma −4.96 to −5.32 mm), VPM (ventral posteromedial thalamic nucleus, bregma −1.58 to −2.46 mm), VPMPC (ventroposterior medialis parvocellularis, bregma −1.94 to −2.30 mm), CeA (central amygdaloid nucleus, bregma −0.94 to −1.82 mm), PSTN/CbN (parasubthalamic nucleus/calbindin nucleus, bregma −2.06 to −2.54 mm), AI (agranular insular cortex, bregma −0.94 to +1.54 mm), GI/DI (granular/dysgranular insular cortex, bregma −0.94 to +1.54 mm), Hipp (hippocampus, bregma −1.22 to −2.18 mm), BLA (basolateral amygdaloid nucleus, bregma −1.70 to +1.82 mm), VTA (ventral tegmental area, bregma −2.92 to +3.88 mm), Acb (accumbens nucleus, bregma +1.70 to +0.98 mm), mPFC (medial prefrontal cortex, bregma +1.98 to +1.54 mm), Arc (arcuate nucleus, −1.46 to −2.46 mm caudal to bregma), and Hbn (habenula, bregma −1.22 to −2.18 mm), according to the atlas of Paxinos & Franklin (2001) [19]. Free-floating sections were then collected in 0.1 M phosphate buffer, pH 7.4, and processed for c-Fos immunohistochemistry. Sections were incubated overnight with rabbit anti-c-Fos (1:20,000, Calbiochem®, Paris, France) primary antibodies diluted in 0.1 M phosphate buffer at pH 7.4, containing 0.3% Triton X100 and 3% normal goat serum (*v*/*v*). Subsequently, sections were incubated for 2 h at room temperature with the biotinylated goat antirabbit secondary antibodies (1:4000, Vector laboratories, Burlingame, CA, USA). The formed antigen–antibody complexes were visualized through the avidin–biotin–horseradish peroxidase procedure (Vectastain Elite ABC kit; Vector Laboratories, USA), using 3,3′-diaminobenzidine (0.04%) as the chromogen. Sections were mounted on gelatin-coated slides, dehydrated, and coverslipped with DePeX (VWR International Ltd., Poole, UK) mountant.

2.3. Quantification of c-Fos Immunopositive Neurons

Sections were analyzed under a 40× objective of a light-optical microscope (Nikon Eclipse E600, Nikon Instruments Inc., Melville, NY, USA) equipped with a digital camera (Nikon Digital

Sight DS-Fi1). c-Fos immunoreactive nuclei were quantified on photomicrographs of the regions of interest (ROI) using the imaging software Image J (National Institutes of Health, Bethesda, MD, USA). A cell was considered as labeled (positive) for c-Fos when the brown-black DAB-staining was unambiguously darker than the background, and this included all cells from low to high intensities of staining. Thresholds over the background section and the size of the particles were determined by the experimenter. The entire region for each area was traced, and mean densities of c-Fos-immunopositive neurons (number of c-Fos positive cells/mm^2) for each ROI were calculated according to their respective areas.

2.4. Statistical Analysis

Data are presented as means ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism version 5.00 for Windows (GraphPad Software, La Jolla, CA, USA). For the immunohistochemical experiment, the Kolmogorov–Smirnov test revealed parametric (normal) distribution for the c-Fos data for most brain regions. Therefore, differences between groups were assessed using unpaired one-sided *t*-tests. The level of significance was set at $p < 0.05$.

3. Results

3.1. Lingual LA Stimulation Triggers Neuronal Activation of the Canonical Central Cerebral Gustatory Reward Pathway

Figure 1A shows the c-Fos expression in the NST, arcuate nucleus (Arc), and hippocampus (Hipp) in LA-stimulated and control mice. Quantitative analysis of the immunohistochemical data revealed that oral LA application produced a robust increase in c-Fos expression in most brain regions explored along the putative pathway for gustatory lipid perception (Figures 1A,B and 2). More precisely, unpaired one-sided *t*-tests show a significant effect of the treatment in the NST ($t(10) = 2.252$; $p = 0.0240$), PBN ($t(9) = 3.992$; $p = 0.0016$), VPMPC ($t(9) = 3.153$; $p = 0.0058$), CeA ($t(10) = 2.380$; $p = 0.0193$), PSTN/CbN ($t(9) = 3.037$; $p = 0.0070$), and VTA ($t(10) = 2.309$; $p = 0.0218$), but the differences did not reach statistical significance either at the cortical level (AI ($t(10) = 0.3142$; $p = 0.3799$), GI/DI ($t(10) = 0.05505$; $p = 0.4786$), mPFC ($t(10) = 0.8558$; $p = 0.2061$) and HIPP ($t(9) = 0.3831$; $p = 0.3553$)) or in the non-gustatory-related thalamus VPM($t(9) = 1.377$; $p = 0.1009$), BLA ($t(10) = 0.2419$; $p = 0.4069$), Acb ($t(10) = 0.06695$; $p = 0.4740$), Hbn ($t(10) = 0.4021$; $p = 0.3480$) or Arc ($t(9) = 1.052$; $p = 0.1602$) (Figures 1A,B and 2).

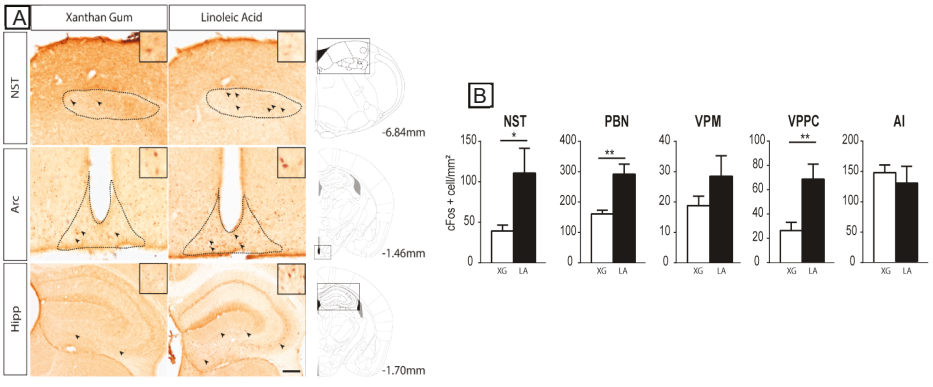

Figure 1. Linoleic acid deposition on the tongue induces c-Fos expression in the major cerebral structures of the canonical gustatory pathway. (**A**) Typical photomicrographs of the NST, Arc, and Hipp, showing c-Fos immunoreactivity in mice subjected to oral stimulation with linoleic acid or xanthan gum (XG, 0.3%, *w*/*v*) to mimic the texture of lipids. The dotted lines circumscribe the regions

of interest. Arrowheads point to representative c-Fos immunopositive nuclei. The boxes show higher magnification (×4) of representative c-Fos immunopositive nuclei. Scale bar, 100 µm. The square windows indicate the area shown in the photomicrographs. (**B**) Bar graph representation of the density of c-Fos immunopositive cells (number of c-Fos positive cells/mm^2) in mice subjected to oral stimulation with linoleic acid (LA) or xanthan gum (XG). Values are means ± standard error of the mean (SEM); $n = 6$; for each structure studied, treatment effects on c-Fos expression were assessed using unpaired one-sided *t*-tests. *, $p < 0.05$; **, $p < 0.01$. AI, agranular insular cortex; Arc, arcuate nucleus; Hipp, hippocampus; NST, nucleus of the solitary tract; PBN, parabrachial nucleus; VPM, ventral posteromedial thalamic nucleus; VPMPC, ventroposterior medialis parvocellularis.

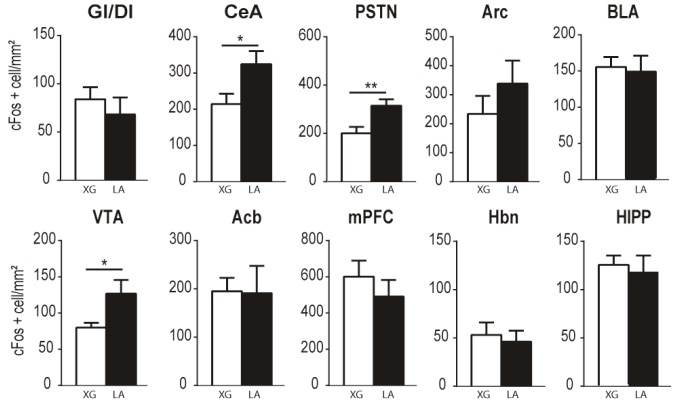

Figure 2. Linoleic acid deposition on the tongue activates c-Fos expression in the major cerebral structures related to emotional and reward traits. Bar graph representation of the density of c-Fos immunopositive cells (number of c-Fos positive cells/mm^2) in mice subjected to oral stimulation with linoleic acid (LA) or xanthan gum as control solution. Values are means ± SEM; $n = 6$; for each structure studied, treatment effects on c-Fos expression were assessed using unpaired one-sided *t*-tests. * $p < 0.05$; ** $p < 0.01$. Acb, accumbens nucleus; Arc, arcuate nucleus; BLA, basolateral amygdaloid nucleus; CeA, central amygdaloid nucleus; GI/DI, granular/dysgranular insular cortex; Hbn, habenula; Hipp, hippocampus; mPFC, medial prefrontal cortex; PSTN/CbN, parasubthalamic nucleus/calbindin nucleus; VTA, ventral tegmental area.

3.2. Lingual LA Stimulation Modulates the Expression of mRNA Encoding BDNF, Zif-268 and Glut-1

The RT-qPCR results show that the relative expression of Zif-268 mRNA was significantly increased ($p < 0.001$) in the NST, arcuate nucleus, and hippocampus by lingual application of LA in mouse brains (Figure 3A). To our surprise, LA induced a nearly fivefold higher increase in Zif-268 mRNA in the NST than controls. LA also resulted in a significantly higher increase ($p < 0.001$) in Glut-1 mRNA expression than control in the nucleus of solitary tract (NST), arcuate nucleus (Arc), and hippocampus (hipp) (Figure 3B). Hence, the increase in Glut-1 mRNA expression was more evident (about a threefold increase) in the NST and hippocampus, as compared to the control solution, after lingual application of LA. The BDNF mRNA expression was increased in the arcuate nucleus and hippocampus, but not in the NTS, after fatty acid lingual application (Figure 3C).

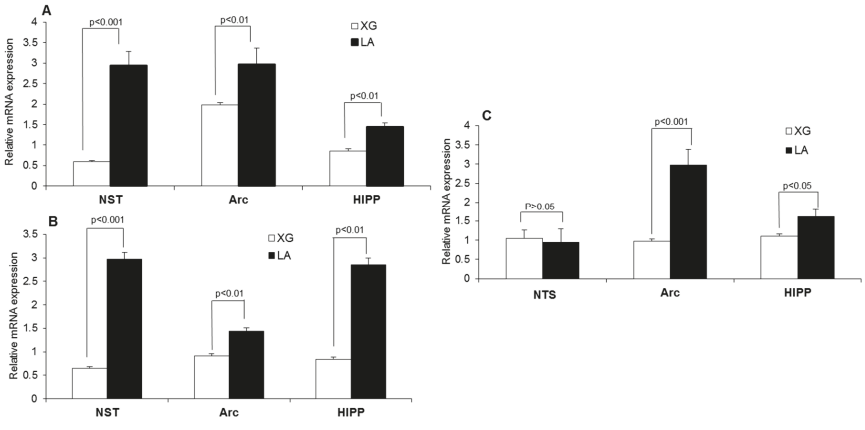

Figure 3. Lingual application of LA modulates brain-derived neurotrophic factor (BDNF), Zif-268, and Glut-1 mRNA expression in the mouse brain. Bar graphs represent the relative increase in mRNA expression (Zif-268 in (**A**), Glut-1 in (**B**), BDNF in (**C**)) in mice subjected to oral stimulation with linoleic acid (LA) or xanthan gum as control solution. Values are means ± SEM; $n = 6$; for each structure studied, treatment effects on Zif-268 and Glut-1 mRNA expression were assessed using unpaired one-sided *t*-tests. NST, nucleus of solitary tract; Arc, arcuate nucleus; Hipp, hippocampus.

4. Discussion

Gene transcription during memory consolidation is a very dynamic and complex process, depending on the type of learning involved. Hence, many types of mRNAs are transcribed, such as for the transcription factors, c-Fos, Zif-268, and the effector genes, like BDNF [20]. The c-Fos, both at mRNA and protein levels, is generally among the first to be expressed and, therefore, referred to as an immediate early gene (IEG) and considered to serve as a marker of the neuronal activity in the neuroendocrine systems [21].

Central gustatory pathways have been well studied in murine models [22]. Branches of the facial (chorda tympani and greater superficial petrosal), glossopharyngeal, and vagus (superior laryngeal) nerves, which establish synaptic contacts with receptor cells in the taste buds, convey taste messages to the first relay nucleus, that is, the rostral part of the nucleus of the solitary tract (NST). The second relay nucleus for ascending taste inputs is the parabrachial nucleus (PBN) of the pons. The third relay station is the ventroposterior medialis parvocellularis (VPMPC) of the thalamus. This thalamic nucleus sends taste information to the insular cortex (IC) [22]. In most of the experiments/observations reported so far, the prominent neurochemical changes in the brain areas take place immediately following training, but in some instances, there are waves from 3 h to 6 h and/or at 24 h following training. In the present study, we were interested in elucidating the early brain responses to short-term application, that is, 30 min, of a long-chain fatty acid, that is, linoleic acid (LA). We applied LA onto the circumvallate papillae as they have been reported to contain more CD36, a lipid receptor, than fungiform papillae [23]. Our results demonstrate that acute lingual application of LA resulted in a significant increase in c-Fos expression in the NST, PBN, and VPMPC in the mouse brain. However, the increase in c-Fos expression in VPM was not significantly higher in fatty-acid-treated mice than control animals, suggesting that VPMPC, but not VPM, is involved in the transfer of lipid taste messages from the PBN to the insular cortical areas (AI, GI/DI), though LA failed to induce c-Fos expression in latter areas of the brain.

The ventral tegmental area (VTA), nucleus accumbens (NAcb), and ventral pallidum (situated between the NAcb and lateral hypothalamus) are the essential components of the brain reward system [24]. Acute application of LA resulted in a significant increase in c-Fos expression in the VTA, which constitutes the mesolimbic dopaminergic system. However, no significant difference in c-Fos expression was observed in the NAcb after the application of the fatty acid. Our observations can be

supported by the study of Dela Cruz et al. [25], who have shown that fat intake is associated with the activation of the VTA in the mouse brain [25]. Reward is closely related to hedonic stimuli and our results, showing c-Fos activation in the CEA and PSTN, support previous reports describing the activation of the PSTN, the major target for projection from the CeA [26] in response to hedonic taste [27].

In order to correlate c-Fos findings, we further quantified the mRNA expression of BDNF, Zif-268, and Glut-1 in three areas of the brain, that is, the NTS, arcuate nucleus, and hippocampus, as peripheral signals go through the NTS and arcuate nucleus to the hippocampus, involved in multimodal learning and memory. BDNF is a small dimeric protein that is widely expressed in the adult mammalian brain and extensively involved in synaptic plasticity and memory processes [28]. We observed that BDNF mRNA was highly induced in the arcuate nucleus and hippocampus, but not in the NTS. In fact, this growth factor is more involved in learning and memory rather than the transfer of peripheral signals, as is the case of the NTS. Alonso et al. [29] have demonstrated that BDNF is involved not only in memory consolidation, but also in long-term memory formation in the CA1 region of the hippocampus. Genoud et al. [30] have clearly shown that BDNF is mandatory to induce formation of activity-dependent synapses in cerebral cortex. However, there are regional and task-dependent differences underlying differential mechanisms of BDNF and its receptor function [28].

Zif-268 belongs to the regulatory transcription factor family, responsible for inducing transcription of late-response genes [31]. Induction of memory in the hippocampus has been shown to increase the expression of Zif-268 mRNA, and *Zif-268* knock-out mice had deficits in long-term memory for socially transmitted food preference and object recognition [32]. As far as Glut-1 is concerned, we would like to state that glucose is an important source of energy for the brain and glucose transporters (Glut) enable passage of glucose across both the endothelial cells of the blood–brain barrier and the plasma membranes of neurons and glia [33]. Glut-1 is present at high levels in brain endothelial cells [34]. We observed that lingual application of LA resulted in a significantly increased expression of Zif-268 and Glut-1 mRNA in the NST and arcuate nucleus, as well as in the hippocampus. Surprisingly, the number of c-Fos immunopositive neurons was not significantly increased in the arcuate nucleus and hippocampus by acute application of LA. We would like to mention that, under some conditions, the concomitant activation of these two markers (c-Fos and Zif-268) is not seen. Barbosa et al. [35] have shown that Zif-268 was increased in the dorsal CA1 region of the hippocampus, while there was no c-Fos activation in the experiments conducted to assess episodic-like memory in rats. Furthermore, we elucidated the Zif-268 expression in the whole hippocampus, and it is possible that, in different subregions, there might be a differential expression of Zif-268 mRNA. It is also worth mentioning that c-Fos is well correlated with neuronal activity, whereas Zif-268 is more related to memorization mechanisms, such as long-term potentiation [36,37]. As far as Glut-1 is concerned, we would like to state that sometimes there is no direct correlation between Glut-1 and c-Fos expression. Glut-1 is activated immediately as per energetic demand of the cells, whereas c-Fos might be activated at a later stage. Indeed, Hauguel-de Mouzon et al. [38] have shown that Glut1 mRNA, but not c-Fos levels, is subjected to the variations in glucose concentrations in human placental cells, and this differential regulation of Glut1 and c-Fos genes could be relevant to, respectively, metabolic and mitogenic pathways. In fact, high glucose concentrations are supposed to upregulate c-Fos expression [39] and downregulate Glut-1 levels [36]. It is possible that, in response to lipid gustatory information coming from tongue to brain, the hippocampus is in high requirement of glucose due to high glucose utilization, and this would result in high GLUT-1 and low c-Fos mRNA expression in this region of the brain.

On the basis of our observations, we provide a schematic representation of the gustatory pathway, depicting the major central synaptic relays and its connections with structures involved in metabolic, reward, learning, and memory processes in response to fatty acid stimulation (Figure 4). Thus, lipid taste perception relies on systematic activation of the major cerebral structures of the canonical gustatory pathway, ranging from the first central synaptic relay NST in the brain stem to the PBN,

reaching the gustatory part of the thalamus (VPMPC), up to the gustatory insular cortical areas (AI, GI/DI) with modulatory influences of the central amygdaloid nucleus (CeA) and the posterior part of the lateral hypothalamus, that is, the parasubthalamic nucleus/calbindin nucleus (PSTN/CbN). It is worth noting that, at this early stage of lipid oral stimulation, the reward circuit is already involved, mainly through the VTA. It is not well understood, in the present study, how the arcuate nucleus (Arc), which is sensitive to peripheral postingestive signals, is activated (as evidenced by high BDNF, Zif-268 and Glut-1 mRNA levels). Indeed, the feeding behavior is also regulated by circulating hormonal signals, released by nutrients in the gut, such as cholecystokinin (CCK) and glucagon-like-peptide 1 (GLP-1), released as a result of postingestive/postoral activation of the gastrointestinal tract. Further physiological studies are required to assess the effects of these and other circulating factors that might regulate fat-eating behavior either via the arcuate nucleus or via the vagal nerve X. Nonetheless, ours is the first report to demonstrate that the application of a long-chain fatty acid like linoleic acid would activate a long chain of events in the conscious brain of the mouse.

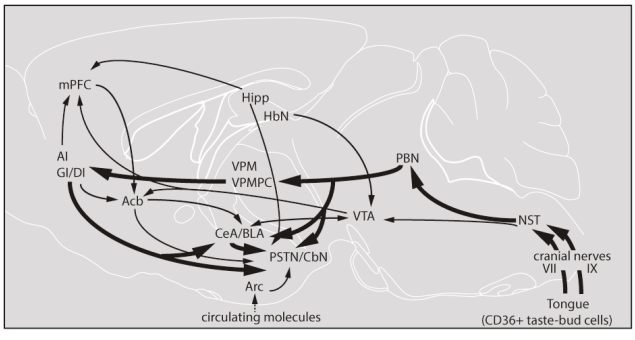

Figure 4. Schematic representation of the gustatory pathway, depicting the major central synaptic relays and their connections with structures involved in metabolic, reward and learning, and memory processes. The lingual application of a long-chain fatty acid will trigger signaling events via CD36, localized in the circumvallate papillae. The gustatory information on dietary lipids will be conveyed to the NST via cranial nerves VII and IX. The NST that serves as the relay structure of the peripheral information will send the gustatory information to different brain areas, as mentioned in the Discussion section. Acb, accumbens nucleus; AI, agranular insular cortex; Arc, arcuate nucleus; BLA, basolateral amygdaloid nucleus; CeA, central amygdaloid nucleus; GI/DI, granular/dysgranular insular cortex; Hbn, habenula; Hipp, hippocampus; mPFC, medial prefrontal cortex; NST, nucleus of the solitary tract; PBN, parabrachial nucleus; PSTN/CbN, parasubthalamic nucleus/calbindin nucleus; VPM, ventral posteromedial thalamic nucleus; VPMPC, ventroposterior medialis parvocellularis; VTA, ventral tegmental area (adapted from Reference [27]).

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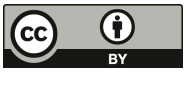

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Review

Umami as an 'Alimentary' Taste. A New Perspective on Taste Classification

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Abstract: Applied taste research is increasingly focusing on the relationship with diet and health, and understanding the role the sense of taste plays in encouraging or discouraging consumption. The concept of basic tastes dates as far back 3000 years, where perception dominated classification with sweet, sour, salty, and bitter consistently featuring on basic taste lists throughout history. Advances in molecular biology and the recent discovery of taste receptors and ligands has increased the basic taste list to include umami and fat taste. There is potential for a plethora of other new basic tastes pending the discovery of taste receptors and ligands. Due to the possibility for an ever-growing list of basic tastes it is pertinent to critically evaluate whether new tastes, including umami, are suitably positioned with the four classic basic tastes (sweet, sour, salty, and bitter). The review critically examines the evidence that umami, and by inference other new tastes, fulfils the criteria for a basic taste, and proposes a subclass named 'alimentary' for tastes not meeting basic criteria.

Keywords: basic tastes; taste; taste reception; umami

1. Introduction

The relationship between individual variation in taste perception, food choice and intake, and ultimately diet related disease, provides a framework for applied taste research. A taste perception arises from the interaction of non-volatile, saliva soluble chemicals with taste receptors on the tongue within the oral cavity. This interaction initiates a signal transduction to processing regions of the brain, resulting in the formation of a taste perception. The taste perception formed could include the perception of one of the basic tastes: sweet, sour, salty, bitter, umami [1], or fat [2]; or a perception of other putative taste qualities including, but not limited to, kokumi (rich, mouthful, thick, delicious taste) [3,4], carbohydrate [5], calcium [6], or metallic tastes [7]. Detection and perception of basic tastes is hypothesised to exist for species' survival throughout evolution to prevent the consumption of potential noxious food, and promote consumption of nutritious food, a nutrient–toxin detection system [8–11].

Basic tastes have perceptual independence, that is, they do not elicit a taste perception similar to that of any other basic tastes and cannot be produced from a combination of other tastes [12–14]. The concept of basic tastes dates back as far 384–322 B.C. when Aristotle originally listed the seven tastes he proposed as basics, these included sweet, sour, salty, and bitter as well as astringent, pungent, and harsh [15]. Throughout history the lists of tastes have been extended, or reduced, depending on the prevailing thought of the time, with the only consistency being the inclusion of sweet, sour, salty, and bitter in basic taste lists [15]. Research during the 1800's separated olfaction and tactile perceptions from tastes. The development and advancement of science and technology, including psychophysical testing led to sweet, sour, salty, and bitter tastes being confirmed as basics as evidenced throughout published literature [16].

Advances in molecular biology, and the recent discovery of taste receptors and ligands has increased the basic taste list to include umami and fat taste [17–20]. The existence of specific taste receptors responsive to a single compound that elicits a taste is often suggested as a key piece of evidence for basic tastes classification, with literature citing this as the key evidence in the case of umami [14]. Due to these recent advances, there is the potential for a plethora of new tastes to be discovered, and potentially classified as a basic taste if receptors on taste cells are found to respond to ligands. The current possibilities include, but are certainly not limited to kokumi, carbohydrate, calcium, and metallic tastes.

Thus, due to advances in knowledge, and the possibility for an ever-growing list of basic tastes, it is pertinent to critically evaluate whether new tastes, including umami, are suitably positioned with the four classic basic tastes (sweet, sour, salty, and bitter). This is of importance for three predominant reasons, first, there is historical and academic relevance to determine whether umami, and by inference other new tastes, should in-fact be considered in the same category as sweet, sour, salty, and bitter. Second, understanding individual variation in taste perception, perceptual associations with other basic tastes, and physiological responses resulting from detection of specific tastants, may enhance our understanding of the complex relationship between taste, dietary choice and intake, and diet related health related outcomes. Third, the classic basic tastes have significant immediate influence on whether to swallow or not swallow a potential food, while the more recent tastes such as umami and fat may have more post-ingestive relevance and determine extent of consumption and ultimately health. The review that follows will critically evaluate the evidence that umami fulfils criteria for classification as a basic taste.

2. Basic Taste Criteria

Criteria that a stimulus must fulfil for it to be classified as a basic taste has been proposed, although these criteria have not been consistent [12,13]. Kurihara and Kashiwayanagi (2000) suggested that for a compound to be considered a basic taste it should fulfil the following criteria. The proposed basic taste is (1) different to any other basic taste; (2) not replicated by combining other basic tastes and; (3) a taste which is commonly consumed and induced by common components of food [13]. The requirement for a basic taste to have an identified receptor was recently added to this set of criteria [14]. The argument could then be put forward that the discovery of a receptor-ligand complex alone is not reason to justify a taste as a basic taste. Whether the detection of the stimulus from that receptor is transduced and forms a unique perceptible experience may be of higher importance. That is, if a taste receptor is identified, but no unique perceptible experience occurs from the activation of that receptor, then is it appropriate to classify the stimulus as eliciting a basic taste?

A more comprehensive set of criteria have been outlined, covering both unique effective stimuli, transduction (receptors), neurotransmission, and finally, perception [12]. These criteria have been used previously to investigate the appropriateness of other new tastes, specifically fat taste; in this review the following criteria will be used to specifically investigate umami as a basic taste [10,12]. We extend this criterion to involve hedonic responses occurring from tasting the effective stimuli. The criteria are as follows:

1. A distinct class of effective stimuli must exist.
2. Detection of effective stimuli must have an evolutionary benefit.
3. Transduction mechanisms that can convert the chemical code of the effective stimuli into an electrical signal, including receptors, must exist.
4. Neurotransmission of this electrical signal to taste processing regions of the brain must occur.
5. Perceptual quality arising from this processing must be independent from other taste qualities.
6. Hedonic responses occur from taste perception.
7. Physiological and/or behavioural responses must occur following the activation of taste bud cells by the effective stimuli.

3. Umami Taste and Unique Class of Umami Effective Stimuli

Umami was initially discovered by Ikeda who isolated glutamic acid from kombu (seaweed), finding that the salts of glutamic acid, particularly the sodium salt, monosodium glutamate (MSG), gave the seaweed its specific flavour (translated in [21]). Thus, free L-glutamate (glutamic acid) is the predominant umami effective stimuli, and MSG is the predominant prototypical umami stimuli used in psychophysical testing. It was later discovered that the taste of L-glutamate could be synergistically increased through the addition of disodium 5'ribonucleic acids, specifically disodium 5'inosinate monophosphate (IMP) and disodium 5'guanylate monophosphate (GMP) [22]. When tasted in isolation IMP elicits a minimal to weak umami taste hypothesised to occur due to the interaction of IMP with subthreshold concentrations of L-glutamate in humans' saliva, demonstrating that IMP and GMP require L-glutamate for an umami taste perception to occur [23]. In human psychophysical studies, the addition of 0.5 mM IMP significantly reduces the concentration of MSG required for participants to reach RT (7.66 mM and 0.20 mM MSG respectively) due to the taste potentiation produced when IMP is applied with MSG [24].

Free L-glutamate is naturally present in high concentrations in a wide variety of foods including certain vegetables (and fruits i.e., tomato), seaweeds, aged cheese, seafood, fish and soy sauce, egg yolks, and human breast milk [14,25–27]. Whereas, IMP is found predominately in animal products such as chicken, pork, beef, and tuna, and GMP in dried mushrooms such as shitake [14,25,26]. The curing, ageing, heat treatment, and fermenting of certain foods results in an increase in free amino acids, including L-glutamate, and often an increase in umami potentiating ribonucleotides (IMP and/or GMP) [14,26]. Specifically, in animal products such as beef, pork, chicken, and fish that contain high concentrations of protein, which is essentially tasteless, proteolysis occurring from fermentation, curing, or heat treatment releases a complex mixture of amino acids, including L-glutamate. As these animal products naturally contain high concentrations of IMP the umami taste potentiation between IMP and L-glutamate can occur [14,26]. For example, during the process of ageing beef the concentration of free L-glutamate has been shown to increase by approximately 33% over eight days, and when this is combined with naturally present IMP, umami taste potentiation can occur [26].

The increase of tastants occurring from fermenting, curing, heat treatment or ageing is not unique to specifically umami taste quality, with kokumi taste (rich, mouthful, thick, delicious taste) peptides similarly increasing through these same processes. Kokumi tasting compounds include certain γ-glutamyl peptides and during the ageing of dairy products [28] and in fermented products including soy or fish sauce [4,29] the concentrations of γ-glutamyl peptides increases. This ageing/fermenting process results in an accumulation of peptides including γ-glutamic acid that forms a peptide bond with an amino group of a non-polar amino acid [30]. Additionally, glutathione (GSH) a tripeptide made up of glutamic acid, cysteine, and glycine, which elicits a kokumi taste, similarly increases in concentration through fermentation of certain foods. Kokumi stimuli including GSH, similar to IMP, elicit little taste in isolation, but when combined with an umami solution enhances the mouthful, continuity, and thickness, thus enhancing the kokumi aspects of the umami solution [4]. Similar to umami effective stimuli, GSH and other γ-glutamyl peptides are common in a number of high protein foods, such as beef, chicken, and ham, and in low protein foods such as tomato juice, and red wine, at concentrations above GSH DTs [4]. This suggests that there is an association between the effective stimuli eliciting both umami and kokumi tastes in food, namely the involvement of glutamic acid derivatives in both umami and kokumi effective stimuli [30].

4. Umami Taste from an Evolutionary Perspective

In humans, the ability to detect chemicals in the oral cavity prior to ingestion, and interpret salient perceptions of sweet, sour, salty, and bitter allows for rapid evaluation of a food, identifying whether it is acceptable (swallow), or unacceptable and potentially harmful (expectorate), which was essential for species survival [11,31–33]. Sweet taste is stimulated by simple carbohydrates, believed to indicate the presence of readily usable energy, eliciting appetitive hedonic responses

and thereby encouraging consumption [11,31]. Similarly, the detection of complex carbohydrates may also encourage consumption by signaling information regarding the carbohydrate and energy content of food [20]. Excess bitterness indicates the presence of potential toxins within food, ultimately encouraging rejection of the food [8,11,31]. Excess sourness can indicate off or spoilt foods, and is avoided to ensure the body's acid–base balance is maintained [8,31]. Salt taste perception is posited to be for maintenance of the body's electrolyte balance [9], for example, at high concentrations salt taste may play a role in the immediate analysis of whether to swallow or expectorate food, perhaps to avoid acute disturbance in the body's osmotic balance [11]. The ability to detect fat taste may be less important for the rapid evaluation of food and more closely related to activating physiological responses related to digestion and food intake regulation [18,34].

Umami taste has previously been hypothesised to signal the presence of amino acids and protein, promoting consumption of certain protein containing foods. Conversely, many foods naturally high in free L-glutamate are not typically high in protein, for example, peas, corn, red grapes, and tomatoes [14,35]. Along the same line, high protein foods, including beef, pork, and chicken do not contain high concentrations of free L-glutamate [26]. As previously mentioned, protein is essentially tasteless, it is the proteolysis of protein within these foods occurring from fermentation, curing, or heat treatment, that releases amino acids and peptides that can stimulate taste responses. Thus, umami taste perception may indicate the presence of accessible, rather than protein bound amino acids, in foods that have been released during proteolysis occurring through various cooking processes [11]. During proteolysis it is important to consider that the amino acids released are not solely umami tasting (L-glutamate), the release of bitter tasting (i.e., L-Leucine, -Phenylalanine, -Tryptophan) and sweet tasting (i.e., L-Glycine, -Alanine, -Proline) amino acids also occurs in different concentrations depending on the specific food [36]. Thus raising the question of whether it is appropriate to designate the evolutionary purpose of umami taste perception to signal protein content of food, when proteolysis in certain foods results in a complex mixture of taste active amino acids, including sweet and bitter tasting amino acids.

Following on from this, nutritional status, specifically protein-calorie deficiency does not appear to feedback onto preferences for umami tasting stimuli, as both malnourished (protein-calorie malnourished) and healthy infants showed preference for soup containing MSG to the same soup without MSG [37,38]. When the soup was provided in combination with casein hydrolysate, which contains a mixture of amino acids where a bitter taste dominates, the malnourished infants preferred the casein hydrolysate soup whereas the healthy infants did not [38]. Protein deficiency in infants increases consumption of protein containing food independent of the taste profile; the hypothesis that umami taste exists to signal the presence of protein is not supported.

Glutamate receptors (T1R1/T1R3 and mGluR1) exist throughout the gastrointestinal tract [39,40], and stimulation of these receptors has been suggested to affect nutrient absorption through regulating satiety hormones including cholecystokinin (CCK) [40–42]. Moreover, consumption of umami stimuli (MSG) appears to be involved in appetite stimulation and satiety regulation regardless of the macronutrient (i.e., protein and carbohydrate) consumed in human behavioural studies [43] (for further discussion please see section *Behavioural and physiological responses to umami effective stimuli*). Perhaps umami is less involved in the rapid analysis of food in the oral cavity, and more involved in increasing appetite to promote consumption, whilst simultaneously assisting in regulation of protein digestion through signaling mechanisms that promote gastric secretion.

5. Unique Receptor and Neural Transmission of Umami Effective Stimuli

5.1. Unique Receptors for L-glutamate

As previously discussed, taste receptors on the tongue detect saliva soluble, non-volatile chemicals from foods in the oral cavity. Of the basic tastes, sweet, bitter, and umami tastes are mediated via G-protein-coupled receptors, T1Rs and T2Rs, found in type II taste receptor cells [44]. Bitter ligands are

detected by T2R of which there are currently over 25 genes encoding the T2Rs [44,45]. Salty and sour taste have been suggested to be modulated by specialised ion channels. Salty taste has been proposed to involve the selective epithelial type sodium channel (ENaC), and putative sour taste receptors include H+ ions permeating type III sour sensing cells resulting in type III sour cells depolarising and reaching action potential [8,44,45], see Roper et al (2017) for a recent comprehensive review on taste receptor mechanisms.

Umami was widely accepted as a basic taste based on the discovery that the heterodimeric G-protein-coupled receptors, T1R1/T1R3 mediate umami taste detection [17,46,47]. The umami taste heterodimer complex, T1R1/T1R3, shares a common receptor subunit (T1R3) with sweet taste detected by the heterodimeric G-protein-coupled receptors, T1R2/T1R3 [9,44]. T1R1/T1R3 heterodimeric receptor is specific to detecting umami-tasting stimuli (L-amino acids), as it is non-responsive to sweet stimuli but responsive to umami stimuli (MSG and L-glutamate) *in vitro* [17,46]. T1R1/T1R3 was confirmed to respond to umami-tasting stimuli upon the discovery that T1R1, T1R3, and T1R1/T1R3 knockout mice lack, or have attenuated taste responses to umami stimuli (MSG) [47], and human T1R1/T1R3 receptors responded when L-glutamate was applied *in vitro* [46]. Although, studies have found in T1R1 and T1R3 knockout mice that a reduced, but not abolished, taste response to umami stimuli (MSG and MPG) occurs, indicating that other receptors responding to umami stimuli exist [48–51].

When investigating the umami taste synergism occurring from the mixing of IMP/GMP with MSG, T1R3 knockout mice had only moderately reduced taste responses, both neural and behavioural, although the contribution of Na+ was not eliminated in this study, so remaining taste responses in these knockout mice is likely due to the Na+ [48]. Zhao and colleagues showed, in independently generated T1R3 knockout mice, that when the contribution of Na+ was reduced with amiloride, the T1R3 knockout mice lacked responses to IMP with MSG, where responses in control mice remained, highlighting the importance of the T1R3 subunit in umami taste synergism [47]. Similarly, in T1R1 knockout mice the umami synergy when IMP was applied with MSG was abolished [52]. All of this shows that the T1R1/T1R3 umami receptors are important, if not essential, for the synergistic effect of IMP/GMP when applied with MSG, but for MSG in isolation an umami taste response, albeit reduced, remains in the absence of the T1R1/T1R3 umami receptors [53]. This suggests that additional receptors respond to umami taste stimuli, which was supported by studies finding putative umami receptors, metabotropic glutamate receptor 1 and 4 (mGluR1, mGluR4) were activated by concentrations of umami stimuli (MPG) commonly found in food in an *in vitro* assay [51], and mGluR4 knockout mice had reduced neural responses *in vivo* to umami stimuli (MPG) [54].

Finally, the discovery of single nucleotide polymorphisms on human TAS1R1, and TAS1R3 receptor genes, and their association with individual variation in umami (MSG, MPG, and MSG+IMP) taste perception phenotypes, provided further evidence for T1R1/T1R3 contributing to umami taste detection in humans [24,55,56].

5.2. Neural Responses to Umami Stimuli

When taste receptor cells detect chemicals in the oral cavity a neurotransmitter (ATP) is released onto afferent gustatory fibres, three predominant gustatory afferent nerves transmit information from taste buds to the brain [8]. The 7th cranial nerve, chorda tympani (CT), innervates the anterior two thirds of the tongue, and the 9th cranial nerve, glossopharyngeal (GL), innervates the posterior third, and the 10th cranial nerve, vagus nerve, similarly innervates the posterior of the tongue. The information transmitted for umami taste is then processed in the primary and secondary gustatory cortex [57].

Studies investigating responses of the CT in both wild-type mice (not genetically modified), and T1R3 knockout mice, have shown that there are two predominant fibre groups in the CT [58]. These fibres noted are sucrose best (S) and MPG best, or L-glutamate best (M) fibres, each of these fibres have sub-groups (S1, S2, and M1, M2) [58]. S1 and M1 show synergism between L-glutamate and IMP,

whereas S2 and M2 do not display this synergism [58]. In T1R3 knockout mice S1 fibres were lacking, and no synergistic effect between MPG and IMP was observed [58]. Similarly, whole CT responses in T1R3 knockout mice showed the synergism between IMP mixed with MSG is attenuated [48], or eliminated [47], demonstrating the importance of the T1R3 subunit for the synergistic effect between L-glutamate and IMP in the CT nerve. In response to MSG in isolation, T1R3 knockout mice showed reduced CT responses only at the highest MSG concentration [48]. This reduced response did not occur in the GL nerve, indicating that perhaps other receptors mediate umami responses from the GL nerve, for example, the mGluR4 receptor [48]. Supporting this, mGluR4 knockout mice displayed reduced responses to umami stimuli (MPG) in both the CT and GL nerves, these receptors may not be innervated by S1, or S2 fibres [54]. Whether the transduction of umami taste results in a uniquely perceptible experience will be discussed below.

6. Perceptual Independence of Umami Taste

Perception is input from the senses giving rise to a conscious experience of the particular stimulus [11]. Basic tastes should elicit perceptions independent to other basic tastes, and should not be produced by combination of existing basic tastes, or other sensory systems, such as the somatosensory system (i.e., mouthfeel or mouthfullness) [10,12]. The detection of a compound by taste receptors, and transduction to gustatory processing areas may produce a taste perception, but this taste perception may not always be a perceptually salient experience.

An important point to note regarding perceptual independence of umami is that the compound responsible, L-glutamate, is not used in the glutamic acid form as it is sour, so the sodium salt form is primarily used in psychophysical studies, meaning some potential overlap with salt taste (please see *Umami and salty* section).

Describing the perception arising from tasting umami effective stimuli becomes difficult due to the absence of a clear set of lexicon for describing umami, thus, whether umami is perceptually salient is not clear. Throughout the literature, a multitudinous lexicon has been used to describe umami taste, ranging from meaty, savoury, brothy, mouthfullness, and delicious [35,55,59]. Familiarisation or a learning effect for umami taste perception is not consistent within the literature, for example, a learning effect for umami hypotasters occurred after repeated exposure [60], contrary to this, umami taste sensitivity either increased or decreased depending on participants' age over repeated measures [61]. Using familiarisation or repeated exposure for improving perceptual salience of umami taste requires further research, as the current literature is inconclusive. The question that remains is whether a basic taste should require familiarisation for a perceptually salient experience to occur? Additionally, the common description of umami flavour as 'mouthfeel' [62] implies a tactile component to umami taste, similar to the description used for kokumi taste. Descriptions for umami taste cited within the literature are similar to those used to describe kokumi taste. Kokumi descriptions include deliciousness, rich, continuity, and mouthfullness [63]. Although kokumi is not a basic taste, there are similarities in both effective stimuli (glutamic acid derivatives) and descriptions of perceptual experiences arising from tasting both kokumi and umami stimuli suggests these taste qualities have perceptual similarities.

Considering L-glutamate is the predominant umami stimuli that is detected by glutamate receptors [17], it may be suitable to predict that foods containing high concentrations of free L-glutamate would ultimately lead to an experience that is perceived, and described, as umami or savoury. Although, it is important to note that taste perception of whole foods is indeed complex, with contribution of many tastants within the one food resulting in the overall taste perception produced. Nevertheless, in foods including seaweed and specific mushrooms, for example shitake, that contain high concentrations of L-glutamate, these are typically described as umami tasting. Contrary to this, there is a number of natural foods containing high concentrations of free L-glutamate that are not described as having an umami taste, for example peas (200 mg/10 g), corn (140 mg/100 g), red grapes (184 mg/100 g), or tomatoes (140 mg/100 g) [14,64,65]. Similar to sweet and sour tastants in the

previously mentioned foods, the presence of L-glutamate in these foods is an important compound to produce the overall flavour of these foods, rather than eliciting a clear perceptible umami taste [36,66].

Umami (MSG) has been shown to exhibit partially independent taste perception, as previous studies using multidimensional scaling have found that umami lies perceptually outside of the four basic tastes (sweet, sour, salty, and bitter) (cited in [25]), and that the taste perception of umami is predominately due to the anion (L-glutamate), albeit a small effect of the cation needs to be considered [23]. Perceptual associations between umami and salty taste exist as thresholds for the two tastes were found to correlate in participants classified as umami hypotasters [60]. Perceptual associations may similarly exist for umami and sweet tastes possibly due to the shared taste receptor subunit (T1R3). For example, rodents have reduced discrimination ability between sucrose and MSG when the sodium in MSG is neutralised using a salt taste blocker (amiloride) [49]. Furthermore, in humans, perceptual associations have been found between umami and sweet tastes in umami hyposensitive participants [67]. Therefore, it is pertinent to consider the perceptual relationship between umami taste and basic tastes, specifically salty and sweet tastes.

6.1. Umami and Salty

Glutamate in isolation from the sodium ion is glutamic acid, and has been described as having a sour taste [14]. The sodium salt of L-glutamate, MSG, produces an umami taste, and is the predominant prototypical umami stimulus used in psychophysical testing [60,67–69]. MSG potentiating 5′ribonucleotides are similarly tasted in their disodium salt form [13,22] complicating the perceptual independence of umami from salt taste in psychophysical testing [60,67–69]. Participants confuse umami with salty taste [68], and food (soup) containing MSG+IMP has been perceived as saltier, but not more savoury, than soups without MSG+IMP [43]. To overcome the sodium component of MSG, MPG is used in some psychophysical studies as a sodium free umami stimuli [55], although potassium also imparts salty, bitter, and metallic tastes, thus for all psychophysical testing L-glutamate requires a cation to produce a perceptible umami taste [14,23].

Through measuring DT and suprathreshold intensity for umami (MSG) and salty (NaCl), Lugaz, Pillias and Faurion (2002) found that 27% of their study population were classified as putative umami hypotasters. This proportion of umami hypotasters is consistent throughout the literature, with 28% of female participants [67], and 21% of participants [55], having a reduced ability to discriminate between 29 mM MSG and 29 mM NaCl. Using a filter paper disk method, 24% of female Japanese subjects had umami taste thresholds above 50 mM MSG, and were considered hypotasters [69]. The remainder of participants were classified as either semi-discriminators [67], or were able to discriminate between NaCl and MSG at the level of significance and were considered umami tasters [55,60]. Although a very low percentage of the population, 3.5% of a French [60], 3.2% of a German, and 4.6% of a Norwegian population [70] had no ability to discriminate between 29 mM NaCl and 29 mM MSG. These participants were unable to taste the L-glutamate in MSG and were considered non-tasters. Whether a similar proportion of subjects would be found to be umami non-tasters and tasters in non-European populations, or populations with high MSG intake, requires further research.

In individuals with an ability to taste L-glutamate, umami and salty taste perception are independent, conversely, in participants considered umami hypotasters, umami and salty taste perception are associated. Lugaz and colleagues (2002) found a positive correlation (r = 0.75) between individual salty (NaCl), and umami taste (MSG) thresholds in hypotasters, indicating that the hypotasters were likely to be perceiving only the sodium cation of the MSG. Along the same line, Pepino and colleagues (2010) found that participants classified as umami hypotasters (referred to as non-discriminators), perceived significantly more saltiness, and significantly less savouriness in MSG at suprathreshold concentrations, than umami tasters. There was no significant difference between umami tasters and hypotasters umami (MSG) DT [67], suggesting different mechanisms may mediate umami DT and suprathreshold taste dimensions supporting Lugaz and colleagues' (2002) findings that thresholds and intensity perception for umami do not necessarily co-vary. Associations between

umami DT and salty DT were not investigated, therefore, it is unknown if an association would have occurred between salty and umami DT in this female population group [67].

The difficulty with confirming umami taste perception independent from salt taste perception lies in the sodium cation in MSG. This can be overcome in part by the use of MPG, potassium, however has salty, bitter, and metallic taste characteristics [71], and therefore MPG taste perceptions cannot be solely attributed to L-glutamate. The contribution of the sodium ion in MSG can be reduced with the addition of IMP, nevertheless, IMP is tasted in disodium form and although IMP is added to MSG/MPG at subthreshold NaCl concentrations, the presence of sodium cannot be completely negated. Perceptual associations between umami and salty tastes appear to occur specifically in participants classified as umami hypotasters, but not in umami tasters. That is, for umami hyposensitive or non-tasting participants they are predominately sensing the sodium cation within MSG, resulting in associations between salty and umami taste perception for these participants.

6.2. Umami and Sweet

Umami and sweet taste share a common taste receptor subunit, T1R3, therefore there is potential for perceptual associations to exist [67,69]. Mice are capable of discriminating between MSG and sucrose [49], but when amiloride (a sodium blocker) is applied to neutralise the Na+ in MSG, a significant reduction in discrimination ability occurs [49]. Although significantly reduced, discrimination ability of the mice was still above chance, nevertheless, MSG and sucrose have some perceptual associations in rodents when the perceptual influence of Na+ is eliminated [49].

Interestingly, in human studies, umami hypotasters (non-discriminators) (27%, n = 16), have both significantly lower umami, and significantly lower sweet taste perception at suprathreshold concentrations, compared to umami tasters (discriminators) [67]. At DT, no association between umami (MSG) and sweet (sucrose) DT was found, again indicating different mechanisms may mediate suprathreshold and DT taste perception [67]. Similarly, umami (MSG) hypotasters (23.8%, n = 10) have a significantly lower sweet taste sensitivity at RT than umami tasters (n = 32) [69]. This suggests a relationship between umami and sweet taste perception. Contrary to these studies, Chen and colleagues (2009) found no significant difference in sweet taste intensity ratings in umami (MPG) insensitive (n = 5), and umami sensitive (n = 5) subjects. Mixed results could be attributed to different prototypical umami stimulus used (MPG or MSG), or due to the relatively low number of participants (n = 10), compared to previous psychophysical studies [67,69]. Similar to the associations between salty and umami taste, sweet and umami taste perception has been found to be perceptually associated in participants considered umami hypotasters, but not in umami tasters. Although the literature is not consistent, the associations found in some studies could conceivably be due to the shared receptor subunit between sweet and umami tastes, T1R3.

There is enough evidence to question whether umami is perceptually salient, particularly owing to the lexicon used to describe umami taste perception, and the similarities of these descriptions to kokumi taste. Perceptual independence of umami from salty, and sweet, is also unclear as associations exist between umami and salty, and umami and sweet tastes specifically in umami hypotasters. These associations are found across multiple taste dimensions, including DT, RT, and suprathreshold intensity.

7. Umami and Hedonics

MSG in an aqueous solution does not taste pleasant, however, when added to a complex food such as broth, it enhances palatability. For example, in infants the presence of high concentrations of L-glutamate in a breast milk matrix may increase the milks palatability and acceptability [25], and MSG added to a food matrix (soup) is preferred, but in an aqueous solution MSG is aversive [37]. As previously mentioned, free L-glutamate and IMP/GMP are naturally present in a range of foods. Across many cuisines mixtures of foods containing high concentrations of free L-glutamate are combined with foods containing high concentrations IMP/GMP thereby promoting the umami taste synergism and palatability [1]. For example, in Italian cuisine the combination of parmesan

(1200 mg/100 g L-glutamate) and beef/pork mince sauce (70 mg and 200 mg/100 g IMP respectively) or parmesan and tomato (120 mg/100 g L-glutamate). Or in Asian cuisines the combination of fish sauce (ranging from 620–1380 mg/100 g L-glutamate) and meat or fish products (ranging from 70–285 mg/100 g IMP) is frequently seen (see [25] for further examples). This combining of foods for enhanced palatability does not often come independent of sodium and kokumi peptides. Taking the previous example, parmesan contains high concentrations of sodium, and kokumi peptides [72], as does soy sauce [73]. Likewise, in studies where ingredients were omitted to determine key taste active compounds within a food, sodium, and free L-glutamate (along with other taste active amino acids) were common key tastants (reviewed in [74]).

The combination of added MSG and salt (NaCl) increases the acceptance of some foods, including various soup/stocks [75] and rice dishes [76] at certain ratios (usually between 0.1% and 0.8% by weight [66]) depending on the foodstuff and culture. For example, in European populations this may be higher (between 0.6 to 1.2%), possibly owing to the reduced familiarity of umami taste in Western populations [77]. The addition of MSG to improve palatability has been successfully used to reduce the sodium concentration in food without implicating the sensory properties of the foods [75,76], thus, displaying that in certain foods L-glutamate, IMP, sodium and kokumi effective peptides all contribute to the development of flavour and palatability in commonly consumed foods globally.

8. Relationship between Receptor, Perception, and Behavioural Responses of Umami and Sweet Taste

Perceptual associations between sweet and umami taste exist and may be due to behavioural factors including MSG and sucrose consumption [69,78], potentially owing to expression or sensitivity of the shared common receptor subunit T1R3 [17]. *In vitro* when MSG and sucrose are co-applied to sweet taste receptor cells, the response of the sweet taste receptor cells to sucrose is weakened [79]. Response from sweet taste receptor cells is also significantly weakened when glutamyl dipeptides are co-applied with sucrose [79]. When the umami tasting compounds are applied with lactisole, which inhibits activation of T1R3, a more severe reduction in the response from sweet taste receptors occurs. If umami tasting compounds and lactisole interacted with the same transmembrane domain of the T1R3, a synergistic reduction would not be expected, as the two stimuli would be competing for the same transmembrane domain. This suggests an interaction between umami peptides and MSG with sweet taste receptors, preventing sweet substances binding to an alternative domain, potentially T1R2 extracellular domain rather than the T1R3 domain [79].

Interestingly, in a human intervention study, prolonged consumption of MSG significantly reduced female participants' umami suprathreshold intensity perception, and similarly reduced (trending towards significant, $p = 0.06$) sweet taste suprathreshold intensity perception [78]. Similarly, Kubota and colleagues (2018) found that umami hypotasters also had a decreased sweet taste sensitivity, and consumed more sugar than umami tasters, although causation cannot be inferred between umami perception, sweet taste perception and sugar intake. It was not investigated whether umami hypotasters simply had a lower taste sensitivity overall compared to umami tasters, although this is unlikely as no significant differences in bitter taste sensitivity between umami tasters, and non-tasters was found [69].

Increased consumption has been linked with decreased receptor expression for other basic tastes, for example, increased consumption of fat was associated with decreased fat taste perception and decreased expression of fat taste receptor CD36 [18,80]. It would be interesting to know if increased intake of umami and sweet tastes decreases both taste perception of sweet and umami tastes and expression of the shared receptor subunit, T1R3. Alternatively, it is possible that increased intake of L-glutamate may decrease expression of T1R1, mGluR1, or mGluR4 taste receptors, although this does not account for the reduction in sweet taste perception found in previous psychophysical studies [69,78]. Considering umami stimuli may interact with the T1R2 extracellular domain [79], it would be interesting to investigate the influence of oral exposure to umami on T1R2 receptor

expression. Although further research into receptor expression is required, dietary intake of both sweet and umami stimuli appear to influence both umami and sweet taste perception in a similar direction, showing an interesting association between receptor, perception, and intake for umami and sweet tastes.

9. Behavioural and Physiological Responses to Umami Effective Stimuli

A key aspect of taste and taste receptor activation is the physiological responses initiated from oral taste receptor activation, and the influence on behavioural responses in human studies, for example increasing satiation and satiety [41,81]. The commencement of digestion is initiated through the secretion of saliva, the presence of MSG in the oral cavity stimulates a strong response of salivary release through a vagal efferent activation, assisting in initiating this digestion [40,82]. L-glutamate is not only detected in the oral cavity, but also in the gastrointestinal tract where T1R1/T1R3 are found [40,42,83]. T1R1/T1R3 heterodimer has been suggested to affect nutrient absorption through regulation of a peptide transporter through the activation by L-glutamate (reviewed in [42]). Daly and colleagues (2013) found in rodents' gastrointestinal tract T1R1/T1R3 are expressed and activation by L-glutamate results in CCK secretion *in vitro*, which is enhanced by IMP. CCK is involved in digestive processes, including slowing gastric emptying, and has also been suggested to inhibit food intake, thus has a satiety-like action through activating vagal afferent fibres that innervate the stomach and upper intestine (reviewed in [84]).

This enhanced satiety-like action from glutamate consumption has been demonstrated in human behavioural studies where the return of hunger after eating is slowed down after participants consume soup containing MSG, compared to soup without MSG [85], and soup containing protein and MSG compared to other treatments [86]. Similarly, in infants, consumption is decreased and satiation and satiety is increased when infants are fed formula supplemented with MSG, compared with standard cow's milk formula with the same concentration of protein [87]. Masic et al (2014) postulated that umami flavour may play a role in the satiating effects of protein, through sensory-nutrient interactions. Conversely, pre-load soups all containing MSG + IMP in conjunction with either low-energy, high-energy carbohydrate, or high-energy protein, all reduced consumption at a subsequent test meal compared to the same pre-load soups without added MSG + IMP [43]. The presence of MSG + IMP alone reduced consumption, irrespective of the protein content of the preload soup [43]. It is plausible that the presence of MSG + IMP enhanced the post-ingestive release of CCK in the gastrointestinal tract, influencing gastric emptying for all soup pre-load conditions, enhancing satiety, and reducing subsequent intake, although this requires further research. The effect of MSG on satiety is not consistent in the literature, as pre-load soups containing MSG improved energy compensation at a subsequent test meal but did not reduce hunger ratings or total energy intake compared to pre-load soups without MSG [88]. This indicates the importance of IMP in conjunction with MSG, for satiety-like responses and potentially CCK secretion, and considering MSG and IMP are often consumed together in animal protein and other common food combinations, this provides evidence for the importance of umami taste detection and perception in physiological processes and behavioural outcomes.

Interestingly, when a combination of tastants (sweet, bitter, and umami) were infused directly into the duodenum, increased satiety and decreased hunger responses were observed, as was a reduction in consumption of an *ad libitum* meal [89]. When umami was infused in isolation this reduction in hunger and increase in satiety was still observed, but not when sweet or bitter were infused alone, demonstrating the results from the combination of tastants were predominately driven by umami, with the exception of reducing energy intake at an *ad libitum* meal. Interestingly, the infusion of all individual tastants and combination of tastants did not influence the secretion of gastrointestinal peptides in comparison to the placebo infusion. It would be interesting to investigate the interaction of umami stimuli with taste receptors in the oral cavity without subsequent consumption, and whether individual variation in umami taste sensitivity is associated with the previously discussed physiological (satiety hormone release) and behavioural responses (satiety, satiation and intake). Although further

research is required, the consumption of MSG and IMP and the discovery of glutamate receptors in the gastrointestinal tract provides evidence for the role of umami effective stimuli detection stimulating physiological responses which may translate into behavioural responses in human studies.

10. Summary—Is Umami A Basic Taste?

For the past 3000 years four tastes (sweet, sour, salty, and bitter) have been included in all lists of basic tastes, predominantly based on perception. While these lists changed significantly in other attributes listed, often dependent on current thinking at that time, sweet, sour, salty, and bitter have been consistent. The recent advancements of technology and knowledge has led to the discovery of taste receptors and ligands, extending this basic taste list to include umami and fat as basic tastes. Thus, the basic taste list has grown and has the potential to include a plethora of other tastes including kokumi, carbohydrate, calcium and metallic tastes. With the potential of an ever-growing list of basic tastes in the current day it is pertinent to evaluate the current evidence and the 'moment in time' approach to naming basic tastes. It seems reasonable for new basic tastes, including umami, to consider if they belong in the same category as sweet, sour, salty, and bitter. Below is a summary of the evidence of umami as a basic taste, including an overview of basic and new tastes, against the proposed taste criteria, see Figure 1.

1. *Having an evolutionary or adaptive advantage*: Yes. Umami taste appears to have a biphasic effect due to its involvement in appetite stimulation and then digestion regulation. This occurs through both increasing satiety [43], and the presence of glutamate receptors in the gastrointestinal tract stimulating the release of digestive hormones [40–42], providing evidence for umami taste perception existing for evolutionary purposes.
2. *A distinct class of effective stimuli must exist:* Yes. Unique umami effective stimuli found in food includes free L-glutamate, and 5′ribonucleotides, and the prototypical umami taste stimuli are the salts of glutamic acid, MSG or MPG, and disodium salts of IMP and GMP [14,23]. There are a number of foods high in free L-glutamate that would not commonly be described as umami, raising the question of whether high concentrations of naturally occurring L-glutamate elicits an umami like taste in all foods [14,64,65]. Although, common food processing such as curing, and ageing, can increase free L-glutamate and IMP in certain foods, enhancing the umami taste through the glutamate and IMP synergism [26]. Finally, there is similarity between kokumi and umami stimuli, predominately due to the involvement of glutamic acid derivatives [30].
3. *Transduction mechanisms that can convert the chemical code of the stimulus into an electrical signal is required, including receptors:* Yes. Glutamate taste receptors have been identified (T1R1/T1R3, mGluR1, and mGluR4), and these respond to umami stimuli [17,46,47]. This glutamate taste receptor heterodimer (T1R1/T1R3), shares a receptor subunit with the sweet taste receptor (T1R2/T1R3) which has been hypothesised to relate to the perceptual associations that has been found between sweet and umami taste [69,78].
4. *Neurotransmission of this electrical signal to processing regions of the brain must occur:* Yes. Neurotransmission of signals transduced from glutamate receptors occurs, interestingly evidence suggests that different stimuli (MSG, MPG, MSG+IMP) are transduced by different gustatory afferent nerves (CT, GL) for umami taste.
5. *Perceptual experience arising from this processing must be independent from other taste qualities:* No. Studies using multidimensional scaling have found that umami lies perceptually outside of the four basic tastes (sweet, sour, salty, and bitter) (cited in [25]), and individual variation in taste perception across multiple taste dimensions has been established. Nevertheless, prototypical umami stimuli (L-glutamate or IMP/GMP) require cations to produce an umami taste, regardless of whether this cation is sodium or potassium, the additional taste that is imparted is difficult to negate in psychophysical testing. Studies have found perceptual associations with umami and salty taste, specifically in participants considered umami hypotasters at DT [60], and increased

saltiness perception of MSG at suprathreshold concentrations [67]. For umami and sweet taste, associations have similarly been found at DT [67] and RT [69], possibly owing to the shared receptor subunit T1R3. Considering current research finds perceptual associations between umami, other basic tastes (salty and sweet) and putative tastes (kokumi), it is relevant to question umami's classification as a basic taste. Perhaps umami taste fits into a taste classification with other basics (fat) or putative tastes including carbohydrate, kokumi, metallic, and calcium tastes that do elicit a taste perception when presented at high enough concentrations in the oral cavity but this is not necessarily a unique or perceptually salient taste experience.

6. *Hedonic response from tasting umami stimuli:* Yes. Although in aqueous solution MSG is not pleasant in taste, when mixed to certain foods it enhances palatability. The combination of L-glutamate, IMP, sodium, and often kokumi peptides is important in enhancing palatability of certain foods and is found across many cuisines globally.
7. *Physiological effects must occur following activation of taste bud cells:* Yes. Free L-glutamate is not only detected in the oral cavity, but also in the gastrointestinal tract where glutamate taste receptors (T1R1/T1R3) are present [40,42,83]. Glutamate taste receptor heterodimers have been suggested to affect nutrient absorption through regulation of a peptide transporter and glucose transporter through the activation of T1R1/T1R3 by L-glutamate (reviewed in [42]), which also results in CCK secretion *in vitro* [41]. Although the findings in the literature is mixed, behavioural studies have shown that consumption of MSG and particularly MSG+IMP influences satiety, satiation, and food intake, possibly owing to the secretion of digestive peptides upon stimulation of glutamate receptors in the gastrointestinal tract.

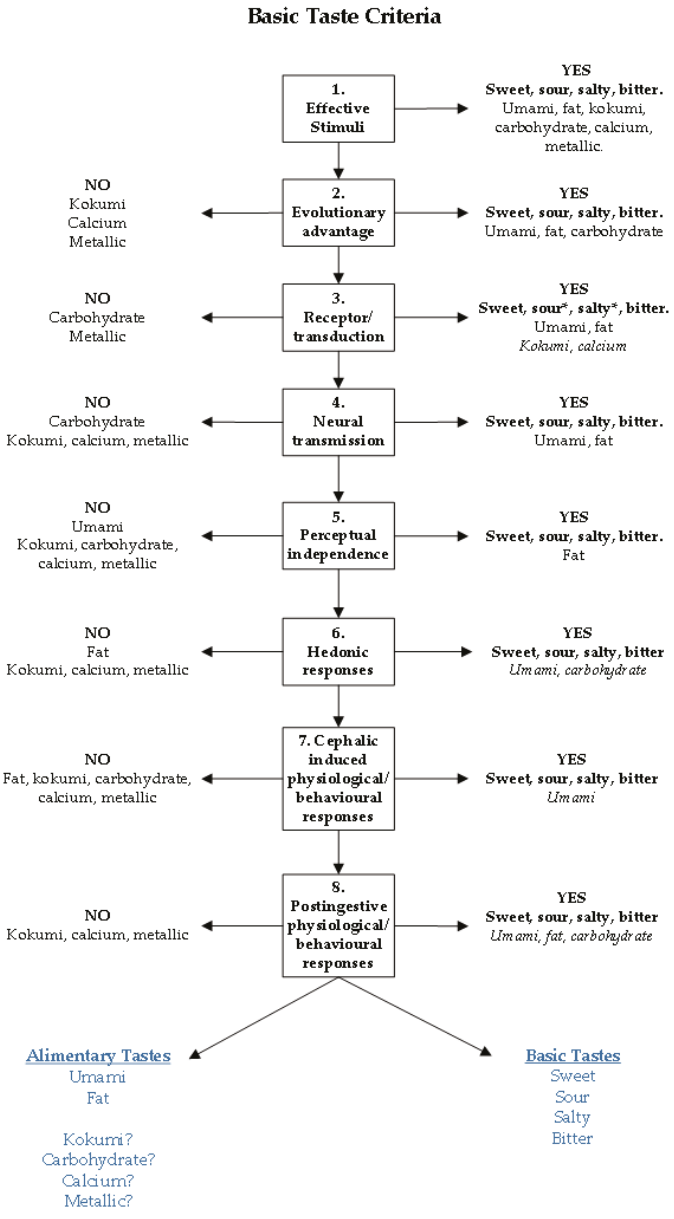

Figure 1. Criteria for tastes to fulfil to be classified as either basic tastes, or within a new taste subgroup. At the first criteria that a taste does not fulfil it is placed on the left-hand side of the model in the 'NO' section, those that fulfil the criteria remain on the right-hand side in the 'YES' criteria. * ENaC knockout mice have eliminated taste and neural responses to NaCl providing evidence for ENaC as the salt taste receptor [90], human studies have not yet confirmed the ENaC channel for salt taste detection. For the receptor criterion the ENaC receptor for salt taste, albeit in mice, has supporting evidence. * Type III sour sensing cells have been shown depolarise and reach action potential due to influx of H+ ions, providing evidence for sour taste detection, the specific proton channel responsible for this remains to be confirmed [45].

11. A New Class for New Tastes: Alimentary Taste

Current advances in knowledge and technology has led to the discovery of taste receptors, which has broadened the stimuli that could potentially be considered basic tastes, including kokumi, calcium, and likely many more to be discovered, for example, receptors responding to carbohydrate and metallic taste ligands. So, this 'moment in time' list of basic tastes has begun to expand with the addition of umami and fat, and others on the horizon such as carbohydrate and kokumi. Should kokumi, fat, or even umami be classified in the same category as sweet, sour, salty, and bitter, all of which have lingered throughout history? Is it enough to have identified receptors on taste cells for new tastes to be considered a basic taste, if the activation of these receptors does not result in a perceptually independent (umami) or perceptually salient (umami and fat) experience? An example of the identification of receptors with an absence of perceptual salience is fat taste, conceivably umami may fall into a similar category. Due to their unquestionable perceptual salience, sweet, sour, salty, and bitter have importance for immediate decision making; do we ingest or reject, that is, these tastes are critical during pre-ingestive taste detection. Many of the new and putative tastes may have far greater importance on post-ingestive consequences of nutrients that are detected not only in the oral cavity, but throughout the alimentary canal. Perhaps it is important, particularly in the context of applied taste research, that we consider umami and fat in a new subgroup of tastes. We propose a new structure of taste classification, with the four traditional tastes remaining as basic tastes due to their critical function during pre-ingestive taste detection, and new tastes becoming 'alimentary' tastes, including umami and fat, which have greater importance for post-ingestive functioning.

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Distorted Taste and Impaired Oral Health in Patients with Sicca Complaints

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Abstract: Senses of smell and taste, saliva flow, and dental status are considered as important factors for the maintenance of a good nutritional status. Salivary secretory rates, chemosensory function, burning mouth sensation, halitosis and dental status were investigated in 58 patients with primary Sjögren's syndrome (pSS), 22 non-Sjögren's syndrome sicca (non-SS) patients, and 57 age-matched healthy controls. A significantly greater proportion of patients with pSS and non-SS had ageusia, dysgeusia, burning mouth sensation, and halitosis compared to controls. Patients with pSS had significantly lower olfactory and gustatory scores, and significantly higher caries experience compared to controls. Patients with pSS and non-SS patients had significantly lower unstimulated and stimulated whole saliva secretory rates compared to controls. The findings indicated that several different aspects of oral health were compromised in both, patients with pSS and non-SS, and this may affect their food intake and, hence, their nutritional status. Although non-SS patients do not fulfill Sjögren's syndrome classification criteria, they have similar or, in some cases, even worse oral complaints than the patients with pSS. Further studies are needed to investigate food preferences, dietary intake, and nutritional status in these two patient groups in relation to their health condition.

Keywords: taste; smell; dysgeusia; burning sensation; halitosis; saliva; caries; primary Sjögren's syndrome; non-SS sicca syndrome

1. Introduction

Nutritional status is closely associated with health status, and decline in dietary intake can lead to weight loss and increased risk for disease [1]. The senses of smell and taste are important for nutrition—smell is vital in identifying potential dietary substances in the environment, while taste is instrumental in voluntary ingestion and early digestion of these dietary substances [2]. Saliva and nasal mucus are important for maintaining normal function of the taste buds imbedded in the oral epithelium and olfactory cells found in the nasal cavity [3]. Patients with reduced salivary secretion are known to have taste and smell abnormalities [3,4]. Furthermore, nutritional status is impaired in patients with taste and smell disorders [5]. Most studies showing taste and smell abnormalities in patients with dry mouth are reported from patients with Sjögren's syndrome. Little is known about

patients having similar symptoms of severe dry mouth and dry eyes, but not fulfilling the classification criteria for Sjögren's syndrome.

Sjögren's syndrome (SS) is an autoimmune connective tissue disorder of the exocrine glands, primarily the salivary and lacrimal glands [6]. A long-lasting inflammatory process in glandular tissue can lead to the loss of glandular cells, resulting in reduction or, in the worst cases, even complete loss of saliva and tear secretions [7]. The disorder has an unknown etiology, and mainly affects women [8]. The female to male ratio has been reported to be nine to one [8].

To be classified for SS diagnosis, patients have to fulfill at least four out of six classification criteria [9]. These criteria include symptoms of dry mouth and dry eyes; reduced tear secretion; reduced saliva secretion; histopathology of minor salivary glands showing infiltrates of lymphocytes; and the presence of autoantibodies directed against Ro/SSA (anti-Sjögren's-syndrome-related antigen A, also called anti-Ro) and/or La/SSB (anti-Sjögren's-syndrome-related antigen B, also called anti-La) [9]. As long as either serological or histopathological tests are positive, the presence of any four out of six symptoms indicates SS. If three out of four objective symptoms are present, it also justifies classifying the patient with SS. Patients complaining of dry eyes and dry mouth, but not fulfilling all the required criteria, are referred to as non-Sjögren's syndrome sicca (non-SS) patients.

Sjögren's syndrome can be subdivided into primary and secondary Sjögren's syndrome. Primary Sjögren's syndrome (pSS) is a diagnosis given to patients with manifest symptoms of dryness in the absence of other connective tissue diseases. Secondary Sjögren's syndrome (sSS) describes patients with symptoms of dryness, in the setting of another connective tissue disease or chronic inflammatory process, such as rheumatoid arthritis, systemic lupus erythematosus, diagnosed prior to developing SS symptoms [10]. The prevalence of pSS has been reported to range from 0.03% to 2.7% worldwide when different classification criteria were applied [11]. When applying the criteria of the American–European Consensus Group, the prevalence of pSS in the Norwegian population is estimated at 0.05% [12].

Patients with pSS and non-SS display a wide range of similar symptoms; among these are xerostomia—the subjective sensation of oral dryness. Symptoms of dry mouth often include frequent feeling of thirst, feeling of dryness in the mouth and throat, and ulcers may occur in the oral cavity [13]. Patients with dry mouth often have problems with decreased taste sensitivity and chewing in addition to difficulties with articulation [14]. Although, patients categorized as non-SS have similar complaints as patients with pSS, there is a risk that they do not receive appropriate medical care by the health authorities because of lacking diagnosis of SS.

Olfactory and gustatory disorders, also known as chemosensory disorders, are the disorders affecting the senses of smell and taste. Chemosensory disorders are categorized into quantitative and qualitative disorders, depending on whether the senses are reduced or distorted, respectively. Following this categorization, olfactory disorders are classified into anosmia (complete loss of smell), hyposmia (reduced ability to smell), and dysosmia (distorted sense of smell) [15]. Similarly, gustatory disorders are classified as ageusia (complete loss of taste), hypogeusia (reduced ability to taste), and dysgeusia (distorted taste, for example, metallic taste perception) [16]. Patients with a normal sense of smell and taste are categorized as normosmic and normogeusic, respectively. Other oral disorders, like halitosis/oral malodor and burning sensation/numbness in the oral cavity, are often observed in patients with chemosensory disorders [4]. About 50% of patients with chemosensory disorders have reported a negative impact on (i) appetite and body weight, (ii) quality of life, and (iii) psychological well-being [17].

There is evidence that patients with SS have a poor dental status [18]. In a cross-sectional study of Chilean SS-patients, as many as 60% had dental caries, a higher prevalence than the general population [18]. However, in another study, no significant differences could be detected in the dental caries experience of Swedish SS patients compared to dry mouth controls [18,19]. Patients with pSS are also reported to have a significantly higher dental caries experience, also called DMFT (DMFT: decayed, missing, and filled teeth) than healthy controls, mainly due to a higher number of filled and missing teeth [20]. A change in a patient's dental caries status has been suggested as one of several potential markers of the extent of autoimmune-mediated salivary gland dysfunction in pSS [20].

The aim of this study was to compare salivary flow, olfactory and gustatory function, burning mouth sensation, halitosis, and dental status in patients with pSS, non-SS sicca patients, and healthy age-matched controls, to gain more insight into the oral status of non-SS sicca patients.

2. Materials and Methods

2.1. Participants

The study was conducted at the Dry Mouth Clinic at the University of Oslo (UiO), Norway, and was approved by the Norwegian Regional Committee for Research Ethics (REK 2015/363). Another study has previously been published with the same REK number which includes 31 female patients with pSS and 33 gender-matched controls [4]. The present study presents an additional patient group (22 non-SS patients), and a higher number of patients, in both pSS group (58 patients with pSS) and healthy control group (57 healthy controls). Moreover, different parameters have been investigated in the two studies. The data presented in this study has not been published before. Participant characteristics are presented in Table 1. Written informed consent was obtained from all participants prior to examination. Most patients with pSS were referred from the Department of Rheumatology at Oslo University Hospital (OUS), where they were classified according to the American–European Consensus Group criteria (13). Non-SS patients were referred to the last author J.L.J for salivary gland biopsies [13]. They all had sicca complaints, but anti-Ro/SSA were absent, and the histopathology of their salivary gland biopsies were not consistent with pSS. The exclusion criteria for controls were mouth and eye dryness, chronic diseases, and use of medications that could affect the salivary glands. The participants were instructed to refrain from eating, drinking, and smoking one hour prior to examination. The assessments of salivary secretory rates, olfaction, gustation, oral malodor, and dental status were carried out by a team of calibrated dental practitioners and specialists.

Table 1. Participant characteristics.

	Non-SS (*N* = 22)	**pSS (*N* = 58)**	**Controls (*N* = 57)**	***p*-Value**
Age (year)				
Mean ± SD Range	52.0 ± 10.4 34–76	52.9 ± 13.4 26–75	49.7 ± 16.5 20–79	NS
Gender				
Female % (*N*) Male % (*N*)	100 (22) 0	96.5 (56) 3.5 (2)	73.7 (42) 26.3 (15)	<0.001
Ethnicity				
Caucasian % (*N*) Non-Caucasian % (*N*)	90.9 (20) 9.1 (2)	98.3 (57) 1.7 (1)	93.0 (53) 7 (4)	NS
Height (cm)				
Mean ± SD Range	166.7 ± 5.3 158–178	169.5 ± 7.1 153–190	170.9 ± 7.1 157–187	0.049
Weight (kg)				
Mean ± SD Range	72.3 ± 16.3 51–120	71.6 ± 13.8 49–120	69.1 ± 11.7 50–90	NS

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's syndrome patients; Fischer's-exact test, One-Way ANOVA, NS = Not Significant.

2.2. Saliva Assessment

Summated Xerostomia Inventory-Dutch (SXI-D) version was used to assess participants' self-reported perception of dry mouth [21]. SXI-D is a shortened version of the Xerostomia Inventory

(XI) [22] and consists of five statements that are used to determine the severity of xerostomia. The SXI-D sum score ranges from 5 to 15, where 15 = very severe problems related to xerostomia. Thereafter, unstimulated (UWS) and chewing-stimulated (SWS) whole saliva were collected from all participants to determine salivary secretory rates. Unstimulated whole saliva was collected first for 15 min, and then SWS for 5 min. Saliva samples were weighed and secretory rates were calculated for UWS and SWS (g/min = mL/min). UWS secretory rate was considered normal if $\geq$ 0.1mL/min, and SWS secretion rate was considered normal if $\geq$ 0.7 mL/min [23].

2.3. Olfactory Assessment

Self-reported perception of sense of smell was obtained prior to olfactory testing. Participants were asked to score their own subjective smell perception on a visual analogue scale (VAS) from 0 to 10, where 0 = no smell perception, and 10 = very good smell perception. Cognitive olfactory function was measured using twelve-stick identification test (Burghart Messtechnik, Wedel, Germany). The participants were instructed to choose from four possible answers on a multiple choice-scoring card. The answers were recorded, and the data were summarized for each participant. A normative classification [24] was used to define anosmic (score 0–5), hyposmic (score 6–9), and normosmic (score 10–12) participants.

2.4. Gustatory Assessment

Self-reported perception of sense of taste was obtained prior to gustatory testing. Participants were asked to score their own subjective taste perception on a visual analogue scale (VAS) from 0 to 10, where 0 = no taste perception, and 10 = very good sense of taste. Gustatory function was evaluated using taste strips (Burghart Messtechnik, Wedel, Germany) with four basic taste qualities; sweet, sour, salty, and bitter, each tested at 4 different concentrations. The taste qualities were presented in a random manner, starting with the weakest concentrations. This protocol resulted in a total of 32 values for each participant, as both sides of the tongue were tested. A normative classification [25] was followed to distinguish between ageusic (score 0–12), hypogeusic (score 13–18), and normogeusic (score 19–32) participants.

2.5. Assessment of Dysgeusia, Burning Mouth Sensation, and Halitosis

A questionnaire was designed for use in this study to assess participants' experience of dysgeusia, burning mouth sensation (BMS), and halitosis (Table 2). The present questionnaire is a modified version of a questionnaire that we have published in a previous study [4]. Both patients with pSS and non-SS reported that they had periods when their disease symptoms were more pronounced ("bad periods") and periods when the symptoms were less pronounced ("good periods").

2.6. Oral Malodor Assessment

Self-reported perception of halitosis was obtained prior to oral gas sampling. Participants were asked to score their own subjective perception of oral malodor on a scale from 0 to 5, where 0 = no appreciable odor, and 5 = extremely foul odor. Halitosis was measured using both organoleptic and objective methods. The organoleptic measurements were performed by instructing the participants to exhale briefly through the mouth at three different distances (100, 30, and 10 cm) from the nose of the organoleptic judge. The level of malodor was recorded using the same scale as for the self-reported perception of halitosis [26]. Levels of volatile sulfur compounds (VSC) in the mouth air of the participants were measured by gas chromatography (GC: OralChroma™, Nissha FIS, Inc., Osaka, Japan). Mouth air samples from the participants were obtained using a standardized procedure according to the user manual. A 1.0 mL syringe was inserted into the oral cavity until the stopper was in contact with the lips and the syringe could be held gently between the teeth without the tongue touching the tip of the syringe. After the syringe was held in this position for 30 s, a mouth air sample was withdrawn using the syringe, and was immediately injected into the OralChroma™.

Analysis of VSC started automatically, and the levels of hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH) determined. The olfactory threshold levels (in parts per billion, ppb) indicating oral malodor were considered either high (H_2S > 112 ppb and CH_3SH > 26 ppb) or low (H_2S < 112 ppb and CH_3SH < 26 ppb), as recommended by the manufacturer and used in other studies [27].

Table 2. Questionnaire used to assess participants' complaints of dysgeusia, burning mouth sensation, and halitosis, and their impact on quality of life.

	Dysgeusia						
1.	Do you experience bad taste on the tongue?			Yes			No
2.	If yes, can you describe the taste?	Metallic	Sour	Rotten	Bitter	Other	
3.	How often do you experience bad taste?	Constantly	Daily	Sometimes	In bad periods *	Other	
4.	Is the bad taste related to meals?	During meals	In between meals	Constantly			
	Burning Mouth Sensation						
5.	Do you experience burning mouth sensation?			Yes			No
6.	Where in your mouth do you experience burning sensation?	Whole tongue	Anterior tongue	Lips	Palate	Other	
7.	How often do you experience burning sensation?	Constantly	Daily	Sometimes	In bad periods *	Other	
8.	Is the burning sensation related to meals?	During meals	In between meals	Constantly			
9.	Do you have to refrain from certain food items due to burning sensation?			Yes			No
10.	If yes, what kind of food items do you have to avoid?	Spicy	Sweet	Sour	Salty	Bitter	
	Halitosis						
11.	Do you have complaints of bad breath?			Yes			No
12.	How often do you have these complaints?	Constantly	Daily	Sometimes	In bad periods *	Other	
	Quality of Life (QoL)						
13.	Which of the disturbances have a negative impact on your QoL?	Burning mouth	Reduced taste/smell	Distorted taste	Bad breath	Dry Mouth	

* Bad periods: periods when disease symptoms are more pronounced.

2.7. Dental Assessment

Self-reported perception of dental health and general health was obtained from the participants prior to clinical and radiological examination of the teeth. Participants were asked to score their own subjective assessment of their dental and general health status on a scale from 0 to 5, where 0 = very poor, and 5 = excellent. A thorough dental examination, consisting of clinical and radiological examination of the oral cavity, was conducted by general dental practitioners. The number of decayed, missing, or filled teeth (DMFT) and only filled teeth (FT) were recorded [23].

2.8. Statistical Analyses

Descriptive statistical analysis was performed, and the results are presented in percentages, median/interquartile range (IQR)/ranges. Normality of continuous variables was tested on histogram, Q–Q plot, and by Shapiro–Wilk test. Due to the low sample size and non-normal distribution of the continuous variables, Kruskal–Wallis ANOVA and Mann–Whitney *U* test was used to detect median differences of continuous, numerical variables between the two or three groups (control, non-SS, pSS). Chi-square ($\chi 2$) test and Fischer's-exact test was used to test the differences of the distribution of categorical variables. Point-biserial and Spearman correlations were used to measure the strength and direction of the association between the one continuous and one dichotomous variable, and between two continuous variables respectively. All differences were considered significant at $p < 0.05$. Statistical Package for STATA (Stata version 14.0; College Station, TX, USA) and SPSS (SPSS version 24, IBM, Armonk, NY, USA) were used for the statistical analyses.

3. Results

3.1. Dysgeusia, Burning Mouth Sensation, and Halitosis

Self-reported complaints of dysgeusia, burning mouth sensation, and halitosis in the three groups are shown in Table 3. The completion rate for Yes/No questions in the questionnaire was 100% in the three groups. The frequency of dysgeusia, burning sensation, and halitosis was significantly higher in the non-SS and pSS groups versus controls, and these self-reported complaints showed significant association with the disease ($p < 0.001$).

Table 3. Overview of self-reported complaints of dysgeusia, burning mouth sensation, and halitosis in the three groups.

	Non-SS (*N* = 22)	pSS (*N* = 58)	Controls (*N* = 57)	*p*-Value
Dysgeusia % (*N*)	77.3 (17)	60.3 (35)	3.5 (2)	<0.001
Burning Mouth Sensation % (*N*)	59.1 (13)	50.0 (29)	3.5 (2)	<0.001
Halitosis % (*N*)	59.1 (13)	37.9 (22)	1.8 (1)	<0.001

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients; Chi-square test.

Fifteen patients with non-SS, thirty-one patients with pSS and one participant in the control group, who experienced dysgeusia, answered further questions. Metallic taste dysgeusia was the most common complaint both in the non-SS and pSS groups. Other taste distortions were described as "rotten" and "bitter", in addition to "other" taste distortions which the participants were not able to describe in words. Distorted taste was significantly more common in the non-SS and pSS groups, compared to controls ($p < 0.001$) (Table 4).

Table 4. Participants experiencing distorted taste.

	Non-SS (*N* = 22)	pSS (*N* = 58)	Controls (*N* = 57)	*p*-Value
Metallic % (*N*)	63.2 (12)	20.4 (10)	0	
Rotten % (*N*)	15.8 (3)	16.3 (8)	0	<0.001
Bitter % (*N*)	0	14.3 (7)	0	
Other % (*N*)	0	12.2 (6)	1.8 (1)	

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients; Fischer's-exact test.

Some patients with pSS and non-SS described that they had good and bad periods, where the disease symptoms were less pronounced in good periods and more pronounced in bad periods. The duration of good and bad periods varied between individuals. Dysgeusia was experienced either "constantly", "daily", "sometimes, or "in bad periods". The perceived distorted taste was significantly more frequent in the non-SS and pSS groups, compared to controls ($p < 0.001$) (Table 5).

Table 5. Overview showing how often participants experienced distorted taste.

	Non-SS (*N* = 22)	pSS (*N* = 58)	Controls (*N* = 57)	*p*-Value
Constantly % (*N*)	9.1 (2)	10.3 (6)	0	
Daily % (*N*)	9.1 (2)	12.1 (7)	0	<0.001
Sometimes % (*N*)	18.2 (4)	20.7 (12)	1.8 (1)	
In bad periods % (*N*)	31.8 (7)	8.6 (5)	0	

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients; Fischer's-exact test.

When answering the question "is bad taste related to meals?", some participants reported dysgeusia "during meals", others experienced it "in between meals", while some reported "constant" lingering of bad taste in the mouth. Only patients with pSS reported that bad taste was more pronounced "during meals", resulting in foul-tasting meals. One of the patients with pSS reported that even water had a metallic taste. Moreover, "constant" perception of dysgeusia was also found only in the pSS group (Table 6).

Table 6. Participants reporting whether dysgeusia was related to meals.

	Non-SS (*N* = 22)	pSS (*N* = 58)	Controls (*N* = 57)	*p*-Value
During meals % (*N*)	0	6.9 (4)	0	
In between meals % (*N*)	9.1 (2)	13.8 (8)	1.8 (1)	<0.001
Constantly % (*N*)	0	10.3 (6)	0	

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients; Fischer's-exact test.

The response rate for the multiple choice questions for burning mouth sensation among the participants experiencing burning mouth, was almost 30% in the non-SS group, 65% in the pSS group and 50.0% controls. Majority of these participants experienced a burning sensation on the "whole tongue", while some patients experienced this only on the "anterior tongue". Only one patient with pSS experienced a burning sensation on the "lips and palate" in addition to the tongue. A significantly higher proportion of participants experienced burning mouth sensation on the tongue, compared to controls ($p < 0.001$) (Table 7). An overview of how often participants in the three groups experienced burning mouth sensation is shown in Table 8.

Table 7. Location of burning mouth sensation experienced in the oral cavity.

	Non-SS (*N* = 22)	pSS (*N* = 58)	Controls (*N* = 57)	*p*-Value
Whole tongue % (*N*)	40.9 (9)	36.2 (21)	1.8 (1)	
Anterior tongue % (*N*)	9.1 (2)	3.4 (2)	0	<0.001
Lips, palate% (*N*)	0	1.7 (1)	0	

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients; Fischer's-exact test.

Table 8. Overview showing how often participants experienced burning mouth sensation.

	Non-SS (*N* = 22)	pSS (*N* = 58)	Controls (*N* = 57)	*p*-Value
Constantly % (*N*)	9.1 (2)	5.2 (3)	0	
Daily % (*N*)	13.6 (3)	10.3 (6)	0	<0.001
Sometimes % (*N*)	4.5 (1)	19.0 (11)	1.8 (1)	
In bad periods % (*N*)	22.7 (5)	3.4 (2)	0	

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients; Fischer's-exact test.

For some of the patients complaining of burning mouth sensation, it was reported to be worst "during meals" in the non-SS and pSS groups (Table 9). Twenty-seven percent of non-SS patients and 24% of patients with pSS reported that they had to refrain from food items like spicy food, sour food items, sour fruits, and beverages like soft drinks, juices, and wine, because of burning mouth sensation.

Table 9. Participants reporting whether burning sensation was related to meals.

	Non-SS (*N* = 22)	pSS (*N* = 58)	Controls (*N* = 57)	*p*-Value
During meals % (*N*)	22.7 (5)	25.9 (15)	0	
In between meals % (*N*)	9.1 (2)	1.7 (1)	0	<0.001
Constantly % (*N*)	4.5 (1)	1.7 (1)	0	

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients; Fischer's-exact test.

Among participants complaining of halitosis, non-SS patients were more affected than patients with pSS, while none of controls complained of oral malodor. Table 10 shows how often participants experienced oral malodor. Some patients reported that they avoided drinking tea or coffee because of perceived risk of getting halitosis. When answering "which of the disturbances have a negative effect on your quality of life?", both non-SS patients and patients with pSS reported burning mouth sensation and distorted taste as major factors affecting their quality of life.

Table 10. Overview showing how often participants experienced halitosis.

	Non-SS (*N* = 22)	pSS (*N* = 58)	Controls (*N* = 57)	*p*-Value
Constantly % (*N*)	4.5 (1)	8.6 (5)	0	
Daily % (*N*)	18.2 (4)	6.9 (4)	0	
Sometimes % (*N*)	13.6 (3)	8.6 (5)	0	<0.001
In bad periods %	13.6 (3)	3.4 (2)	0	
(*N*)After meals % (*N*)	4.5 (1)	1.7 (1)	0	

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients; Fischer's-exact test.

3.2. Gustatory Function

The results of the Kruskal–Wallis ANOVA and the Mann–Whitney *U* test showed that the measured median gustatory scores (median (IQR), range) were significantly lower in the pSS group (20.0 (16.0–26.0), 2.0–32.0) than in the control group (26.0 (22.0–28.0), 12.0–32.0) ($p = 0.001$). No significant differences were observed between the non-SS (24.0 (20.0–26.0), 2.0–32.0) and the control group (Figure 1a). Participants' self-reported taste scores also revealed a significantly lower mean perception of taste in the pSS group (7.0 (5.0–9.0), 0.0–10.0) compared to the control group (8.0 (8.0–10.0), 3.0–10.0), ($p = 0.009$). No significant difference was found comparing the non-SS group (8.0 (5.0–9.0), 3.0–10.0) with controls (Figure 1b). Chi-square tests showed that a significantly higher percentage of pSS and non-SS patients had ageusia compared to controls (Table 11).

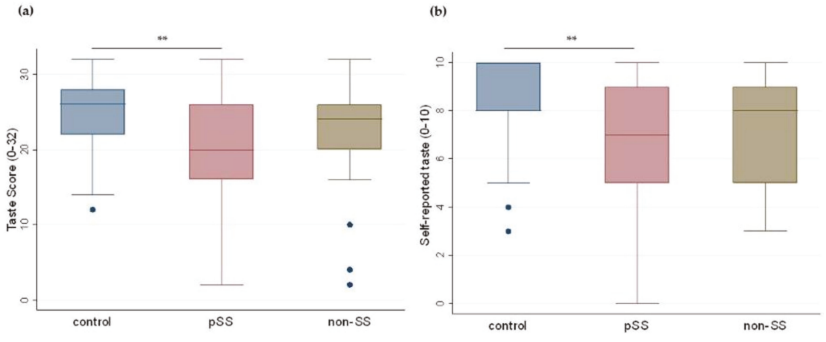

Figure 1. Measured and self-reported taste score in the three groups. Boxplots illustrating (**a**) measured taste scores and (**b**) participants' self-reported taste score in controls, primary Sjögren's patients (pSS), and non-Sjögren's sicca patients (non-SS). (Kruskal–Wallis ANOVA and Mann–Whitney *U* test; ** $p < 0.01$.). Dots in the figures represent the outliers.

Table 11. Percentage of participants with ageusia (no taste perception) and hypogeusia (reduced taste perception) among participants.

	Non-SS (*N* = 22)	pSS (*N* = 58)	Controls (*N* = 57)	*p*-Value
Ageusia % (*N*)	14.3 (3)	15.5 (9)	1.8 (1)	0.031 *
Hypogeusia % (*N*)	9.1 (2)	25.9 (15)	12.3 (7)	0.084

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients; Chi-square test. * $p < 0.5$.

3.3. Olfactory Function

The results of the Kruskal–Wallis ANOVA and the Mann–Whitney *U* test showed that the measured median olfactory scores (median (IQR), range) were significantly lower in the pSS group (10.0 (9.0–11.0), 0.0–12.0) than in the control group (11.0 (9.0–11.0), 3.0–12.0), ($p = 0.007$). No significant differences were observed between the non-SS (10.0 (9.0–11.0), 6.0–16.0) and the control group (Figure 2a). Participants' self-reported smell scores did not reveal any significant differences between the three groups (Figure 2b).

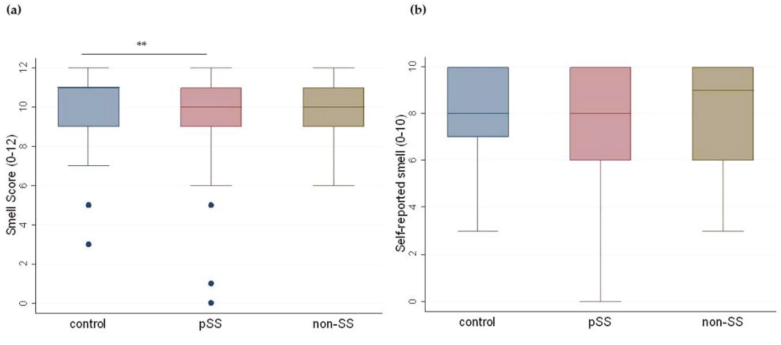

Figure 2. Measured and self-reported smell score in the three groups. Boxplots illustrating median, interquartile ranges (IQRs), and ranges of (**a**) measured smell scores (0–12) and (**b**) participants' self-reported smell scores (0–10) in controls, primary Sjögren's patients (pSS), and non-Sjögren's sicca patients (non-SS). (Kruskal–Wallis ANOVA and Mann–Whitney *U* test; ** $p < 0.01$.). Dots in the figures represent the outliers.

3.4. Oral Malodor Results

Gas chromatographic analysis (median (IQR), range) revealed the following H_2S-values (ppb): control group (33.5 (8.7–141.0), 0.0–2885.0), pSS group (27.5 (15.7–96.2), 0.0–458.0), and non-SS group (41.0 (13.5–84.0), 0–803.0). The results for CH_3SH (ppb) were as follows: control group (8.0 (3.0–26.5), 0–193.0), and for the pSS and non-SS groups were (6.0 (2.0–13.2), 0–75.0) and (5.0 (0–13.2), 0–83.0), respectively. There were no significant differences in H_2S and CH_3SH levels between the groups.

There was no significant correlation between the self-reported perception of halitosis and the organoleptic measurements. The self-reported perceived halitosis scores (median (IQR), range) for the control group were (0.0 (0.0–1.0), 0–3), while the scores for the pSS group and non-SS group were (1.0 (0.0–2.0), 0–4) and (2.0 (1.0–3.0), 0–5), respectively. Organoleptic judge scores were (0.0 (0.0–1.0), 0–2) for the control group, (0.0 (0.0–1.0), 0–3) for the pSS group, and (0.0 (0.0–1.0), 0–2) for the non-SS group. No significant differences were found between groups in self-reported perception of halitosis and organoleptic measurements.

3.5. Saliva and SXI-D

The results of the Kruskal–Wallis ANOVA and the Mann–Whitney *U* test showed that the UWS secretory rates (mL/min) were significantly lower in the pSS group (0.1 (0.0–0.1), 0.0–0.4) and non-SS group (0.1 (0.0–0.2), 0.0–0.6) compared to the control group (0.3 (0.2–0.4), 0.0–0.8), ($p < 0.001$) (Figure 3a). Also, SWS secretory rates were significantly lower in the pSS group (0.7 (0.4–1.0), 0.0–1.5) and non-SS group (0.9 (0.6–1.3), 0.3–1.8) compared to controls (1.6 (1.1–2.4), 0.5–3.5), ($p < 0.001$) (Figure 3a). The results of participants' self-reported perception of xerostomia showed significantly higher SXI-D scores in both the pSS group (12.0 (10.0–14.0), 6.0–15.0) and the non-SS group (12.0 (11.0–14.0), 9.0–15.0) compared to controls (6.0 (5.0–7.0), 5.0–9.0), ($p < 0.001$) (Figure 3b). No significant differences were observed between pSS and non-SS groups, for either salivary secretory rates or SXI-D score.

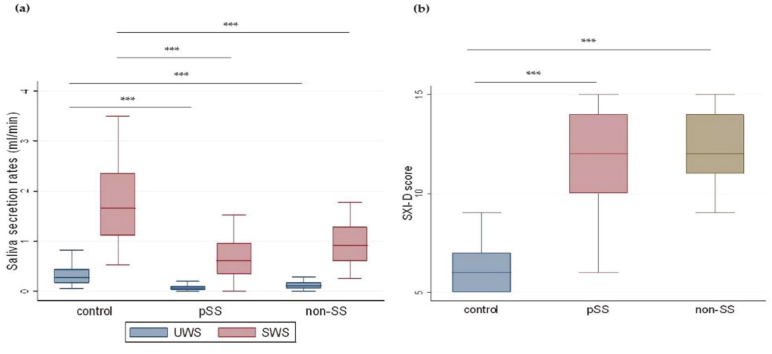

Figure 3. Measured saliva secretory rate and self-reported perception of xerostomia in the three groups. Boxplots illustrate median, IQRs, and ranges of (**a**) saliva secretion rates (mL/min) and (**b**) SXI-D: Summated Xerostomia Inventory-Dutch scores in controls, primary Sjögren's patients (pSS), and non-Sjögren's sicca patients (non-SS). (Kruskal–Wallis ANOVA and Mann–Whitney *U* test; *** $p < 0.001$.)

Pathologically low saliva secretory rates for UWS ($\leq$ 0.1 mL/min) and SWS ($\leq$ 0.7 mL/min were analyzed among the participants. A significantly higher proportion of patients with pSS had saliva secretory level below the threshold level for both UWS and SWS (Table 12). Furthermore, moderate, significant correlations were found between salivary secretory values (USW and SWS) and dysgeusia, burning mouth sensation, halitosis, taste score, DMFT, and FT, when all the participants were considered together (Table 13).

Table 12. Correlations between pathologically low UWS/SWS and the three groups.

	Non-SS (N = 22)	pSS (N = 58)	Controls (N = 57)	p-Value
UWS (below threshold) % (N)	59.1 (13)	74.1 (43)	8.8 (5)	<0.001 ***
SWS (below threshold) % (N)	31.8 (7)	65.5 (38)	5.3 (3)	<0.001 ***

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients. UWS: unstimulated whole saliva, below threshold (≤0.1 mL/min); SWS: stimulated whole saliva (≤0.7 mL/min); Chi-square test. *** $p < 0.001$.

Table 13. Correlations between UWS and SWS and dysgeusia, burning mouth sensation, halitosis, taste score, DMFT and FT.

	UWS (N = 137) r	SWS (N = 137) r
Dysgeusia	−0.37 ***	−0.37 ***
Burning Mouth Sensation	−0.29 ***	−0.39 ***
Halitosis	−0.27 **	−0.27 **
Taste Score	0.21 *	0.21 *
DMFT	−0.30 ***	−0.27 **
FT	−0.26 **	−0.21 **

non-SS: non-Sjögren's sicca patients, pSS: primary Sjögren's patients. UWS: unstimulated whole saliva, SWS: stimulated whole saliva, Pearson's point-biserial correlation coefficient. *** p <0.001, ** $p < 0.01$; * $p < 0.05$.

3.6. DMFT/FT

Caries experience as measured by DMFT (median (IQR), range) was significantly higher in the pSS group (18.0 (11.0–23.0), 0.0–28.0) compared to the control group (12.0 (6.5–18.0), 1.0–27.0), ($p = 0.005$). The DMFT in the non-SS group (16.0 (12.8–19.3), 0.0–28.0) did not differ from that of the control group ($p = 0.3$) or the pSS group ($p = 1.0$) (Figure 4a).

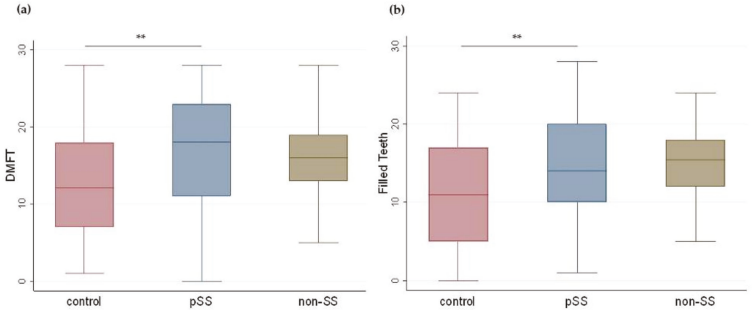

Figure 4. DMFT and FT results from the three groups. Boxplots illustrate median, IQRs, and ranges of (**a**) DMFT: decayed, missing, and filled tooth surfaces and (**b**) FT: filled teeth in controls, primary Sjögren's patients (pSS), and non-Sjögren's sicca patients (non-SS). (Kruskal–Wallis ANOVA and Mann–Whitney U test; ** $p < 0.01$.)

Similarly, the FT component of the DMFT index (median (IQR), range) was significantly higher in the pSS group (14.0 (10.0–20.0), 1.0–27.0) than the control group (11.0 (5.0–17.0), 0.0–24.0), ($p = 0.030$). The FT score in the non-SS group (15.5 (11.8–18.2), 0.0–24.0) did not differ from that of the control group ($p = 0.241$) or the pSS group ($p = 1.0$) (Figure 4b).

3.7. General Health Status and Dental Status

Statistically significant differences were found in self-reported general health status (median (IQR), range) between patients with pSS (2.0 (1.0–3.0), 0–4.0), non-SS patients (1.5 (1.0–2.0), 0–3.0), and controls (4.0 (3.0–4.0), 2.0–4.0), $p < 0.0001$ (Figure 5b). Similar statistically significant differences were found between patients with pSS (2.0 (1.0–3.0), 0–4.0), non-SS patients (1.0 (1.0–2.0), 0–3.0), and controls (3.0 (3.0–4.0), 2.0–4.0), $p < 0.0001$, when participants scored their own dental health status (Figure 5a). Spearman's test showed that when all participants were considered together, participants self-reported dental health status was found to be significantly, negatively correlated to dental status DMFT ($r = -0.27$, $p = 0.001$) and FT ($r = -0.18$, $p = 0.04$). Furthermore, significant positive correlations were found between participants' dental and general health status ($r = 0.58$, $p < 0.001$).

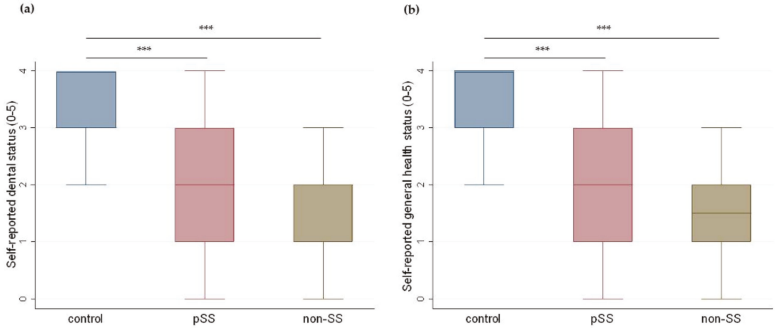

Figure 5. Self-reported dental and general health status in the three groups. Boxplots illustrate median, IQRs, and ranges of (**a**) self-reported dental status (0–5) and (**b**) self-reported general health status (0–5) in controls, primary Sjögren's patients (pSS), and non-Sjögren's sicca patients (non-SS). (Kruskal–Wallis ANOVA and Mann–Whitney *U* test; *** $p < 0.001$.)

4. Discussion

The present study revealed that the non-SS patients have similar or even worse oral health than patients with Sjögren's syndrome. In general, patients with sicca symptoms, suspected to have SS but not fulfilling the classification criteria for SS, far outnumber the patients who fulfill the criteria. Still, only patients who fulfill the criteria are usually included in studies [28]. Thus, non-SS patients are left both without a diagnosis and are often not considered to be of interest for researchers. Therefore, the main focus in this study was the oral health status of the sicca patients without a Sjögren's diagnosis.

In the present study, we found that complaints of dysgeusia, burning mouth sensation, and halitosis were common in the non-SS group. It has previously been shown that patients with pSS have a high percentage of complaints of dysgeusia, burning sensation on the tongue, and halitosis, and that about 50% of patients with pSS report these disorders [4]. In the present study, when comparing non-SS patients with patients with pSS, it was found that non-SS patients had a much higher occurrence of dysgeusia, burning mouth sensation, and halitosis. In the literature, there are no studies available to compare our current findings with results from other studies on non-SS patients. To our knowledge, this is the first study comprehensively evaluating oral health in patients with sicca symptoms without an SS diagnosis. Since many of the patients avoided certain food items due to problems with dysgeusia and burning sensations, it may affect their dietary intake. This is consistent with the literature, where decreased appetite has been reported in 30% of patients and decreased enjoyment of food in 70% of patients complaining of dysgeusia [29]. About 60% of dysgeusia patients have been reported to change their eating patterns and 40% to modify their use of seasonings [29].

In addition to distorted taste, reduced taste function was observed in both non-SS and patients with pSS. Ageusia, a condition characterizing complete absence of taste perception, is a very seldom

condition and accounts for less than 1% of patients referred to taste and smell research centers [5,17]. When taste function was measured, almost 15% of non-SS and patients with pSS were found to have ageusia in this study. Loss of appetite has been reported in patients suffering from ageusia [5]. Furthermore, about 10% of non-SS patients and 26% of patients with pSS were found to be hypogeusic. The incidence of this taste disorder is also low in the general population, and some but not all patients suffering from hypogeusia report decreased enjoyment of food and decreased appetite [5]. However, hypogeusia in combination with other disorders, like dysgeusia and burning sensations, might exacerbate the changes in dietary intake [30]. Therefore, dietary intake monitoring and counselling is very important in those patients with pSS and non-SS patients that suffer from both qualitative and quantitative taste disorders.

Smell and taste disorders are common in the general population, however, patients are frustrated due to the lack of appropriate medical attention and care [17,31]. This may partly be a result of a lack of knowledge and focus on appropriate tools required to assess disorders involving chemical senses among medical practitioners. In this paper, we present a novel questionnaire that can be used to assess (i) patient's chemosensory and trigeminal disorders, (ii) their duration, (iii) their effect on food preferences, and (iv) the effect on patient's quality of life. This questionnaire may be helpful for nutritionists and other health professionals in getting an overview of patients' oral disturbances. This will further be beneficial in managing patients' dietary intake. The questionnaire consists of questions with yes and no answers, supplemented with multiple choice questions, and with the option "other", for open-ended answers. It is easy to fill in and not time-consuming. Therefore, it is practical for use both in clinical and research settings. One of the limitations in the present study is that we did not attain a full rate of completion of the questionnaire, as it was first introduced when we realized that patients were having major issues with dietary intake due to their oral health complications. Further studies are needed to validate this questionnaire.

A large proportion of patients reported dietary limitations because of either dysgeusia, burning mouth sensation, halitosis, dry mouth, or a combination of these different oral problems. A synergetic relationship between oral health and nutrition has been suggested [32], in other words, the relationship may be considered as a positive feedback or a vicious circle. Oral conditions, caused by either local or systemic diseases, impact the functional ability to eat and vice versa, and decline in dietary intake can lead to progression of oral diseases [32]. However, little is known about dietary implications and oral disorders in patients with dry mouth symptoms without SS diagnosis. Further studies are needed to gain better insight into mechanisms leading to oral disorders in this group of patients.

In the present study, there were no significant differences in salivary secretory rates between the two patient groups, indicating that both patient groups have similar problems with dryness of the mouth. Results from self-reported mouth dryness scores and measured salivary secretory rates were also well correlated in this study, indicating severe mouth dryness. Furthermore, significant associations were found in this study, among participants with pathologically low salivary flow rates and oral disorders (chemosensory disturbances, trigeminal disorders, halitosis, and DMFT), consistent with other studies [3,33,34]. These oral disturbances can affect the integrity of the oral cavity and, hence, lead to malnutrition [32].

Patients with pSS had a significantly higher number of decayed, filled, or missing teeth compared to non-SS patients and controls. The dental treatments performed on patients included dental fillings, crowns, and bridges. The reason behind extensive dental treatment may be related to low salivary secretory rates, presence of oral disorders, and/or dietary preferences. Interestingly, non-SS patients share similar symptoms with patients with pSS regarding salivary flow rates and oral disorders, but the same degree of dental treatment was not observed in this group. Other systemic, inflammatory causes in SS may therefore be considered as a potential cause of high caries experience in patients with pSS. These findings are consistent with other studies where dental caries status has been suggested as one of several potential markers of the extent of autoimmune-mediated salivary gland dysfunction in pSS [20].

About 60% of non-SS patients and 40% of patients with pSS reported halitosis when answering the questionnaire. However, no significant differences were observed between these two groups regarding subjective and objective measurements of oral malodor. Halitosis experienced in these patients could therefore neither be confirmed by organoleptic assessment, nor by analysis of VSC levels measured by gas chromatography. The difficulty in the self-assessment of breath odor has been discussed by Rosenberg [35]. Furthermore, organoleptic assessments may assess foul-smelling gases other than those containing sulfur (VSC), and this may explain the difference between organoleptic scores and VSC levels measured by GC. These findings are consistent with other studies where clinicians have reported that one-third of the patients seeking treatment for halitosis do not actually have genuine halitosis [36]. The presence of taste and smell dysfunction has been suggested as an alternative explanation for halitosis [36], which might also be the case in the patient groups in this study.

The main limitation of this study is the small sample size, especially for non-SS patients. The prevalence of SS has been reported to be 0.05% in the Norwegian population [12]. The low prevalence of SS is also reflected in our study with low sample sizes of both patients with pSS and non-SS. For reasons not clear to us, non-SS patients were more difficult to recruit to the study than patients with pSS. Another limitation of this study is the lack of assessments of dietary intake and body composition of the participants. We continue the inclusion of patients in these categories in our studies at the Dry Mouth Clinic and plan to introduce more dietary assessments in the future.

5. Conclusions

In conclusion, this study demonstrated significantly high occurrence of dysgeusia, burning mouth sensation, halitosis, reduced taste, and mouth dryness in non-SS patients and patients with pSS. Impaired smell function and caries experience were more severe in patients with pSS than non-SS patients. Associations were found between participants' self-reported dental health status and general health status indicating a clear synergy between oral and general health.

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Exploring Drivers of Liking of Low-Phenylalanine Products in Subjects with Phenyilketonuria Using Check-All-That-Apply Method

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Abstract: The aim of the present study was to apply the Check-all-that-apply (CATA) method in an ambulatory context involving subjects with phenylketonuria (PKU) to obtain a sensory description and to find the drivers of liking of low-phenylalanine products (Glycomacropeptide vs. L-amino acids formulas). 86 subjects with PKU (age range: 8–55 years) evaluated 8 samples: 4 L-amino acid formulas and 4 Glycomacropeptide (GMP) formulas, flavored with neutral, chocolate, strawberry and tomato aromas. Participants were asked to indicate which sensory attributes characterized each formulations and to score the overall liking. Significant differences were found regarding liking scores ($F = 65.29$; $p < 0.001$). GMP samples flavored with chocolate and strawberry, described as sweets, with a mild and natural taste and odor, were the most appreciated. Overall, GMP formulas obtained higher liking scores compared to L-amino acid formulas. Tomato flavored samples, described as bitter, salty, with artificial color, with strong taste and odor, obtained the lowest scores. In conclusion, CATA questionnaire seems to be a suitable method also in ambulatory context since this approach suggested that different foods and beverages with GMP could be developed to improve dietary treatment compliance of subjects with PKU from school age onwards.

Keywords: acceptability; food development; sensory attributes; CATA; dietotherapy; aromas

1. Introduction

Phenylketonuria (PKU; Online Mendelian Inheritance in Man, OMIM 261,600) is an autosomal recessive disorder of phenylalanine (Phe) metabolism [1], primarily due to mutations in Phe hydroxylase (PAH) gene, which facilitates conversion of the essential amino acid Phe to tyrosine. Loss of PAH activity results in increased Phe concentrations in the blood (hyperphenylalaninaemia, HPA) and therefore in toxic concentrations in the brain. The main goal of treatment for PKU is to maintain the blood Phe within safe limits to prevent mental retardation and ensure normal growth and life with good health through adulthood. The dietary treatment usually begins immediately after confirmation of PKU diagnosis in newborns and should be continued throughout their lifetime in patients with untreated phenylalanine levels more than 600 μmol/L. Compliance with treatment is adequate in infancy and childhood, however difficulties in maintaining a PKU diet in adolescent and adulthood are reported [2]. Patients with PKU have to avoid foods rich in protein (e.g., meat, fish and dairy products), thus the diet consists mainly of low-protein natural foods (vegetables, fruits) and special low protein products, such as bread and pasta with a protein content <1 g/100 g. The required

amount of daily protein is obtained from Phe-free protein substitutes providing essential amino acids in suitable proportions [3]. The number of protein substitutes (mixtures of amino acids that are free from or low in Phe) available for PKU patients is increasing constantly over time [4,5].

Even if improvements in the palatability, presentation, convenience and nutritional composition of substitutes have helped to improve long-term compliance with PKU diet, the acceptability of these substitutes remains a critical point, thus further improvement in this area is needed.

Casein Glycomacropeptide (GMP) is a protein derived from cheese whey that is rich in specific essential amino acids but it is the only known natural protein that in its purified form is free of tyrosine, tryptophan and phenylalanine. Therefore, GMP could provide an alternative protein source for PKU individuals when manufactured to sufficient purity to ensure the absence of Phe [6].

Studies in literature that consider the sensory analysis of low protein recipes for PKU dietotherapy are scarce. Moreover, these researches generally focused only on the overall acceptability of GMP products applying unsuitable hedonic methods [7,8]. Indeed, in the mentioned studies PKU subjects' sample size was not appropriate to perform a hedonic evaluation, since the subjects involved were less than the required number. The lack of empirical studies regarding the patients' satisfaction of the low-phenylalanine products and the requirement of new approaches for dietary management of PKU, reinforce the need to evaluate methods for studying PKU patients' perception about the sensory characteristics of low protein products. In this context, the Check-all-that-apply (CATA) questionnaire could be an alternative approach for this purpose, since it has been proposed as a valid and rapid method for obtain a descriptive profile from consumers [9]. Indeed, with this exploratory approach a larger number of attributes, compared to other sensory evaluation such as the Just-about-right scale performed on a set of well-known products focusing on few attributes, can be evaluated to identify those with greatest impact on hedonic product performance [10].

To our knowledge, there are no studies using valid method to evaluate and obtain a sensory characterization of low-phenylalanine products, especially in an ambulatory context.

The first aim of this study was to investigate the liking of low-phenylalanine products comparing L-amino acid formulas and GMP formulas. The second aim was to obtain a sensory description of the desirable and undesirable sensory properties of these products. For this purpose, the CATA questionnaire was used to identify PKU subjects' drivers of liking.

2. Materials and Methods

2.1. Participants

A total of 86 subjects with PKU (range age: 8–55 years), 45 males and 41 females, were recruited among patients referred to the Department of Pediatrics, San Paolo Hospital (Milan, Italy).

All participants had a biochemical diagnosis confirmed by genetic investigation and 6 participants were late diagnosed while for all others neonatal screening was made. The characteristics of the population are summarized in Table 1.

All participants were following a low-phenylalanine diet (84% of the participants were following a diet mainly based on L-amino acid formulas, 7% of the participants were not using a medical integration, 8% of the participant were following a diet based on both L-amino acid formulas and GMP, only one participant was using GMP). PKU children were defined as compliant to the diet when the annual mean Phe levels, monitored monthly by the Guthrie test, was within the range 120–360 mmol/L in childhood (<12 years) and 120–600 mmol/L in adolescence and adult age (>12 years) [2].

Table 1. Characteristics of participants.

Variable	PKU [a] 8–12 years (*n* = 18)		PKU > 13 years (*n* = 68)	
	Mean (SD [b])	**Median (25th–75th Centile)**	**Mean (SD)**	**Median (25th–75th Centile)**
Metabolic Control Phe (μmol/L)	268.5 (72.4)	272.6 (204.2–277.4)	569.0 (325.4)	471.9 (338.2–738.5)
Anthropometry [c]	Childhood and adolescence (*n* = 30)		Adult (*n* = 56)	
	Mean (SD)	Median (25th–75th centile)	Mean (SD)	Median (25th–75th centile)
BMI [d] (kg/m^2)			22.9 (4.6)	21.8 (19.7–25.6)
BMI Z-score	0.53 (1.0)	0.44 (−0.32–1.29)		
Underweight (%)		0		10.7
Normal-weight (%)		72.4		67.8
Overweight (%)		17.2		12.5
Obese (%)		10.3		8.9

[a] PKU: phenylketonuria; [b] SD: standard deviation; [c] according to WHO (http://www.who.int/en/); [d] BMI: body mass index.

The exclusion criteria were: pregnancy, food allergies to whey proteins, severe neurological and functional disorders. Every subject was asked for informed consent before making the assessments. The present study was performed according to the principles established by the Declaration of Helsinki after the protocol was approved by the Institutional Ethics Committee (protocol approval n°210).

2.2. Samples

Eight low protein recipes for PKU dietotherapy were used. The GMP formula (GMP_N; nutritional composition: 9 g carbohydrates of which 7 g sugars, 1.4 g fat and 5 g protein per 100 mL) was made using 100 mL of Glytactin RTD^{TM} (Cambrooke Therapeutics, MA, USA) whereas the L-amino acid formula (AA_N; nutritional composition: 43 g carbohydrates of which 3 g sugars, 14 g fat and 5 g protein per 100 g) was made mixing 16.5 g of powder high in L-amino acid (Xphe energy kid neutral, MetaX, Dietetic Metabolic Food: DMF, Limbiate, Monza Brianza, Italy) and water to reach a final volume of 100 mL.

The flavored versions were prepared by adding 2 g of flavoring powder to these neutral formulations. In particular, strawberry aroma (aroMaxx erdbeere, MetaX, DMF, Limbiate, Monza Brianza, Italy) or tomato and basil aroma (aroMaxx tomate-basilikum, DMF, Limbiate, Monza Brianza, Italy) were added to GMP-base formula (GMP_S and GMP_T; respectively), and chocolate aroma (aroMaxx schoko, MetaX, DMF, Limbiate, Monza Brianza, Italy) or tomato and basil aroma (aroMaxx tomate-basilikum, DMF, Limbiate, Monza Brianza, Italy) were added to L-amino acid formula and water (AA_C and AA_T; respectively). The GMP chocolate flavored sample (GMP_C) were prepared using 100 mL of Glytactin RTD^{TM} Chocolate (Cambrooke Therapeutics, Massachusetts, USA) and the L-amino acid strawberry flavored sample (AA_S) were prepared using 16.5 g of Xphe energy kid erdbeere (MetaX, DMF, Limbiate, Monza Brianza, Italy) and water. Each of these samples provides 5 g/100 mL protein equivalents.

A detailed composition of the samples is reported in Table 2.

Table 2. Compositions of the eight samples.

Samples	Composition
GMP [a] formulas	
GMP_N	50 mL Glytactin 10 RTD [b] neutral + 50 mL Glytactin 15 RTD neutral
GMP_C	50 mL Glytactin 10 RTD chocolate + 50 mL Glytactin 15 RTD chocolate
GMP_S	50 mL Glytactin 10 RTD neutral + 50 mL Glytactin 15 RTD neutral + 2 g strawberry aroma
GMP_T	50 mL Glytactin 10 RTD neutral + 50 mL Glytactin 15 RTD neutral + 2 g tomato and basil aroma
L-amino acid formulas	
AA_N	16.5 g Xphe energy kid neutral + water
AA_C	16.5 g Xphe energy kid neutral + 2 g chocolate aroma + water
AA_S	16.5 g Xphe energy kid erdbeere + water
AA_T	16.5 g Xphe energy kid neutral + 2 g tomato and basil aroma + water

[a] GMP: glycomacropeptide; [b] RTD: ready to drink.

The eight samples were presented as beverages to participants following a randomized and balanced order for each participant. Approximately 30 mL of each sample were presented to the participants monadically in plastic cups labelled with three-digit codes. Water was available for rinsing the palate.

2.3. Experimental Procedure

All the evaluations were performed in a quiet room and all the participants were tested at the same time (10:30–12:30). They were asked to refrain from consuming anything but water for 2 h before the test (hungry state). For each sample, subjects had to score their overall liking and to answer a check-all-that-apply (CATA) questionnaire.

2.4. Liking Assessment

Participants were asked to taste the products monadically and to express their liking scores. Children (aged 8–12 years) were asked to express their liking through a vertical 7-point facial hedonic scale, from "super good" (7) to "super bad" (1) [11], whereas subjects aged between 13 and 65 years rated their liking using a 10cm Visual Analogue Scale (VAS) anchored by the extremes "extremely disliked" (rated 0) and "extremely liked" (rated 10).

2.5. Check-All-That-Apply (CATA) Assessment

The CATA questionnaire consisted on a list of 27 sensory attributes including appearance, odor, taste, flavor and texture terms. Participants were asked to check from the list all the terms that they considered appropriate to describe each of the samples. The terms considered were the following: 10 for the appearance (light brown, dark brown, light yellow, dark yellow, light pink, dark pink, natural color, artificial color, brightness and opaque), 6 for the odor (natural odor, artificial odor, mild odor, strong odor, milk odor and vanilla odor), 8 for the taste/flavor (sweet, bitter, salty, sour, mild taste, strong taste, milk flavor and vanilla flavor) and 3 for the texture (thin, thick and floury). The position of attributes was randomized using the "to assessor" list order allocation scheme [12].

A separate group of 12 untrained PKU subjects aged 20–40 years' old took part in a pilot test, wherein judges used a free listing questionnaire to establish the appropriate terms to describe the samples [13]. They were provided with the eight formulations and for each sample, they were asked to pay attention to the sensory characteristics and to write all terms for describing their color, appearance, odor, taste, flavor and texture. An open discussion followed the development of lexicon. Then the experimenters finalized the list of terms, selecting the most mentioned and the most common word in order to avoid synonymous [14].

2.6. Data Analysis

A mixed ANOVA was carried out on overall liking data considering 'samples' (GMP_N; GMP_S; GMP_C; GMP_T; AA_N; AA_S; AA_C and AA_T), 'gender' (women and men), 'age' (young: <21 years old; adults: ≥21 years old) and their two-way interaction ('sample' × 'gender'; 'sample' × 'age') as fixed factors. Liking provided by children where adjusted by using a proportion in order to have results comparable to those provided by adults. In order to examine how the adherence to the dietotherapy affects the liking of the evaluated low-phenylalanine formulas a model was constructed with 'adherence to diet' ('good adherence' = Phe levels 120–360 mmol/L in children < 12 years, and 120–600 mmol/L in adolescence and adult age >12 years; 'scarse adherence' = Phe levels > 360 mmol/L in children < 12years, and >600 mmol/L in adolescence and adult age > 12 years) (van Spronsen et al. 2017) and 'samples category' (GMP and AA) and their two-way interaction as fixed factors. This analysis has also been performed considering only GMP samples which obtained the higher liking scores and the L-amino acid samples flavored with the same aroma. Thus, a model was constructed with 'adherence to diet' and 'samples' (GMP_S; GMP_C; AA_S; AA_C) and their two-way interaction as fixed factors. Participants were added as random factor in all the analyses. When a significant difference ($p < 0.05$) was found, least significant difference (LSD) *post hoc* test was used. These statistical analyses were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA).

For the CATA question, frequency of mention for each term was determined by counting the number of participants that used that term to describe each sample. Cochran's *Q* test was carried out for each of the 27 terms to detect differences in participants' perception of the evaluated samples. Correspondence analysis (CA) was performed to study the relationship between CATA questions and liking data. CA was performed on the frequency table containing responses to the CATA questions, considering the average liking scores by product as supplementary variable. These statistical analyses were performed using XLSTAT-Sensory® software for Windows, Version 2015.6.01 (Addinsoft™, Paris, France). A *p*-value of <0.05 was considered significant.

3. Results

3.1. Liking Assessment

The mean liking scores by samples are provided in Figure 1. The main factor 'samples' was found to have a significant effect on liking ($F_{(7,581)} = 65.29$, $p < 0.001$). Overall, GMP formulas obtained higher liking scores (M = 4.84 ± 0.18) compared to the L-amino acid formulas (M = 3.06 ± 0.18). In particular, GMP_S (M = 6.27 ± 0.26) and GMP_C (M = 6.27 ± 0.26), which were comparable to each other, obtained the highest liking scores. GMP_T (M = 2.06 ± 0.26), AA_T (1.81 ± 0.26) and AA_N (M = 2.15 ± 0.26), which were comparable to each other, obtained the lowest hedonic ratings and were not acceptable since they were below the middle of the scale.

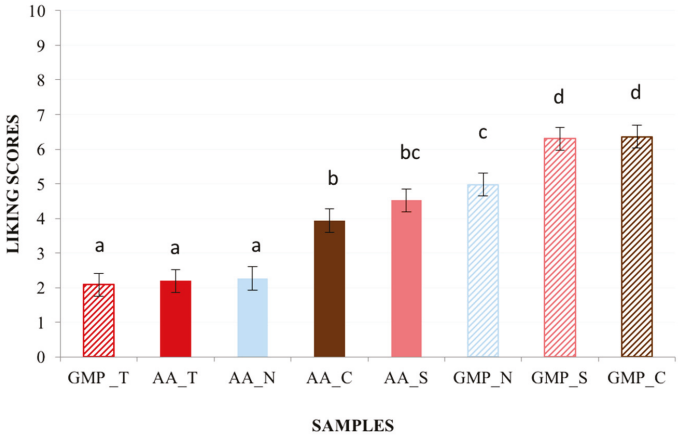

Figure 1. Liking score ± standard error mean (SEM) by samples. Different letters indicate significant differences according to *post hoc* test (Patients $n = 86$).

The main factor 'age' was found to have a significant effect on liking ($F_{(1,83)} = 5.96$, $p = 0.02$). Overall, the young participants provided higher liking scores (M = 4.31 ± 0.21) to the samples compared to the adult participants (M = 3.59 ± 0.21). Moreover, the interaction 'samples' × 'age' had a significant effect on liking scores ($F_{(7,581)} = 2.45$, $p = 0.02$). As shown in Figure 2, considering the less preferred samples (GMP_T, AA_T, AA_N, AA_C) young participants gave significant higher liking scores ($p < 0.05$) compared to adult participants.

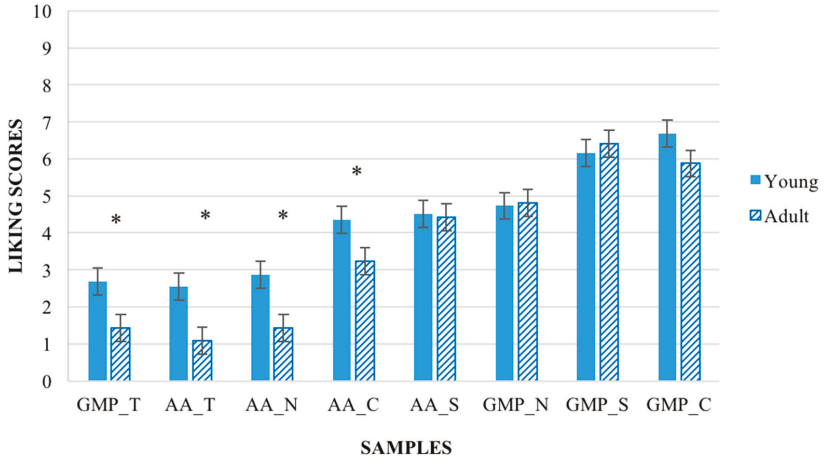

Figure 2. Liking score ± SEM by samples × age. Significant difference for * $p < 0.05$ (Young patients $n = 43$; Adult patients $n = 43$).

The main factor 'gender' and the interactions 'sample' × 'gender' were not significant ($F_{(1,83)} = 0.07$, $p = 0.80$; $F_{(7,581)} = 0.45$, $p = 0.87$, respectively).

A significant effect of the main factor 'adherence to diet' on liking scores was found ($F_{(1,84)} = 6.10$, $p = 0.02$). Generally, participants with 'scarce adherence' to the dietotherapy gave significant lower

liking scores (M = 3,63 ± 0.19) compared to participants with 'good adherence' (M = 4.37 ± 0.23). Moreover, the interaction 'adherence to diet' × 'samples category' had a significant effect on liking ($F_{(1,600)}$ = 5.06, p = 0.02). In particular, as shown in Figure 3, participants characterized by 'scarce adherence' gave significant higher liking scores to the GMP formulas (M = 3.74 ± 0.27) compared to the L-amino acid formulas (M = 2.55 ± 0.24).

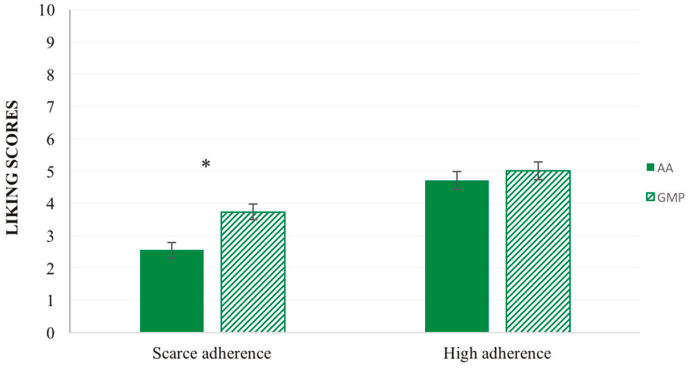

Figure 3. Liking score ± SEM by 'adherence to diet' × 'samples category'. Significant difference for * $p < 0.05$ (Patients with scarce adherence n = 49, Patients with high adherence n = 37).

Considering only GMP samples which obtained the higher liking scores (GMP_C and GMP_S) and the L-amino acid samples flavored with the same aroma (AA_C and AA_S) and the 'adherence to diet' a significant 'sample' effect was found ($F_{(1,256)}$ = 64.83, $p < 0.001$). As shown in Figure 4 patients characterized by a scarce adherence to diet gave comparable liking scores to GMP samples compared to patients with high adherence to the diet. Contrarily, patients with scarce adherence to diet gave significant ($p < 0.05$) lower liking scores to AA samples compared to subjects with high adherence to diet. The main factor 'adherence to diet' and the interaction 'adherence to diet' × 'samples category' were not significant ($F_{(1,84)}$ = 1.99, p = 0.16; $F_{(1,256)}$ = 3.75, p = 0.06, respectively).

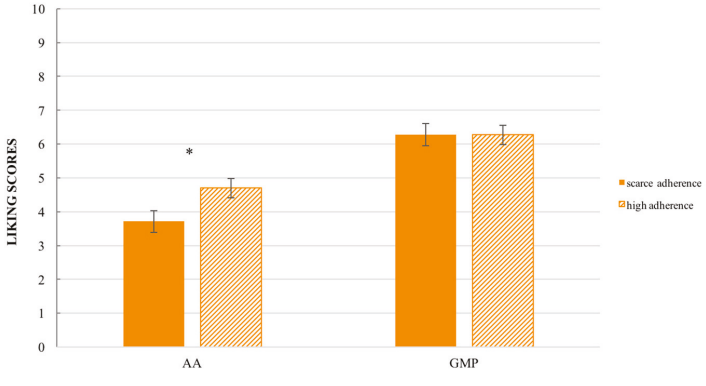

Figure 4. Liking score ± SEM by 'adherence to diet' × 'samples' (AA_C, AA_S, GMP_C, GMP_S). Significant difference for * $p < 0.05$. (Patients with scarce adherence n = 49, Patients with high adherence n = 37).

3.2. CATA Assessment

The frequency table of terms checked by patients to describe the eight samples is reported in Table 3.

As shown in Table 3 significant differences were found in the frequency for 25 out of 27 terms within the five categories considered, suggesting that participants perceived differences between samples in terms of their sensory characteristics. The sensory attributes that were not useful in order to discriminate samples were 'artificial color' and 'natural odor'. Indeed, looking at the frequency of mention of these attributes these terms were used quite homogeneously and were checked by less half of the respondents, indicating that the participants' consensus was low.

Table 3. Frequency mention of sensory attributes associated with each samples.

Sensory Modality	Sensory Attributes	Frequency of Mention							
		Samples							
		AA_C	AA_S	AA_N	AA_T	GMP_C	GMP_S	GMP_N	GMP_T
Appearance	Artificial color $^{n.s.}$	19	26	17	19	18	18	19	25
	natural color ***	11	10	27	10	20	10	23	8
	light yellow ***	0	0	14	1	22	0	34	3
	dark yellow ***	0	0	1	0	21	0	4	4
	brightness *	8	20	14	15	13	10	19	14
	light brown ***	5	0	0	62	25	0	3	61
	dark brown ***	81	0	0	14	3	0	0	7
	opaque ***	28	9	14	15	22	14	20	17
	light pink ***	0	79	0	0	0	23	0	4
	dark pink ***	0	6	0	0	0	60	0	2
Odor	artificial odor *	32	28	36	33	19	22	23	31
	mild odor ***	20	34	12	5	27	29	31	7
	milk odor ***	4	3	16	1	25	7	30	7
	vanilla odor ***	2	9	4	2	17	9	20	3
	strong odor ***	22	15	21	56	16	17	12	53
	natural odor $^{n.s.}$	13	11	10	8	16	16	12	5
Taste	sweet ***	27	43	9	5	54	70	34	10
	sour ***	13	19	29	24	4	6	4	31
	salty ***	11	9	23	55	4	1	5	49
	bitter ***	32	14	37	32	1	3	13	20
	mild taste ***	17	23	5	3	42	46	38	9
	strong taste ***	42	39	47	59	9	17	18	55
Flavor	milk flavor ***	10	8	7	1	32	12	38	7
	vanilla flavor ***	4	7	4	2	29	5	15	4
Texture	thin ***	18	50	33	24	31	33	44	37
	thick ***	38	4	27	24	17	20	14	13
	floury ***	25	2	18	14	12	10	14	10

$^{n.s.}$, non-significant difference according to Cochran's Q test. significant difference for * $p < 0.05$; *** $p < 0.001$.

3.3. Relating Sensory Profiling (CATA) with Liking

The purpose of this calculation was to establish which sensory attributes are mainly related to the overall liking of the samples and to obtain a perceptual map of the products based on both liking and sensory profiling.

The CA performed on the total frequency participants counts for each attributes resulted in two dimensions accounting for 60.43% of variance in the data. As inferred from the product plot (Figure 5), samples were discriminated according to their flavor, with samples with strawberry aroma (AA_S and GMP_S) in the upper right side of the map while the samples added with tomato aroma were positioned in the upper left side of the map (AA_T and GMP_T). Looking at the lower part of the map GMP formulas without aroma (GMP_N) and the chocolate one (GMP_C) are well distinguished from the L-amino acid formulas with the same aromas (AA_N and AA_C).

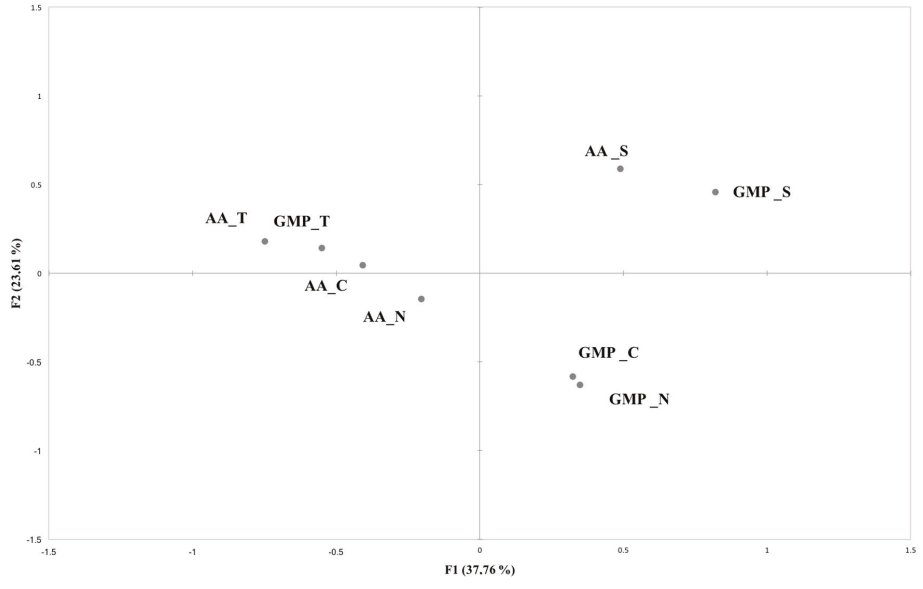

Figure 5. Products plot obtained from Check-all-that-apply (CATA).

The relation between sensory terms and overall liking of the eight samples is reported in Figure 6.

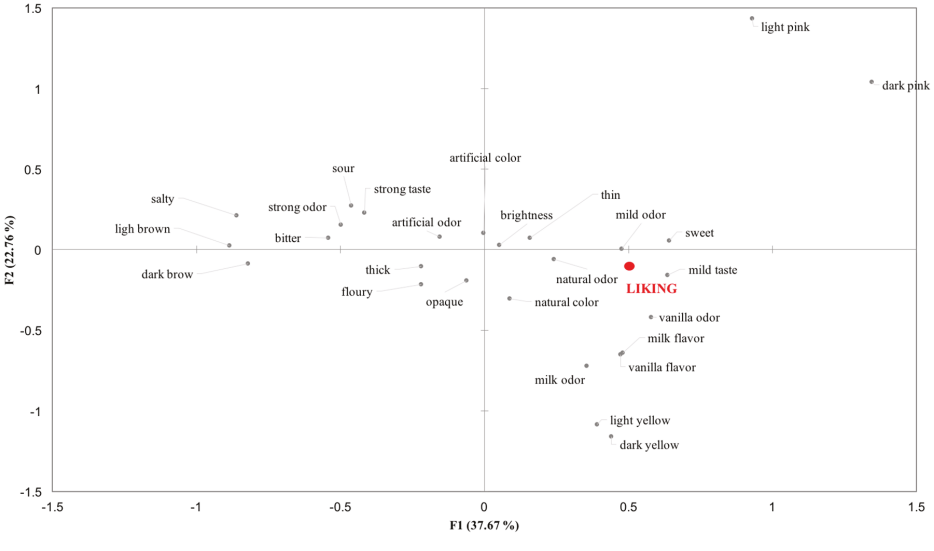

Figure 6. Attributes plot obtained from CATA total frequency counts and liking. Underline terms described samples that obtained higher liking scores while terms in italic describe mainly the disliked samples.

Comparing Figures 5 and 6, it is possible to see that participants liking was oriented toward GMP_C and GMP_S on the right side of the map, which were mainly associated with the sensory

attributes 'sweet taste', 'mild taste' and 'mild odor', and 'natural odor'. Liking was negative related to 'bitter taste', 'strong taste', 'salty', 'strong odor', 'artificial odor', 'light brown' and 'artificial color', which described the samples that obtained the lowest liking scores (GMP_T, AA_T and AA_N).

4. Discussion

The aim of the present study was to perform the Check-all-that-apply (CATA) method in an ambulatory context involving subjects with phenylketonuria (PKU) to obtain a sensory description and to find the drivers of liking of low-phenylalanine products (Glycomacropeptide vs. L-amino acids formulas).

We demonstrated a greater acceptability of GMP beverages compared to the amino acid formulas currently required as the primary source of protein in the PKU diet [15]. Moreover, GMP samples flavored with chocolate and strawberry, described as sweets, with a mild and natural taste and odor, were the most appreciated. Contrarily, tomato flavored samples, described as bitter, salty, with artificial color, with strong taste and odor, obtained the lowest liking scores.

Liking scores between women and men were comparable to each other while young subjects provided generally higher liking scores compared to the adults. Accordingly, it is well known that subjects become more critical in their food choices and preferences with increasing age [16], maybe due to a greater exposure to a wide range of food products.

Previous studies explored the overall acceptability of GMP products, but no one investigated which sensory properties characterized these products [7,8,17]. None of the mentioned studies measured acceptability with an appropriate method or with a representative sample size. Indeed, Lim and collaborators (2007) showed that a GMP chocolate beverage was significantly more acceptable compared to the same flavored amino acid beverage. However, PKU subjects' sample size was not appropriate to perform a hedonic evaluation, since the subjects involved were less than the required number. Similarly, van Calcar and colleagues [8] concluded that, in a group of only 10 subjects involved in an 8-day inpatient metabolic study, the GMP products were better tasting compared with usual amino acid formulas, both consumed during the treatments. Again, this assumption was not supported by a proper hedonic evaluation, since, besides the small group of subjects recruited, any quantitative sensory evaluation was performed. Recently, Ney and colleagues (2016) in a randomized crossover trial with 30 early-treated phenylketonuria subjects found, using a questionnaire, that GMP samples were generally more acceptable than AA formulas. However, a sensory evaluation was not performed to confirm these results. From a methodological point of view, this is the first study that included an adequate sample of subjects with PKU and fulfilled the requirements to perform a sensory description of products using the CATA approach [9]. In line with the literature data, an appropriate sample size was recruited [18] and a suitable list of CATA questions was used. Indeed, in order to consider consumer heterogeneity and avoiding a dilution effect of the responses, it has been reported that a minimum of 10 to a maximum of 40 terms should be comprised in the list [14,19].

Subjects' CATA counts were significantly different for the evaluated samples suggesting that this technique was able to detect differences in subjects' perception of low protein products. Thus, CATA questionnaire may represent an alternative and rapid method for the description of products' sensory characteristics, when it is difficult to apply traditional sensory descriptive analyses (e.g., sensory profile). This new approach has been already successfully applied to evaluate sensory perception of a food product in particular context or with specific group of subjects [20,21]. Indeed, De Pelsmaeker and collaborators [20] used this method with 8- to 13-year-old children to obtain emotional profiles of food, and Laureati and collaborators [21] tested CATA questions in a natural context (e.g., school) as an alternative approach to descriptive methods in food product development with a young panel.

Present results support the feasibility of GMP in making a great selection of palatable foods and beverages to improve the taste, variety and compliance of the PKU diet, with a positive impact on it. In addition, it has been suggested the ability of GMP to promote a greater satiety when compared to amino acid-based formulas and to suppress plasma ghrelin levels in individuals with PKU [8,22].

Regarding variety, one the biggest obstacle to following the PKU diet is that the amino acid mixtures are usually available and consumed as a liquid formula [23]. On the contrary, GMP is well suited for use not only in beverages but also in semi-solid foods [24]. Indeed, various low-phenylalanine products such as beverages, pudding and crackers has been developed [15], due to its functional properties including good heat stability and solubility in acid [25].

Moreover, the main goal in PKU treatments is to facilitate long-term dietary compliance and to ultimately improve quality of life and metabolic control for individuals with PKU. Indeed, data in literature shown that compliance with treatment seems to be adequate in infancy and childhood [26] but scarce adherence and difficulties in maintaining PKU diet have been reported in patients above 16 years of age, in whom Phe values were within or below guideline goals [27]. Even if, evidences suggested that GMP is more acceptable than the traditional amino acid mixtures it is not an easy task to change patients' food habits trough these new formulations. Indeed, it is well known that food habits are difficult to be changed and PKU patients are used to drink amino acid mixtures since infancy. PKU is an extreme example of a well-established eating pattern. This suggests that subjects with PKU who are compliant with consuming AA formulas are imprinted with a preference for the taste and emotional components associated with lifelong consumption these formulas [17]. Indeed, Ney and colleagues (2016) showed that patients used to consumed the amino acid mixtures stated that GMP tasted better but that they still craved AA formulas. Moreover, even if it has been shown that in PKU mouse model the ingestion of GMP decreased Phe concentrations in blood and increased the brain tissue, researches are ongoing to evaluate the long-term safety and efficacy of GMP in the nutritional management of PKU [2,28].

The present data suggested that subjects with a scarce adherence to diet preferred generally the GMP formulas compared to the commonly amino acid mixtures, supporting the hypothesis that the implementation of the diet with more appreciated products could maybe enhance their dietary compliance, improving also subjects' health status, especially in adults.

5. Conclusions

In conclusion, the sensory approach through the CATA method could be useful to understand how implements dietotherapy of subjects with PKU, considering their satisfaction as one of the main aspects during the product development. As future perspective, it could be useful for industries to develop new GMP products taking into account the information related to the sensory perception, specially to taste and odor attributes, in order to satisfying at the same time both nutritional and sensory aspects. Thus, the low-Phe formulas should have mild odor and taste, they should be sweet and with a more natural odor compared to the traditional mixtures.

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A Preventive Prebiotic Supplementation Improves the Sweet Taste Perception in Diet-Induced Obese Mice

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Abstract: Orosensory perception of sweet stimulus is blunted in diet-induced obese (DIO) rodents. Although this alteration might contribute to unhealthy food choices, its origin remains to be understood. Cumulative evidence indicates that prebiotic manipulations of the gut microbiota are associated with changes in food intake by modulating hedonic and motivational drive for food reward. In the present study, we explore whether a prebiotic supplementation can also restore the taste sensation in DIO mice. The preference and licking behavior in response to various sucrose concentrations were determined using respectively two-bottle choice tests and gustometer analysis in lean and obese mice supplemented or not with 10% inulin-type fructans prebiotic (P) in a preventive manner. In DIO mice, P addition reduced the fat mass gain and energy intake, limited the gut dysbiosis and partially improved the sweet taste perception (rise both of sucrose preference and number of licks/10 s vs. non-supplemented DIO mice). No clear effect on orosensory perception of sucrose was found in the supplemented control mice. Therefore, a preventive P supplementation can partially correct the loss of sweet taste sensitivity found in DIO mice, with the efficiency of treatment being dependent from the nutritional status of mice (high fat diet vs. regular chow).

Keywords: Obesity; taste; eating behavior; prebiotics; microbiota

1. Introduction

Development of a nutritional obesity is a complex phenomenon depending on multiple causes among which food choice plays a significant role. By determining nutrient quality and acceptability, gustation is considered as an important sensory driver of the food selection and intake. However, the relationships between taste and obesity remains poorly understood. Sweet taste sensitivity is challenged in obese rodents. Rats and mice chronically subjected to an obesogenic high fat diet (HFD) become unable to detect properly low concentrations of sweet solutions during behavioral tests minimizing post-ingestive cues (e.g., neuro-endocrine regulations) [1]. A blunting of both peripheral detection and central perception to sweet stimuli might explain this relative loss of taste sensitivity. Indeed, sucrose-evoked calcium signaling is dramatically decreased in taste bud cells freshly isolated from diet-induced obese (DIO) mice [2]. Similarly, chronic HFD elicits a down-regulation of dopamine and opioid receptors [3,4] in the mesolimbic area leading to a progressive devaluation of the reward value of oral stimuli, as found with abuse drugs [5]. Such a diet-acquired sensory deficiency might explain the tendency of DIO rodents to overeat high rewarding foods [1], probably to gain the desired hedonic satisfaction [3].

This diet-induced gustatory disorder is widely corrected in rodents after bariatric surgery, leading to healthier food choices [6–8]. Understanding how this improvement of the sweet taste sensitivity takes place might open new insights in obesity treatment. In rats, Roux-en-Y gastric by-pass is associated with changes in gut microbiota similar to those found after a prebiotic (P) supplementation [9], known to affect the production of hormones controlling the eating behavior, such as glucagon-like peptide-1 (GLP-1) which has an anorexigenic effect. Interestingly, behavioral responses to sweet compounds (i.e., number of licks per 10 s) are reduced in GLP-1 receptor-null mice (GLP-1 R), as compared to wild-type animals [10]. Intestinal dysbiosis is also associated with a chronic low-grade metabolic inflammation by promoting intestinal permeation of lipopolysaccharides (LPS) derived from the outer membrane of Gram-negative bacteria [11,12]. These endotoxins promote the release of pro-inflammatory cytokines in various tissues by activating members of the Toll-like receptors (TRL). A set of observations suggests that this inflammatory environment might play a role in the change of taste sensitivity in DIO mice. Indeed, taste buds express the TLR4 signaling cascade and, thus, are LPS responsive [13]. Moreover, a chronic consumption of a HFD rich in saturated fatty acids elicits a pro-inflammatory gene profile in the gustatory papillae [14]. Finally, chronic endotoxemia reduces the number of taste buds in obese mice [15]. Collectively, these findings suggest an implication of the intestinal dysbiosis in the impairment of the sweet taste sensitivity observed in DIO mice that could be improved by a prebiotic supplementation.

To explore this hypothesis, the impact of a preventive prebiotic supplementation on the orosensory perception of a sweet stimulus was compared in lean and DIO mice. Specific gut bacteria, known to be involved in the regulation of the gut peptide production and/or the gut barrier function such as *Bifidobacterium* spp. and *Akkermansia muciniphila* [16,17], were analyzed in the caecal content of mice to highlight the prebiotic effect of inulin in our model.

2. Materials & Methods

2.1. Animals

This study was carried out in the strict accordance with European guidelines for the care and use of laboratory animals and protocol approved by the French National Animal Ethic Committee (CNEA n°105). Six-weeks-old C57Bl/6 male mice were purchased from Charles River Laboratories (France). Animals were individually housed in a controlled environment (constant temperature and humidity, dark period from 7 p.m. to 7 a.m.) and had free access to tap water and chow. Experiments took place after a one-week acclimatization period. To study the impact of a preventive prebiotic treatment on the orosensory perception of sucrose during a diet-induced obesity, standard laboratory chow or custom high fat diet (Table 1) were supplemented with 10% prebiotic (P) and mice were split in four groups (n = 8–10): lean controls fed regular chow (C), lean controls fed supplemented regular chow (C+P), diet-induced obese mice (DIO) and supplemented diet-induced obese mice (DIO+P). Mice were fed *ad libitum* for 12 weeks. Inulin-type fructans (P—Fibruline®, Cosucra, Pecq, Belgium) was used as prebiotic. We have chosen a 10% prebiotic enrichment since this supplementation is known to promote metabolic [18] and cognitive benefits [19].

Evolution of the body composition (i.e., fat mass) was determined by nuclear magnetic resonance relaxometry (EchoMRI—Echo Medical Systems, Houston, TX, USA).

Table 1. Composition of the diets.

Contents (% *w*/*w*)	Control Diet *(4RF21 Mucedola)*	Control Diet + Prebiotic	High Fat Diet *(4RF25 Mucedola + palm oil)*	High Fat Diet + Prebiotic
Proteins	18,5	16,65	15	13,5
Carbohydrates				
Starch	53,5	48,15	34,4	30,96
Lipids				
Soybean oil	3,0	2,7	2,4	2,16
Palm oil	0,0	0,0	31,8	28,62
Saturated fatty acids	0,5	0,45	16,7	15,03
Mono-unsaturated fatty acids	0,5	0,45	13,0	11,7
Poly-unsaturated fatty acids	1,3	1,17	4,5	4,05
Prebiotic Inulin-type fructan	0,0	10	0,0	10
Energy (Kcal/100 g)	315,0	315,2	505,8	506,0

2.2. Two-Bottle Choice Tests

Tests were performed for 12 h at the beginning of the dark period in individually housed mice. Animals were food restricted during the duration of the experiment [20]. This protocol provides behavioral data combining orosensory sensations (i.e., oral detection and central perception) and post-ingestive cues. Mice were subjected to a choice between a control solution (0.3% xanthan gum in water to minimize textural influences) or a 1% sucrose in control solution. At the end of the test, fluid intake was measured for each bottle and the preference (i.e., ratio between experimental solution consumption and total intake) was calculated.

2.3. Gustometer

Licking behavior was studied using an original octagonal shaped gustometer of which each side has a computer-controlled shutter giving random access during a short time (10 s in the present study) to a bottle filled with a specific solution. All the bottles (five in the present study) are equipped with a lickometer. This original design, which forces the animal to move to access to the drinking source, allows a simultaneous analysis of the licking behavior, which mirrors the immediate pleasure gained from the consumption of a rewarding stimulus (i.e., "liking") and the motivation to drink (i.e., "wanting"). Concept and procedures are detailed in [21].

In brief, 20 h water-deprived mice were subjected to two training sessions before the taste-testing sessions (30 min, each). During the first training, all the doors were opened so that the mouse had free access to all the bottles filled with water. It was a time of habituation to a new environment. During training two, the mouse learned to drink according to the protocol used during the brief-access taste testing (i.e., random opening of shutters), all the bottles being filled with the control solution (i.e., water). Each mouse had access to a first bottle for 10 s after the first lick. After this trial, all doors remained closed for 10 s before another one was opened among the 4 remaining shutters, in a randomized manner. The program continued until the animal had licked all five bottles. This event constituted a block. At the end of one block, another block started, so that the number of blocks mirrored the motivation for the stimulus. A taste-testing session was performed in water and food deprived mice to explore their licking responsiveness to a set of sweet stimuli (0.01, 0.2, 0.6, 1.0 M sucrose).

2.4. Blood Analysis

Freshly drawn blood samples from fasted animals were centrifuged at 6000 g for 15 min (4 °C). Plasma was collected and kept at −80 °C. Glucose, total cholesterol and triglycerides were assayed in

plasma samples using commercial kits certified for in vitro diagnosis (colorimetric assays, ref#981780, 981786 and 981813) on Indiko device from Thermo (Waltham, MA, USA).

2.5. Gut Microbiota Analysis

Genomic DNA was extracted from the caecal content using a silica membrane-based purification technique with QIAamp DNA Stool Mini Kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions, including a bead-beating step. Total bacteria, *Bifidobacterium* spp. and *Akkermansia* spp. were analyzed by quantitative PCR, as previously described [22].

2.6. Statistics

Results are expressed as Means ± SEM. The significance of differences between groups was evaluated with R software (v3.4.4; The R Foundation, Vienna, Austria). We first checked that the data for each group were normally distributed and that variances were equal. We then carried out either a Student's *t*-test or a Two-way ANOVA with the Tukey HSD post-hoc test. A principal component analysis (PCA), normalized and centered, was done with R software and the R-commander package (v2.4.4) on the different parameters studied.

3. Results

3.1. Prebiotic Supplementation Attenuates the Negative Effects Elicited by a Diet-Induced Obesity

Four groups of mice, subjected for 12 weeks to distinct diets (regulatory chow or obesogenic diet alone or supplemented with 10% P), were used to explore putative changes in orosensory perception of sweet stimuli (Figure 1A).

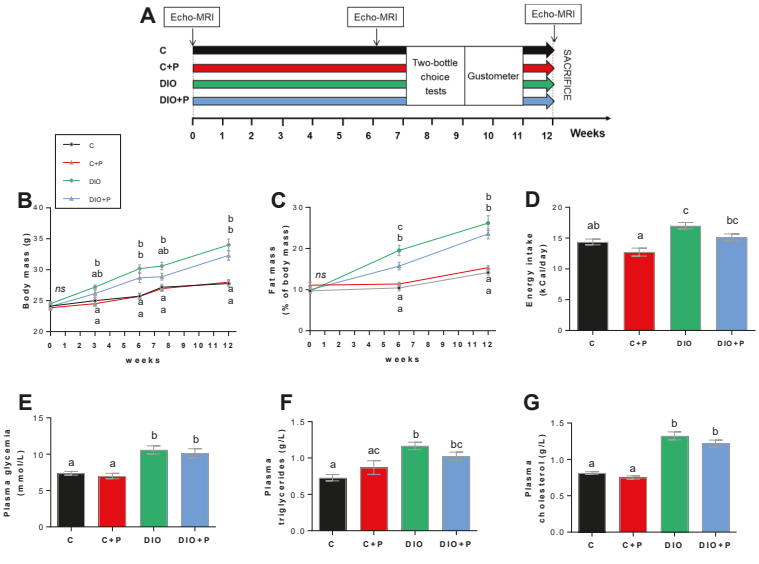


Figure 1. Comparison of body and biochemical parameters in mice subjected for 12 weeks to a regulatory chow or an obesogenic diet alone (C and DIO) or supplemented with 10% Prebiotic (C+P and DIO+P). (**A**) Time course of the experiment; (**B**,**C**) Evolution of the body and fat; (**D**) Daily energy intake; (**E**) Blood glucose; (**F**,**G**) Plasma triglyceride and cholesterol levels. Mean ± SEM, different letters indicate a statistical difference between groups. Significance was achieved at $p < 0.05$. C, C+P, DIO, $n = 10$. DIO+P, $n = 8$.

To verify the efficiency of this experimental design, body mass and composition, energy intake, various blood parameters, cecal tissue mass, mass of cecal content and cecal bacteria were analyzed. As expected, mice fed with the HFD displayed a greater gain in body weight and fat mass than animals fed the regular chow (Figure 1B). Prebiotic addition to the HFD led to a lower body mass gain as compared to DIO mice (Figure 1B) mainly attributable to a diminution in the relative fat mass (Figure 1C). Such a phenomenon was not observed in lean mice (Figure 1B,C). Prebiotic supplementation elicited a slight decrease in the energy intake whatever the diet, but this effect was insignificant (Figure 1D). According to previous data [23], blood glucose, plasma triglycerides and cholesterol levels were increased in DIO mice (Figure 1E–G). Surprisingly in our hands, these systemic changes were not improved in prebiotic-treated mice.

In agreement with the literature [23], chronic prebiotic consumption increased the caecal tissue mass and, in a lower extent, the fecal mass in caecum (Figure 2A,B). Prebiotic supplementation is known to modify gut microbiota composition [24]. To explore whether the bacterial signature was modified by our treatment, cecal bacterial content was studied by qPCR. As shown in Figure 2C, prebiotic supplementation tended to increase the cecal content of total bacteria whatever the diet. When abundance of selected bacterial displaying beneficial health effect was measured, a rise of *Bifidobacteria* and *Akkermansia* abundance was found in the DIO+P group (Figure 2D,E). We have previously described that ITF feeding promotes endogenous GLP-1 production through higher expression of proglucagon in the colon [25,26]. In the present study, we showed that the higher level of its expression was found in the cecal tissue from DIO+P mice (Figure 2F). Collectively, these data demonstrate the efficiency of our prebiotic protocol on DIO mice.

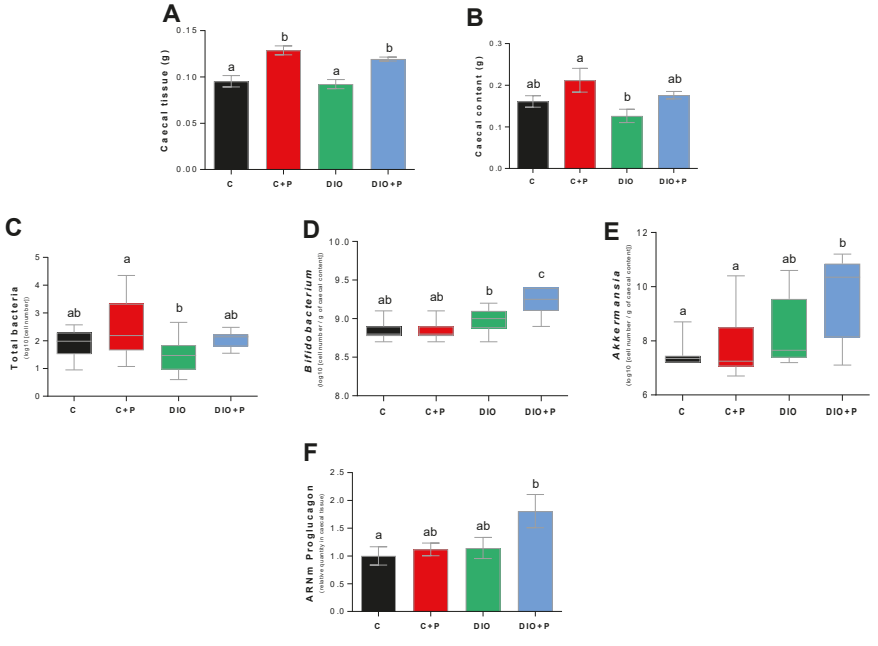


Figure 2. Comparison of bacterial parameters in mice subjected for 12 weeks to a regulatory chow or an obesogenic diet alone (C and DIO) or supplemented with 10% Prebiotic (C+P and DIO+P). (**A**) Cecal tissue mass; (**B**) Fecal mass in caecum; (**C**) total cecal bacteria; (**D**) *Bifidobacterium*; (**E**) *Akkermansia muciniphilla*; (**F**) relative proglucagon mRNA levels in caecum in reference to a housekeeper gene (RPL19). Mean ± SEM, different letters indicate a statistical difference between groups. Significance was achieved at $p < 0.05$. C, C+P, DIO, $n = 10$. DIO+P, $n = 8$.

3.2. The Lower Sucrose Preference Found in DIO Mice Was Improved in Presence of Prebiotic

The preference for a sweet stimulus was explored using a two-bottle choice test. Consistent with previous published data [27], DIO mice subjected to a long-term (12 h) two-bottle preference test showed a significant lower intake (Figure 3A) and preference for 1% sucrose solution than C or C+P groups (Figure 3B), suggesting a diet-induced modification of the orosensory perception of the sweet sensation. The prebiotic attenuated the effect of the DIO. Indeed, sucrose intake was higher in DIO-P group than DIO mice, this change improving their preference score to a level similar to C and C+P mice (Figure 3B).

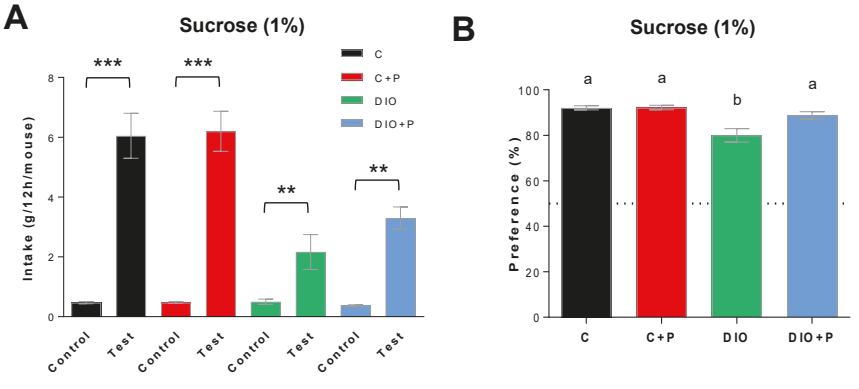


Figure 3. Two-bottle choice test analysis of orosensory perception of a sweet stimulus in mice subjected to a regulatory chow or an obesogenic diet alone (C and DIO) or supplemented with 10% Prebiotic (C+P and DIO+P). Animals were simultaneously subjected for 12 h to a control solution (0.3% xanthan gum in water, *w*/*v*) and a test solution containing 1% sucrose (*w*/*v*) in the control solution. (**A**) Final consumption of control and experimental solution; (**B**) Preference i.e., ratio of the final consumption of control or experimental solution upon the final total liquid intake. Mean ± SEM. (**A**) Student *t* test: ** $P < 0.01$, *** $P < 0.001$; (**B**) 2-way ANOVA + Tukey HSD: different letters indicate a statistical difference between groups. Significance was achieved at $p < 0.05$. C, C+P, DIO, $n = 10$. DIO+P, $n = 8$. The dotted line represents the 50% preference.

3.3. Prebiotic Supplementation Improves the Licking Behavior in Response to Sucrose Stimulus in DIO Mice

Licking behavior was studied by using the gustometer FRM8 [21]. Training sessions failed to reveal any behavioral differences between mice suggesting that DIO and/or prebiotic supplementation did not affect animal adaptability to a new environment (training 1—Figure 4A) neither their ability to learn how the device works (training 2—Figure 4B). The fact that the number of total licks was similar between groups attests that mice did not present any oromotor or mobility defect (Figure 4C).

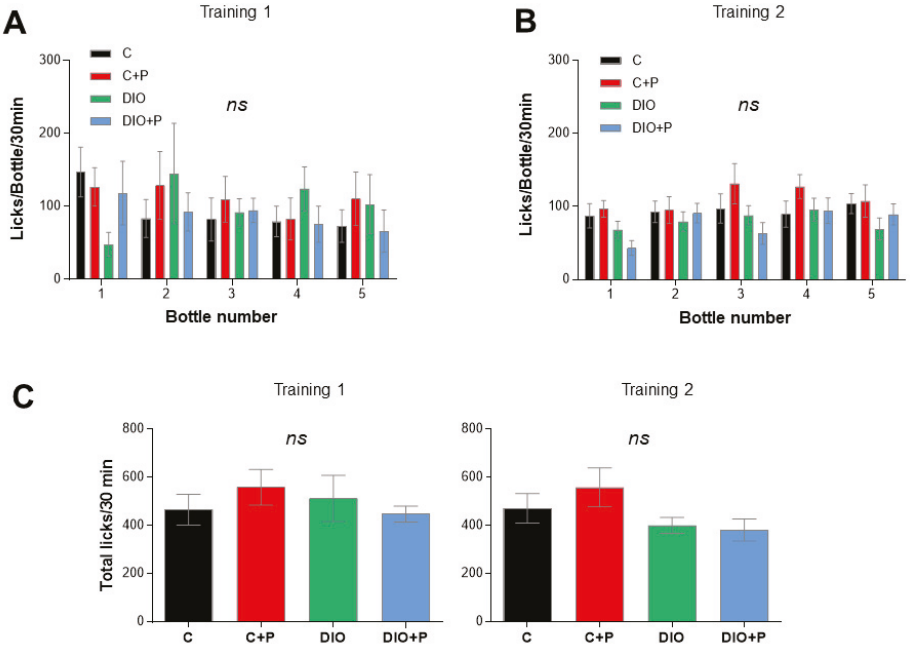


Figure 4. Gustometer analysis: training sessions in mice subjected to a regulatory chow or an obesogenic diet alone (C and DIO) or supplemented with 10% Prebiotic (C+P and DIO+P). 20 h water-deprived mice were subjected to 2 training sessions before the taste-testing sessions (30 min, each). (**A**) Training 1: Each mouse had a free access to the 5 bottles filled with water in order to determine the licking rate/bottle/30 min. It is time of adaptation to a new environment; (**B**) Training 2: Each learned to drink water according to the protocol used during the brief-access taste testing, i.e., a random and intermittent opening of shutters. The licking rate/bottle/30 min was determined; (**C**) Total licks for 30 min during the training 1 and 2. Mean ± SEM. 2-way ANOVA + Tukey HSD. ns non-significant. C, C+P, DIO, $n = 10$. DIO+P, $n = 8$.

In control mice, licking activity (licks/10 s) showed a typical dose-response curve in response to growing concentrations of sucrose, with a maximal frequency around 60 licks/10 s (Figure 5A). According to previous data [1], the number of licks/10 s elicited by sweet solutions was dramatically reduced in DIO mice as compared to controls (Figure 5A). Consistent with this observation, total licks (i.e., immediate pleasure gained from the consumption of a rewarding stimulus or "*liking*") and number of blocks (i.e., motivation to drink, "*wanting*") during the taste testing session (30 min) were significantly lower in DIO mice as compared to controls (Figure 5B,C). Altogether, these data confirm the existence of substantial differences in the licking responses to a sweet stimulus between C and DIO mice. A licking rate improvement was found in the prebiotic supplemented groups (Figure 5A). In response to the higher sucrose concentration, DIO+P mice displayed a similar licking response than C group, (Figure 5A), suggesting that this prebiotic treatment is able to counteract partially the orosensory deficit found in DIO mice. Despite of this significant improvement, only an upward trend in the total number of licks differentiated the DIO+P group from the DIO group (Figure 5B), the number of blocks remaining unchanged (Figure 5C).

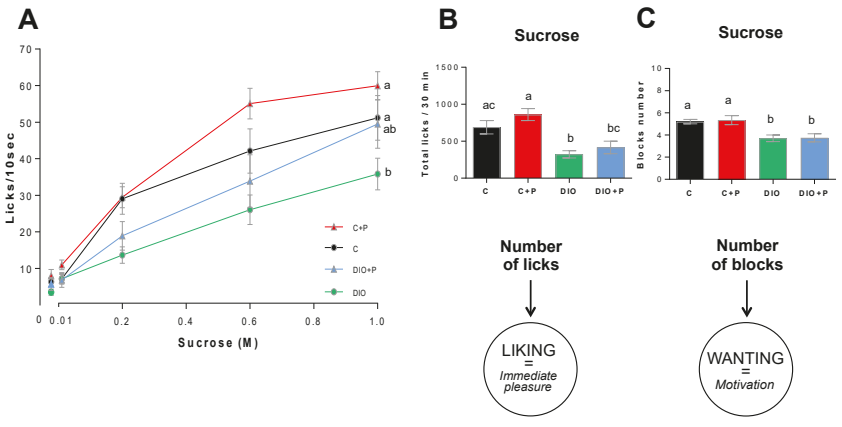


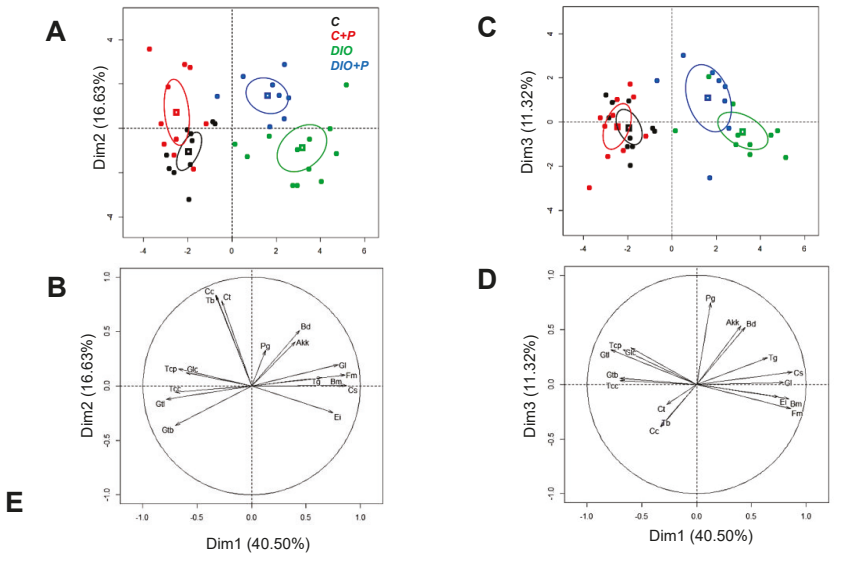
Figure 5. Gustometer analysis of orosensory perception in response to various concentration of sucrose in mice subjected to a regulatory chow or an obesogenic diet alone (C and DIO) or supplemented with 10% Prebiotic (C+P and DIO+P). (**A**) Brief-access taste testing responses (licks/10 s) of naïve mice to control solution (0.3% xanthan gum in water) and ascending concentrations of sucrose (0.01, 0.3, 0.6, 1.0 M). Random access to bottles was computer controlled. Zero on the x-axis represents the licking rate obtained in response to the control solution; (**B**,**C**) Total number of licks (representative of the "Liking" component) and number of blocks (representative of the "Wanting" component) performed for 30 min. Mean ± SEM. 2-way ANOVA + Tukey HSD, significance was achieved at $p < 0.05$. Different letters indicate a statistical difference between groups, ns non-significant, C, C+P, DIO, $n = 10$. DIO+P, $n = 8$.

3.4. Prebiotic Supplementation Brings the DIO Group Closer to Control Group

In order to better delineate the global impact of a prebiotic supplementation in control and DIO mice, a multivariate analysis (principal component analysis—PCA) was performed from variable values at the time of behavioral phenotyping (Figure 1A). Values on the *x*-axis represent the component score for the dimension 1, and those on the *y*-axis for dimensions 2- and 3, accounting for 40.50, 16.63 and 11.32% of the inertia (total = 68.45%), respectively.

Confidence ellipse analysis highlighted that C and C+P groups were partly overlapping suggesting that prebiotic supplementation did not induce any discriminant change in lean mice according to the variables studied (Figure 6A,C). They were mostly defined on the dimensions 1 and 2 by variables related to efficient taste sensations (positive correlation with high number of licks, blocks and preference) and cecal bacteria content (Figure 6B,E).

As expected, they were clearly different from the DIO group, which was mainly characterized by variables linked to obesity (cholesterol, body mass, fat mass, energy intake, glycaemia and triglycerides—Figure 6E) on the dimension 1. The third group representing the DIO+P group was found in an intermediate position between lean and obese mice suggesting that the prebiotic supplementation improved the health status of DIO mice (Figure 6A,C). Interestingly, on the dimensions 2 and 3, the DIO+P group was positively correlated with variables linked to the high caecal bacteria content and related to taste sensations (Figure 6D,E). In brief: DIO+P mice exhibited a healthier pattern than DIO mice and were closer to C & C+P mice.



C		C+P		DIO		DIO+P	
Gtl	Gustometer total licks	Gtl	Gustometer total licks	Cs	Cholesterol	Cc	Caecal content
Gtb	Gustometer total blocks	Gtb	Gustometer total blocks	Fm	Fat mass	Tb	Total bacteria
Tcc	Two choice consomption	Tcc	Two choice consomption	Bm	Body mass	Ct	Caecum tissue
Tcp	Two choice preference	Tcp	Two choice preference	Gl	Glycemia	Bd	Bifidobacterium
Glc	Gustometer licks curves (AUC)	Glc	Gustometer licks curves (AUC)	Ei	Energy intake	Akk	Akkermansia
Cc	Caecal content	Cc	Caecal content	Tg	Triglycerides	Pg	Proglucagon
Tb	Total bacteria	Tb	Total bacteria	Bd	Bifidobacterium	Glc	Gustometer licks curves (AUC)
				Akk	Akkermansia		

Figure 6. Principal component analysis performed from studied variables in mice subjected to a regulatory chow or an obesogenic diet alone (C and DIO) or supplemented with 10% Prebiotic (C+P and DIO+P). (**A**) Confidence ellipse analysis. Cluster distribution along the dimension 1 & 2. Each dot represents a mouse; (**B**) Arrows represent the direction of each variable in the 2-dimensional PCA space; (**C**) Cluster distribution along the dimension 1 & 3. Each dot represents a mouse; (**D**) Arrows represent the direction of each variable in the 2-dimensional PCA space; (**E**) Variables significantly representative of the 4 clusters (ranking in descending order of importance) and their respective abbreviations.

4. Discussion

Deciphering the functional links between the nutritional obesity, taste sensitivity and eating behavior is a challenge that might open new corrective nutritional and/or pharmacological interventions to fight obesity. Nevertheless, how diet-induced obesity affects the taste perception is not yet fully understood.

Chronic consumption of an obesogenic diet (e.g., *saturated high fat diet*) leads to a shift in the gut microbiota composition and a progressive accumulation of body fat. These changes might promote a chronic low-grade inflammation (e.g., endotoxin release and production of pro-inflammatory cytokines) and a new endocrine balance (e.g., drop of GLP-1 and rise of leptin) decreasing the orosensory acuity (at taste bud level) and related reward response (corticomesolimbic level). Taken together these alterations might promote an over-consumption of energy-dense foods to compensate the sensory deficit.

According to our present knowledge, the following scenario has been proposed [28]. Gut dysbiosis and increased fat mass elicited by the chronic consumption of an obesogenic diet, induce a low-grade inflammation (e.g., LPS release and proflammatory cytokines) associated with an endocrine unbalance

(e.g., low GLP-1 and high leptin levels). Collectively, these systemic changes might blunt both the taste acuity (taste bud level) and the taste-driven reward behavior (corticomesolimbic level), modifying food choices to reach an expected hedonic satisfaction. This new gustatory phenotype might lead to a preferential consumption of highly palatable foods, rich in sugar and fat, and thus to a growing obesity (Figure 7). Especially, the putative role of the gut microbiota on the taste efficiency remains to be established.

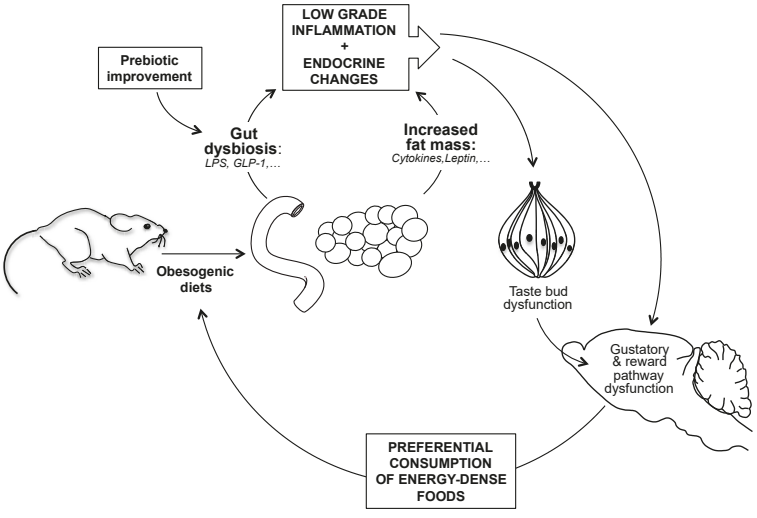


Figure 7. Functional relationships between diet-induced obesity and taste sensitivity in the mouse: Working model.

In the present study, we explored the impact of a preventive prebiotic supplementation on the orosensory responsiveness to a prototypical tastant (i.e., sucrose) in mice fed a standard chow or an obesogenic diet. Multivariate analysis clearly shows that the prebiotic supplementation poorly affects studied variables in lean mice, C and C+P groups being partially overlapping in contrast to what was found in obese (DIO) mice. Indeed, DIO+P group appeared as an independent cluster found in an intermediate position between lean and obese mice, suggesting that addition of prebiotic partially counteracts the deleterious effects of HFD. Interestingly, DIO+P group was found to be mainly depicted by variables related to gut microbiota and taste sensations. Addition of 10% inulin, not only corrected the negative effects of HFD on gut microbiota in DIO mice, but also improved their orosensory response to sweet stimuli. This last prebiotic-mediated change was found using two complementary behavioral approaches (two-bottle choice test and gustometer exploration). Collectively, our behavioral data reported a partial covery of the sweet taste sensitivity in DIO+P mice, as compared to controls, in relation with a better hedonic response to sweet stimuli (i.e., rise of lick number = "liking") without motivational change (i.e., same block number = "wanting") resulting in a correction of the preference deficit observed in DIO mice. A distinct impact of P supplementation on "liking" and "wanting" component of the food reward was also reported using an operant-responding performance test in DIO mice [29]. Altogether, our data raise the question of the implied mechanisms.

GLP-1 is produce in the gut by the enteroendocrine L Cells via a differential post-transcriptional processing of the proglucagon gene. This peptide hormone regulates the digestive tract (ileal brake), glucose homeostasis (incretin action) and appetite/satiation (anorexigen effect) [30]. It is known that P increase the number of endocrine L cells in the jejunum and in the colon of rodents and promote the production and release of the active forms of GLP-1, thereby decreasing glycaemia [31]. Several lines of evidence support an implication of GLP-1 in the improvement of sweet taste sensitivity after

microbiota manipulation. Firstly, GLP-1 R is found both in afferent fibers innerving the gustatory papillae [32] and mesolimbic areas involved in the reward pathway (i.e., ventral tegmental area—VTA and nucleus accumbens—NAc) [33]. Secondly, GLP-1 R-null mice display a reduced response to sweet tastants during behavioral tests, suggesting that the GLP-1 signaling enhances the sweet taste sensitivity in this species [32]. Thirdly, GLP-1 reduces hedonic value of food by suppressing VTA and NAc dopamine signaling [34]. Finally, a rise of the proglucagon mRNA levels was specifically found in the caecum from DIO+P mice. Therefore, an involvement of prebiotic-induced GLP-1 release in regulation of sweet taste sensitivity is likely. Further studies combining GLP-1 R-null mice and prebiotic manipulations are required to fully establish the causal role of GLP-1 in the prebiotic impact on the gustation.

Obesity-induced endotoxemia is an alternative, but not exclusive, possible cause of implication of gut microbiota in the taste dysfunction since gustatory papillae are LPS-sensitive and display a pro-inflammatory gene profile in DIO mice [14]. However, a chronic infusion of LPS at a level similar to that observed in DIO mice was not sufficient to alter the spontaneous preference for oily solution in lean mice subjected to two-bottle choice tests. Although, the response to a sweet tastant was not analyzed in this study, it is likely that a LPS-induced low-grade endotoxemia alone does not explain the change in the orosensory perception observed in DIO mice [14].

In conclusion, this study brings the first demonstration that a gut microbiota manipulation can affect the orosensory perception of sweet compounds. These results are consistent with a recent study revealing that human volunteers shifting their habits towards a diet based on the daily consumption of P-rich vegetables showed a reduced desire to eat sweet, fatty, and salty foods, together with an increased hedonic attitude for some inulin-rich vegetables [30]. The present study also helps to reveal the complexity of the homeostatic system responsible for the proper functioning of the taste system whose disruption, undoubtedly, contributes to the establishment of nutritional obesity.

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Conflicts of Interest: None to disclose.

Abbreviations

DIO	diet-induced obesity
GLP-1	glucagon-like peptide-1
GLP-1 R	GLP-1 receptor
HFD	high fat diet
LPS	lipopolysaccharides
P	prebiotic
PCA	principal component analysis
TLR	toll-like receptor
VTA	ventral tegmental area
NAc	nucleus accumbens

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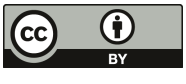

nutrients

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Article

Intraintestinal Delivery of Tastants Using a Naso-Duodenal-Ileal Catheter Does Not Influence Food Intake or Satiety

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Abstract: Intraduodenal activity of taste receptors reduces food intake. Taste receptors are expressed throughout the entire gastrointestinal tract. Currently, there are no data available on the effects of distal taste receptor activation. In this study, we investigate the effect of intraduodenal and/or intraileal activation of taste receptors on food intake and satiety. In a single-blind randomized crossover trial, fourteen participants were intubated with a naso-duodenal-ileal catheter and received four infusion regimens: duodenal placebo and ileal placebo (DPIP), duodenal tastants and ileal placebo (DTIP), duodenal placebo and ileal tastants (DPIT), duodenal tastants and ileal tastants (DTIT). Fifteen minutes after cessation of infusion, subjects received an *ad libitum* meal to measure food intake. Visual analog scale scores for satiety feelings were collected at regular intervals. No differences in food intake were observed between the various interventions (DPIP: 786.6 ± 79.2 Kcal, DTIP: 803.3 ± 69.0 Kcal, DPIT: 814.7 ± 77.3 Kcal, DTIT: 834.8 ± 59.2 Kcal, $p = 0.59$). No differences in satiety feelings were observed. Intestinal infusion of tastants using a naso-duodenal-ileal catheter did not influence food intake or satiety feelings. Possibly, the burden of the four-day naso-duodenal-ileal intubation masked a small effect that tastants might have on food intake and satiety.

Keywords: satiety; tastants; food intake; intraduodenal infusion; intraileal infusion; overweight; weight management

1. Introduction

Obesity is considered a major healthcare problem with worldwide obesity almost being tripled since 1975 [1]. Therefore, there is an increasing need for non-invasive therapies for weight management. Gastrointestinal (GI) hormones, such as cholecystokinin (CCK) and glucagon-like peptide-1 (GLP-1), have been shown to reduce food intake and hunger after intravenous administration [2–4]. Therefore, the GI-tract is an interesting target for non-invasive therapies to reduce food intake and induce satiety/satiation.

Intestinal macronutrient infusion decreases food intake and induces the release of CCK, GLP-1, and peptide YY (PYY) [5]. This mechanism is commonly referred to as intestinal- or ileal brake [6,7]. A recent review proposed a proximal to the distal gradient in the small intestine, where a more profound effect on food intake can be found after distal compared to proximal macronutrient

infusion [8]. Previous studies have demonstrated that besides macronutrients, substances referred to as tastants are able to activate certain taste receptors in the GI-tract which are coupled to enteroendocrine cells (EEC), and can trigger the release of satiety hormones (i.e., CCK, GLP-1, and PYY) [9–13]. These taste receptors can be found throughout the entire GI-tract. Expression levels for the various taste receptor differ throughout the gut. Table 1 gives a simplified visual representation of the relative expression of taste receptors throughout the human gut based on current literature [14–17].

In a recent study, van Avesaat et al. have shown that duodenal infusion of a combination of sweet, bitter, and umami tastants significantly decreased *ad libitum* meal intake, whilst increasing satiety and decreasing hunger feelings. These effects were not accompanied by changes in systemic levels of GLP-1, PYY, and CCK [18]. Up to now, no data are available on the effect of activation of taste receptors in the more distal small intestine. Since one of the functions of taste receptors in the gut is to sense food being present in the lumen, it should be investigated whether the beforementioned proximal to distal gradient found for the intestinal brake is operative for taste receptor activation.

Therefore, in the present study, we compared the effects of intraduodenal infusion versus intraileal infusion of a combination of tastants (sweet, bitter, and umami) on *ad libitum* food intake, satiation, and GI-complaints in healthy subjects. Since sweet and umami taste are sensed by various subtypes of the taste receptor family 1 (TAS1R) and bitter taste is sensed by the taste receptor family 2 (TAS2R), the combination will activate a wide range of taste receptors. We hypothesized that infusing tastants at both infusion sites (duodenum and ileum) will decrease food intake and increase satiation to the greatest extent when compared with infusion of placebo or single port infusion. Infusing in solely the duodenum or the ileum will also decrease food intake and increase satiation when compared to placebo, albeit to a lesser degree than infusing at both infusion sites simultaneously. Furthermore, we expect intraileal delivery of tastants will decrease food intake and increase satiation to a greater extent when compared with intraduodenal delivery of tastants.

Table 1. A simplified visual representation of the relative expression of taste receptors and gustducin throughout the human GI-tract.

	Stomach	Duodenum	Jejunum	Ileum	Colon
TAS1R1 (Bezencon et al. [14])	++	+	++	+	+/−
TAS1R2 (Bezencon et al. [14])	−	++	+	+/−	+
TAS1R2 (Young et al. [15])	−− $^{\$}$	+	++ $^{\#}$	N/A	N/A
TAS1R3 (Bezencon et al. [14])	+	++	++	+	+
TAS1R3 (van der Wielen et al. [16])	N/A	+	+	+	+
TAS1R3 (Young et al. [15])	+ $^{\$}$	++	++ $^{\#}$	N/A	N/A
TAS2R102–TAS2R144 (Gu et al. [17]) *	N/A	+	+	+	N/A
Gustducin (Bezencon et al. [14])	−−	++	++	+	−
Gustducin (Young et al. [15])	− $^{\$}$	+	++ $^{\#}$	N/A	N/A

Expression levels are relative to each other and a simplified visual representation with ++ indicating very high expression, + indicating high expression, +/− indicating medium expression, − indicating low expression, and −− indicating very low expression. $^{\$}$ Young et al. displayed the stomach as fundus, body, and antrum. For details, please refer to Young et al. [15]. $^{\#}$ Young et al. displayed jejunum as proximal jejunum and distal jejunum. For details, please refer to Young et al. [15]. N/A: not available. * T2R family is expressed throughout the entire small intestine in a comparable fashion with some subtypes more abundant proximally and some distally. For details, please refer to Gu et al. [17].

2. Materials and Methods

This study was approved by the Medical Ethics Committee of the Maastricht University Medical Center+ (MUMC+), Maastricht, The Netherlands, and performed in full accordance with the Declaration of Helsinki (latest amendment by the World Medic Association in 2013) and Dutch Regulations on Medical Research Involving Human Subjects (WMO, 1998). This study was registered in the US National Library of Medicine (http://www.clinicaltrials.gov, ID NCT03140930). All subjects gave written informed consent before screening.

2.1. Subjects

Healthy men and women were recruited by local advertisements. Inclusion criteria were age between 18 and 65 years, a body mass index (BMI) between 18 and 25 kg/m^2, with a stable weight over the past six months (<5% body weight change). Exclusion criteria were gastrointestinal complaints, history of chronic or severe disease, use of medication influencing endpoints within 14 days prior to testing, administration of investigational drugs which interfere with this study, major abdominal surgery, dieting, pregnancy or lactation, excessive alcohol consumption (>20 alcoholic consumptions per week), smoking, weight <60 kg, non-tasters of sweet, bitter or umami stimuli, evidence of monosodium glutamate (MSG)-hypersensitivity.

Prior to testing, screening was performed where abovementioned inclusion and exclusion criteria were checked, and a taste perception test was performed. Subjects tasted quinine (0.5 mmol/L), Reb A (50 mmol/L), MSG (50 mmol/L), and tap water blindly and had to indicate their sense of taste. Subjects had to identify each taste correctly in order to be eligible for the study. Furthermore, their length and weight were measured to calculate their BMI.

A sample size calculation was based on the difference in meal intake between duodenal infusion of a combination of tastants and duodenal infusion of placebo as reported by van Avesaat et al. [18]. Using a difference in means of 64 Kcal, a standard deviation of difference of 63, a power of 80%, and an alpha of 1.67%, a total number of 13 subjects were needed. An alpha of 1.67% was used to correct for multiple testing.

2.2. Study Design

In this single-blind randomized, placebo-controlled crossover study, subjects received the combination of tastants (sweet, bitter, and umami) and/or placebo (tap water) in the duodenum and/or the ileum for four consecutive test days. This results in four combinations which were infused on the various test days: duodenal placebo and ileal placebo (DPIP), duodenal tastants and ileal placebo (DTIP), duodenal placebo and ileal tastants (DPIT), duodenal tastants and ileal tastants (DTIT).

2.3. Catheter Positioning

A 305 cm long silicon 9-lumen (8-lumen, 1 balloon inflation channel, the outer diameter of 3.5 mm) custom-made naso-ileal reusable catheter (Dentsleeve International, Mui Scientific, Mississauga, Canada) was used for intubation.

One day prior to the first test day, subjects arrived at 7:40 AM at the Maastricht University Medical Center+ (MUMC+) after an overnight fast. If preferred by the subject, local anesthesia of nasal mucosa using xylocaine (10% spray, AstraZeneca, Zoetermeer, The Netherlands) was applied. After placement of the catheter in the stomach, the catheter was guided through the pylorus and into the duodenum under intermittent fluoroscopic control. Progression of the catheter from duodenum to ileum was performed as described earlier [19]. Fluoroscopy was used to check the positioning of the catheter on the first and the last test day. Radio-opaque markers were added to the infusion ports on the catheter, which accounted for the determination of the catheter position. On all test days, intestinal fluid was sampled from various infusion ports, and pH was measured using pH strips (MColorpHast™, Merck, Darmstadt, Germany) in order to estimate the catheter positioning.

2.4. Preparation and Infusion of Tastants

The combination of three tastants was infused in the duodenum, the ileum, or both the duodenum and the ileum. In order to prevent side effects from occurring, 75% of acceptable daily intake (ADI) of these tastants was infused. 540 mg Rebaudioside A (Reb A, Stevija Natuurlijk, Drachten, The Netherlands), 75 mg Quinine (Arnold Suhr, Hilversum, The Netherlands), and 2 g Monosodium Glutamate (MSG, Ajinomoto, Hamburg, Germany) were dissolved in 120 mL tap water and was used as tastant mixture for infusion, as was done by van Avesaat et al. [18]. All tastants used were non-caloric and yielded no nutritional value. The placebo infusion consisted of 120 mL of tap water. A magnetic stirrer was used to dissolve the tastants. The mixture was infused over a 60-min period with an infusion rate of 2 mL/min. This was consistent with the infusion rate of van Avesaat et al. mimicking the slow influx from the stomach to duodenum and slow transit through the gut in the ileum.

2.5. Protocol

On each test day, after an 8 h overnight fast, subjects arrived at 8:00 AM at the MUMC+. Subjects were instructed to consume the same habitual meal on the evening prior to testing. Hereafter, at t = 0 min, a standardized liquid breakfast meal (250 mL Goedemorgen drinkontbijt (Vifit); energy 145 Kcal per portion, 20.25 g carbohydrates, 8.5 g protein, and 2 g fat) was consumed. One hundred and fifty min (at t = 150 min) after breakfast consumption, a syringe containing the mixture for infusion was connected to the duodenal and ileal infusion port. The infusion was performed in 60 min at an infusion rate of 2 mL/min. Subjects received a standardized *ad libitum* lunch meal (Lasagna Bolognese (Plus supermarket); energy density per 100 g: 152 Kcal, 11 g carbohydrates, 7.1 g protein, and 8.6 g fat) fifteen min (at t = 225 min) after cessation of the infusion. The test meal was offered in excess and subjects were instructed to eat until they felt satiated.

2.6. VAS for Satiation and GI-Complaints

Feelings of satiation-/satiety feelings and GI-complaints (e.g., satiety, hunger, stomach pain, and nausea) were measured using visual analog scales (VAS, 0–100 mm) scores at various time points (t = −30, 30, 90, 150, 165, 180, 195, 210, and 240 min) during the day. Subjects were asked to indicate on a line, anchored at the low end with the lowest intensity feelings, with opposing terms at the high end, which place on the scale best reflected their feeling at that moment [20].

2.7. Statistical Analyses

Data were analyzed using IBM SPSS statistics 24 (IBM Corporation, Armonk, NY, USA). A visual check of the normality of the data was performed. The primary outcome of this study was the amount of food intake in Kcal during an *ad libitum* lunch meal. Secondary outcomes were VAS scores for satiation-/satiety feelings and GI-complaints.

Age, BMI, and gender were calculated by descriptive statistics. Food intake in Kcal and area under the curve (AUC) for VAS scores were compared using a linear mixed model with intervention (DTIP, DPIT, and DTIT, and DPIP), test day and the interaction of intervention × test day as fixed factors. When no significant interaction was found, the interaction was removed from the model to get the best model fit.

For VAS scores, a linear mixed model that included abovementioned fixed factors with the addition of fixed factors time and time × treatment interaction was also performed.

Data are presented as mean ± standard error of the mean (SEM) (unless specified otherwise), and a $p < 0.05$ was considered statistically significant.

3. Results

3.1. Subjects

In total, 19 subjects met the inclusion and exclusion criteria. Two subjects dropped out due to discomfort induced by the naso-ileal catheter, two subjects dropped out due to incorrect position of the catheter on the first test day, and one subject was excluded after not properly following the instructions for the *ad libitum* meal on the first test day. Therefore, 14 healthy volunteers (11 female, age 25.6 ± 10.5 years, BMI 22.3 ± 1.7 kg/m^2) completed the study protocol and were included in the analyses.

3.2. Food Intake

No intervention × test day interaction was found. No differences in *ad libitum* food intake in Kcal were observed after intraduodenal, intraileal or combined infusion of tastants versus placebo infusion (DPIP: 786.6 ± 79.2 Kcal, DTIP: 803.3 ± 69.0 Kcal, DPIT: 814.7 ± 77.3 Kcal, DTIT: 834.8 ± 59.2 Kcal; $p = 0.59$) (Figure 1). Furthermore, as depicted in Figure 2, no trends in individual responses were found.

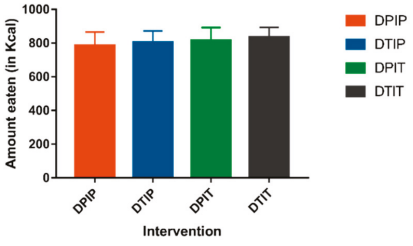

Figure 1. The amount eaten in Kcal (mean + SEM) 15 min after cessation of the infusion of placebo both intraduodenal and intraileal (DPIP), tastants intraduodenal and placebo intraileal (DTIP), placebo intraduodenal and tastants intraileal (DPIT), and tastants both intraduodenal and intraileal (DTIT). Based on a linear mixed model, no difference in food intake was observed between the conditions ($p = 0.59$).

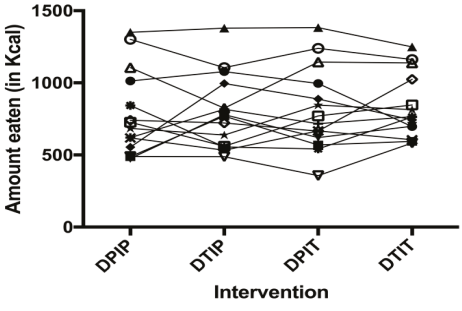

Figure 2. An individual representation per subject of amount eaten in Kcal 15 min after cessation of the infusion of placebo both intraduodenal and intraileal (DPIP), tastants intraduodenal and placebo intraileal (DTIP), placebo intraduodenal and tastants intraileal (DPIT), and tastants both intraduodenal and intraileal (DTIT). Treatment order was randomized for each subject. Each line with a unique symbol represents an individual subject. Based on a linear mixed model, no difference in food intake was observed between the conditions ($p = 0.59$).

3.3. Satiation/Satiety Scores

The mean VAS scores for the desire to eat, hunger, satiety, and fullness are depicted in Figure 3. No differences in area under the curve ($AUC_{150–210}$) for these VAS scores were observed between the

various interventions. Furthermore, no intervention × timepoint interactions were found for these VAS scores.

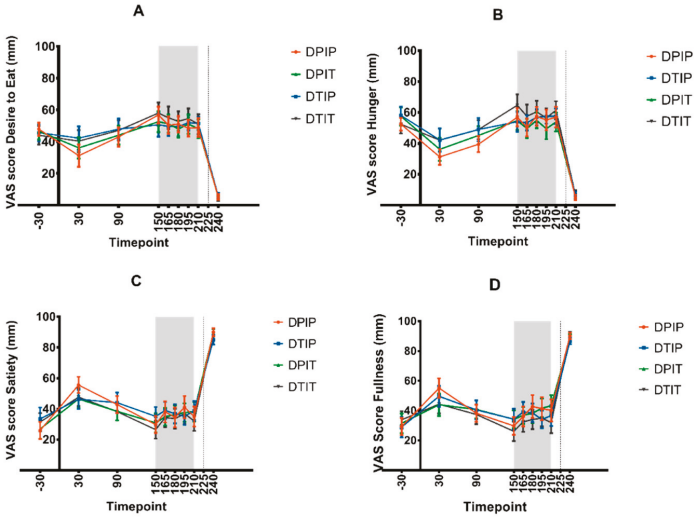

Figure 3. VAS scores for desire to eat (**A**), hunger (**B**), satiety (**C**), and fullness (**D**) (mean + SEM) before, during, and after the infusion of placebo both intraduodenal and intraileal (DPIP), tastants intraduodenal and placebo intraileal (DTIP), placebo intraduodenal and tastants intraileal (DPIT), and tastants both intraduodenal and intraileal (DTIT). VAS scores were measured at t = −30, 30, 90, 150, 165, 180, 195, 210, and 240 min. No VAS scores were taken at t = 225 min. At t = 0 min, subjects received a standardized breakfast, infusion of mixtures was performed from t = 150 until t = 210 min, and *ad libitum* test meal was presented at t = 225. Based on a linear mixed model of mean scores and area under the curve ($AUC_{150–210}$), no differences in desire to eat, hunger, satiety, and fullness were observed between the various conditions.

3.4. GI-Complaints

The mean VAS scores for stomach pain, bloating, and nausea are depicted in Figure 4. No differences in area under the curve ($AUC_{150–210}$) for these VAS scores were observed between the various interventions. Furthermore, no intervention × timepoint interactions were found for these VAS scores.

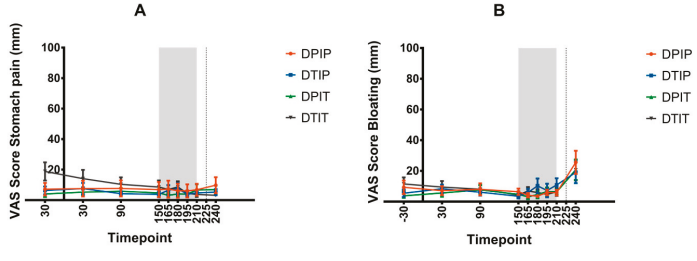

Figure 4. *Cont.*

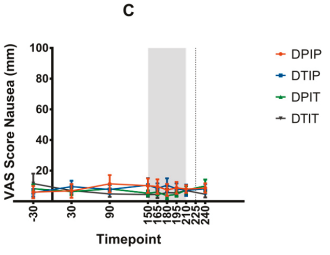

Figure 4. VAS scores for stomach pain (**A**), bloating (**B**), and nausea (**C**) (mean + SEM) before, during, and after the infusion of placebo both intraduodenal and intraileal (DPIP), tastants intraduodenal and placebo intraileal (DTIP), placebo intraduodenal and tastants intraileal (DPIT), and tastants both intraduodenal and intraileal (DTIT). t = −30, 30, 90, 150, 165, 180, 195, 210, and 240 min. No VAS scores were taken at t = 225 min. At t = 0 min, subjects received a standardized breakfast, infusion of mixtures was performed from t = 150 until t = 210 min, and *ad libitum* test meal was presented at t = 225 min. Based on a linear mixed model of mean scores and area under the curve ($AUC_{150–210}$), no differences in stomach pain, bloating, and nausea were observed between the various conditions.

4. Discussion

Our results do not reveal any difference in satiety or food intake between duodenal administration, ileal administration or combined duodenal administration of a tastant mixture (sweet, bitter, and umami) or infusion of placebo. Moreover, no GI-complaints were caused by infusing tastants or placebo into the duodenum and/or the ileum.

Van Avesaat et al. have investigated the effect of intraduodenal infusion of the same tastant mixture on food intake [18]. In that study, intraduodenal infusion of this combination of tastants, in similar study design, using the same amount of tastants significantly reduced food intake by 64 Kcal and was accompanied by changes in satiation/satiety feelings. However, it must be noted that this is a small difference, which on its own might not be clinically significant. Repeating this effect multiple times per day with each meal might result in a clinically significant decrease of caloric intake. This difference in results of food intake between the two studies may be related to differences in study design. In the study of van Avesaat et al., the subjects were intubated with a naso-duodenal catheter on every test day for the administration of tastants. The catheter was removed immediately thereafter before the subjects were presented with the *ad libitum* test meal. In the present study, subjects were intubated for several days with a naso-ileal catheter, and therefore this catheter was present while meals were offered and ingested. We hypothesize that having a naso-ileal catheter in situ for multiple days negatively influences meal ingestion to such a degree that this masks the smaller magnitude of effect that infusion of non-caloric tastants into the intestine has. On the other hand, mean caloric intake showed no major differences between the two studies.

Previous studies from our group investigating the 'intestinal brake' by infusing macronutrients in the ileum have repeatedly shown that infusion of even low doses of macronutrients results in a significant reduction of food intake, ranging between 64–188 Kcal, corresponding to a percentual decrease of 11.7%–32% of caloric intake during a single meal [5,21]. This indicates a negative feedback mechanism on food intake that arises from nutrient sensing. These data demonstrate that magnitude of the effect of macronutrient infusion on food intake is greater than the effects of infusing tastants.

Conclusively, studies investigating differences in food intake should be aware that naso-ileal intubation might mask a small effect. Therefore, other delivery options, such as encapsulation, should be considered in the future.

Results of studies investigating the effects of single tastants on food intake, satiation/satiety, and GI peptides are not consistent. An initial strong decrease of hunger with a steep increase thereafter has been observed after administration of a non-caloric sweetener [22]. Ingestion of low caloric sweeteners did not influence energy intake compared with a control condition (intake of water) [23]. Adding an

umami tastant to a meal did not affect appetite sensations, but has been shown to result in an increase of subsequent food intake [24]. Recently, increased attention has been given to the effects of bitter substances on satiety and food intake. Intake or infusion of bitter substances (quinine, denatonium benzoate) not only reduced antral motility [25,26] but also increased satiety scores and resulted in a significant decrease in food intake [27]. A possible mechanism explaining the strong aversive effects of bitter tastants is that bitter taste is evolutionarily linked to toxic substances, as has been showed by presenting newborn infants with bitter substances [28].

Alleleyn et al. have shown that the inhibition of food intake shows a proximal to the distal gradient, with higher effects observed after distal versus proximal administration of nutrients [8]. Based on our data, such a gradient was not observed for intestinally administered tastants. Intestinal taste receptor expression varies for various taste receptors, where some taste receptors are more profound proximally in the GI-tract, while expression of other taste receptors is higher in the more distal intestine [14–17].

We thought the proximal to distal gradient found for macronutrient infusion might be operable for taste receptor activation, which was clearly not the case. It is possible that taste receptors inhibit food intake in a different fashion than macronutrients. For instance, it has been speculated that taste receptors function by sensing the type of food (i.e., sweet for carbohydrates, umami for amino acids, and bitter for toxic substances) [29]. Since bitter tastants are linked to toxic substances, another working mechanism for bitter tastants could be through an aversive reaction of subsequent food intake.

From an evolutionary perspective, a more pronounced inhibitory or aversive effect for toxic substances could be expected to occur in the most proximal parts of the GI tract. However, there are no data available with respect to activation of oral (bitter) taste receptors on subsequent food intake. It is therefore unclear, whether activation of more proximal taste receptors will reveal more pronounced effects on food intake and satiation/satiety. Consequently, further studies are needed to investigate whether more proximal activation of taste receptors results in a stronger decrease in food intake.

Published data on the role of GI peptides in the regulation of food intake after administration of tastants are not in line. Van Avesaat et al. found a clear effect of intraduodenal administration of tastants on food intake that was not accompanied by changes in GLP-1- or PYY level [18]. Other studies, however, did show a decrease in systemic ghrelin- and motilin levels [25,26] and an increase in systemic CCK levels [27] after administration of a bitter tastant.

A limitation of our study is that the wash-out period consisted of only one day. Prolonging the wash-out period over one day would have resulted in a longer period of naso-ileal catheter intubation increasing the discomfort to our volunteers. No interaction effect between intervention and test day was found on food intake, satiety scores or GI-complaints, indicating that no carry-over effect was present.

Another limitation of the present study was the absence of systemic GI hormone measurements. This would have provided a complete analysis of the effects of intestinal tastant administration on eating behavior. However, van Avesaat et al. showed a decrease in food intake and an increase in satiety scores, which was not accompanied by changes in systemic GI hormone levels [18]. Therefore, no systemic GI hormone measurement was conducted in the present study.

It has to be noted that the ideal duration of administration of the intervention and of the timing between intervention and serving the *ad libitum* meal is unknown. We employed a design similar to that of van Avesaat et al. based on their positive results [18]. Future research protocols should consider these factors.

Studies investigating the effects of tastants on food intake up to now focus on only acute effects in a single *ad libitum* meal. It is not known whether repetitive or chronic administration of tastants will lead to other results. More data are needed on the long-term effects of tastants, especially on daily energy intake.

Author Contributions: The authors' responsibilities were as follows: Conceptualization, D.K. and A.A.M.M.; methodology, T.K., D.K., F.J.T. and A.A.M.M.; formal analysis, T.K.; investigation, T.K., A.M.E.A. and M.v.A.; resources, T.K. and F.J.T.; writing-original draft preparation, T.K.; writing-review and editing, T.K., A.M.E.A., D.K. and A.A.M.M.; supervision, D.K. and A.A.M.M.; project administration, T.K.; funding acquisition, D.K. and A.A.M.M.

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Taste Perception and Caffeine Consumption: An fMRI Study

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Abstract: Caffeine is ubiquitous, yet its impact on central taste processing is not well understood. Although there has been considerable research on caffeine's physiological and cognitive effects, there is a paucity of research investigating the effects of caffeine on taste. Here we used functional magnetic resonance imaging (fMRI) to investigate group differences between caffeine consumers and non-consumers in blood-oxygenation-level-dependent (BOLD) activation during hedonic evaluation of taste. We scanned 14 caffeine consumers and 14 caffeine non-consumers at 3 Tesla, while they rated three tastes: caffeine (bitter), sucrose (sweet), and saccharin (sweet with bitter after taste), in aqueous solutions. Differences in BOLD activation were analyzed using voxel wise independent samples *t*-tests within Analysis of Functional Neuroimage (AFNI). Results indicated that during the hedonic evaluation of caffeine or sucrose, caffeine non-consumers had significantly greater activation in neuronal areas associated with memory and reward. During the hedonic evaluation of saccharin, caffeine consumers had significantly greater activation in areas associated with memory and information processing. The findings suggest caffeine consumption is associated with differential activation in neuronal areas involved in reward, memory, and information processing. Further research on intensity and hedonics of bitter and sweet stimuli in caffeine consumers and non-consumers will be of great interest to better understand the nature of differences in taste perception between caffeine consumers and non-consumers.

Keywords: fMRI; caffeine; taste; memory

1. Introduction

Caffeine consumption is ubiquitous. It currently ranks as the most popular psychostimulant in the world [1]. Eighty-five percent of the United States' population consumes at least one caffeinated beverage daily [2]. Many beverages contain caffeine, including coffee, the most widely consumed beverage after water [3]. Other widely consumed caffeinated beverages are tea and energy drinks, which typically contain a high caffeine content, as well as a high glucose content [2,4]. Despite caffeine's bitter taste and the fact that bitter tastes often discourage intake, coffee and tea remain two of the most widely ingested beverages [5]. Caffeine's widespread consumption warrants a better understanding of its effects.

Evidence supporting caffeine's ability to exert beneficial effects is abundant [6]. When consumed in moderate amounts, caffeine has been reported to decrease fatigue and increase energy [6]. Caffeine has also been reported to increase motor performance on sustained response tasks. For example, participants randomly assigned in a double-blind study to either consume a drink containing 40 mg

of caffeine or placebo, showed enhanced performance on a selective attention task when exposed to the experimental condition [7]. Further, caffeine produces mild autonomic nervous system arousal and improved mood when compared to a non-caffeinated placebo [8]. During a visuomotor task, participants demonstrated increased blood-oxygenation-level-dependent (BOLD) activation in the putamen and insula after consuming 200 mg of caffeine [9]. The putamen is part of the basal ganglia, an area that has been shown to modulate the top-down influence of the prefrontal cortex on sensory processing in humans [10]. Increased activation in the striatum following caffeine consumption suggests that caffeine can act as a cognitive enhancer by modulating these attentional areas [9].

While caffeine consumed at moderate doses may provide consumers with a number of favorable effects, research suggests negative consequences as a result of caffeine consumed at higher doses [8,11]. Increasing caffeine consumption can exert dose-dependent effects on a number of acute autonomic responses, including increased blood pressure [8]. Caffeine consumed at 300–800 mg can induce anxiety, nervousness, and insomnia [11]. Further, withdrawal from caffeine is detectable overnight, and causes fatigue, stress, as well as decreased alertness and clear-headedness in heavy caffeine consumers [12–14].

The motivational desire to ingest a certain food incorporates a combination of flavor, learned associations, and physiological state that integrate to produce a food reward [15,16]. Since bitter taste is typically avoided by many species and may be an adaptation to protect them from adverse physiological effects, repeated consumption of caffeine may be a learned process [5]. The choice to consume caffeine may occur as a result of altered activation in brain areas related to reward pathways, particularly in areas associated with processing food rewards. Previous studies have reported that altered neuronal processing can occur as a consequence of repeated ingestion of a substance [17,18]. For example, habitual consumption of non-nutritive sweeteners has been associated with altered processing of sweet taste in individuals who regularly consume diet soda [17]. When compared to non-diet soda drinkers, diet soda drinkers demonstrated greater activation in areas related to reward processing, such as the dopaminergic midbrain, in response to sweet taste. Diet soda drinkers also exhibited greater activation in orbitofrontal cortex (OFC) Brodmann Area (BA) 47, an area related to pleasantness evaluation, when rating saccharin. Therefore, food consumption choices may be associated with altered neuronal activation.

The effects of caffeine consumption on central aspects of taste perception are not well understood. In addition to caffeine's bitter taste [5], there is some suggestion from psychophysical studies that caffeine, which is an adenosine-receptor antagonist, may influence perception of some sweeteners through its action on adenosine receptors in sweet-sensitive taste cells [5,19,20]. The current study investigates differences between habitual caffeine consumers and non-consumers on brain activation during hedonic evaluation of taste, rather than the acute effects of caffeine consumption or withdrawal from caffeine consumption [21,22].

The purpose of the current study was to test the hypothesis that caffeine consumers and non-consumers may show differential brain activation, assessed with functional magnetic resonance imaging (fMRI), during hedonic evaluation of a bitter taste (caffeine), a sweet taste (sucrose), and a sweet taste with bitter after taste (saccharin). Results suggesting differential brain activation in association with caffeine consumption and different taste stimuli adds to preceding literature regarding caffeine's influences on taste perception. Since caffeine consumption was a defining factor in group membership, it was chosen as the representation for bitter taste. Sweet taste was also chosen as a taste stimulus in response to preceding literature suggesting that caffeine may influence perception of sweet taste [5,19,20]. Saccharin was chosen as the third taste stimulus since it evokes a combination of bitter and sweet taste and may result in differential activation during taste processing in comparison to caffeine and/or sucrose. We aimed to investigate differential brain activation during the hedonic evaluation of taste to determine (1) whether caffeine consumers have greater activation than non-consumers in areas related to reward processing (e.g., nucleus accumbens, OFC BA 10); (2) whether caffeine non-consumers have greater neuronal activation than consumers in memory

pathways, such as areas in the medial temporal lobe (MTL); and (3) whether caffeine non-consumers may rely upon activation of a larger network than consumers in order to perform the task.

2. Methods

2.1. Participants

The current sample (n = 28) consisted of 12 males and 16 females. Participants were divided into one of two groups: caffeine non-consumer (n = 14) and caffeine consumer (n = 14). Participants were divided into these groups based on answers to a survey that was administered after study completion. Participants who reported they not drink caffeinated beverages were labeled as caffeine non-consumers. Participants who responded that they did consume caffeinated beverages constituted the consumers group. Groups were matched on age, body mass index (BMI), and gender. Participants were part of a larger study investigating fMRI and taste processing. The Institutional Review Boards at San Diego State University and University of California, San Diego approved the study. All participants gave informed consent and were given monetary compensation for their participation.

2.2. Screening Session

The current study used the methodology described in detail in Haase, Cerf-Ducastel, Buracas, and Murphy (2007) [23]. All participants completed one screening session and one event-related fMRI session. At the initial screening, participant information, height, and weight were recorded. Participants were screened for metal in their body for the fMRI scan, as well as ageusia and anosmia with forced choice taste and odor threshold measures [24]. Being left-handed was an exclusionary criterion to avoid differential lateral activation in hemispheres due to handedness [25]. Participants who met the study criteria returned to complete one fMRI scan.

2.3. Odor and Taste Threshold Measures

In order to screen for anosmia, odor thresholds for the odor n-butyl alcohol (butanol) were assessed for each nostril monorhinically using a forced choice, ascending methods of limits test [24]. The solutions were in a series of 10; each dilution was one-third the concentration of the solution preceding it. On each trial the participant was presented with two bottles: one containing distilled water and the other containing the odor stimulus. The participant was asked to decide which bottle contained an odor. There was a 45 s inter-stimulus interval between each stimulus delivery to avoid adaptation [26]. If the participant chose the incorrect bottle, a higher concentration was given on the next trial. Once the participant met the criterion of choosing correctly on five successive trials the odor threshold was determined.

In order to screen for ageusia, taste thresholds for sucrose were assessed using a sip and spit, forced choice staircase procedure [24]. Stimuli were presented in 14 concentrations of sucrose, ranging from 0.0032 to 0.36 M in geometrical progression. All stimuli were presented at room temperature in distilled water [24]. The experimenter presented the participants with two cups, one containing distilled water and the other containing sucrose solution. The stimulus was sipped, held in the mouth for 10 s, and expectorated. After the participant sampled 10 ml of water and solution, he (she) was asked to select the stimulus with the sweet taste. The experimenter increased the concentration until the participant consistently (twice in a row) chose the stronger stimulus. This procedure was then reversed to a descending series until the participant failed to choose the correct stimulus. Participants were required to rinse with distilled water before each stimulus to avoid adaptation and waited a minimum of 30 s between each stimulus. Testing continued for five reversals with the mean of the last four reversals taken as the threshold.

2.4. Neuroimaging Procedure

Functional MRI data were collected in order to investigate brain response of caffeine consumer and caffeine non-consumer groups to stimuli during the physiological state of hunger. All scanning sessions occurred in the morning, and participants were instructed to fast 12 h prior to the scan. When stimuli were presented, participants used a joystick to rate pleasantness on a modified general Labeled Magnitude Scale. The scale was projected on a screen visible to the participant through a mirror attached to the head coil [23,27].

2.5. Stimulus Delivery

The stimuli used in this study were pure tastes delivered in aqueous solutions: 0.04 M caffeine, 0.64 M sucrose, and 0.028 M saccharin. These concentrations were chosen based on a previous study from our laboratory reporting how stimulus delivery method impacted the slopes of taste intensity functions for these stimuli [28]. The simulated stimulus delivery system was shown to produce psychophysical functions with slopes that were generally lower than experiments conducted with the sip and spit technique and that were similar to slopes of intensity functions associated with the dorsal flow procedure [28]. The concentrations chosen for the present study reflect the highest concentrations of each stimulus tested in Reference [28].

Stimuli were presented orally and presentations were randomized during functional data acquisition through the use of a computer-controlled delivery system (Figure 1). All taste stimuli were presented while the participant was inside the scanner, where the participant lay supine with a bite bar, which was positioned comfortably between the lips so that the tubes delivered stimuli to the tip of the tongue. Immediately before, during, and after the scan, participants rated the pleasantness and intensity of each stimulus. The taste stimuli and water were delivered at room temperature each through a unique 25-ft long plastic tube, which was connected to a different computer-programmable syringe pump. The pumps were programmed to present 0.3 mL of solution in 1 s.

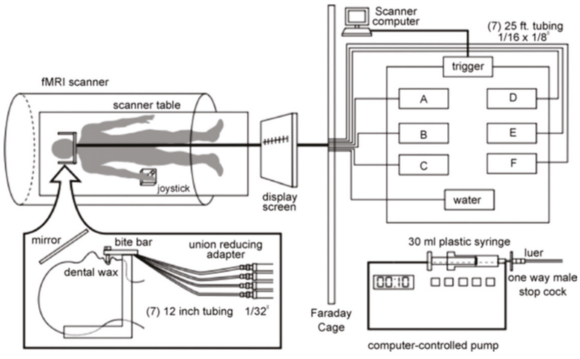

Figure 1. Stimulus delivery paradigm. Reprinted with permission from Haase et al. (2007) [23].

The imaging session consisted of two functional runs. During the functional runs, each stimulus was presented in 0.3 mL of solution for a total of 16 times with a 10 s inter-stimulus interval. Participants were presented with water twice; first as a rinse, and then as a baseline to be used in data analysis. A complete outline of the stimulus delivery protocol used in the fMRI sessions is described in the Journal of Neuroscience Methods [23].

2.6. Imaging Acquisition

Functional MRI sessions took place at the Center for Functional Magnetic Resonance Imaging at the University of California, San Diego. All data were collected using a 3T General Electric Signa Excite short-bore scanner (GE Healthcare, Chicago, IL, USA). Structural data were acquired for anatomical

localization of the functional images. Parameters used to acquire structural images were as follows: T1—weighted whole-brain fast spoiled gradient echo (FSPGR) sequences, field of view (FOV) = 25.6 cm, slice thickness = 1 mm, resolution = 1 × 1 × 1 mm^3, echo time (TE) = 30 ms, Locs per slab = 190, flip angle = 15°. Parameters used to acquire functional images were as follows: T2*—weighted images, 32 axial slices, FOV = 19.2 cm, matrix size = 64 × 64, resolution = 3 × 3 × 3 mm^3, flip angle = 90°, echo time (TE) = 30 ms, repetition time (TR) = 2000 ms.

2.7. Imaging Analysis

Imaging data were processed using FMRIB Software Library (FSL, Analysis Group, FMRIB, Oxford, UK) and Analysis of Functional NeuroImage (AFNI, open source software) [29,30]. Data were preprocessed to correct head movement and alignment as well as to concatenate the runs. Temporal and spatial smoothing of the brain images were also applied. Images were spatially smoothed to four full widths at half maximum (FWHM), auto-masked to remove voxels located outside of the brain, and normalized into Talairach space to control for individual variation in structural differences.

We conducted the analyses within AFNI, using 3dDeconvolve, on each participant's concatenated runs based on the specified contrast (e.g., activation during evaluation of caffeine minus activation during evaluation of water) that accounted for the timing of delivery of the stimulus and the water baseline, which served as a control for identifying non-gustatory intra-oral stimulation [30,31]. Deconvolution estimates the hemodynamic response per voxel in a participant's concatenated runs given the experimental paradigm (i.e., stimulus onset timing) using ordinary least squares regression. The output from 3dDeconvolve contains fit coefficients (i.e., beta weights) for each voxel, indicating the amplitude of the signal model for each contrast, and corresponding *t*-statistics.

Several thresholding steps were taken in an attempt to control for Type I error in all group analyses. Individual voxels were thresholded at $p \leq 0.015$. To protect a whole-brain probability of false positives at an overall alpha of 0.05, group statistical maps were corrected for multiple comparisons at the cluster level using the AFNI program ClustSim [31]. ClustSim uses Monte Carlo simulations to compute the probability of generating a random "significant" cluster of noise (i.e., a false positive) given the individual voxel threshold, the voxel connection radius, the amount of blurring, and the search volume (i.e., overall dataset size). For an overall alpha level of 0.05, a cluster threshold of 21 contiguous voxels was applied. Neuronal activation in the caffeine consumers group during hedonic evaluation of the individual taste stimuli was subtracted from activation in the caffeine non-consumers group.

2.8. Demographic Data Analysis

To examine potential demographic differences between caffeine consumers and caffeine non-consumers, multivariate analyses of variance (MANOVA) were performed using caffeine status as an independent variable. Age, gender group, body mass index (BMI), taste threshold, right odor threshold, and left odor threshold were dependent variables. The results can be found in Table 1.

Table 1. Participant characteristics for caffeine non-consumers and matched caffeine consumers.

	Caffeine Non-Consumers		**Caffeine Consumers**			
Demographics	**Mean**	**SD**	**Mean**	**SD**	**F**	***p***
Age	56.786	15.837	45.00	18.925	3.193	0.086
Gender	0.571	0.514	0.571	0.514	0.000	1.000
BMI	29.564	6.783	29.654	6.208	0.001	0.972
Taste Threshold	0.005	0.005	0.006	0.011	0.169	0.684
Odor Threshold R	6.000	1.797	7.000	1.240	2.935	0.099
Odor Threshold L	6.140	1.875	6.786	1.051	1.252	0.273

BMI: body mass index.

2.9. Psychophysical Data Analysis

The general Labeled Magnitude Scale (gLMS) was used to collect intensity ratings and a modified version of the gLMS was used to collect hedonic ratings for caffeine, sucrose, and saccharin taste before and after each scan [27]. To examine between group differences in psychophysical ratings, a MANOVA was performed using caffeine status as an independent variable. Results are shown in Tables 2 and 3. Repeated measures analyses of variance (RM-ANOVA) were performed to examine possible differences between hedonic and intensity ratings of each taste before and after stimuli were presented during the scan.

Table 2. Hedonic ratings for caffeine non-consumers and matched caffeine consumers.

	Caffeine Non-Consumers		Caffeine Consumers			
Hedonic Ratings	**Mean**	**SD**	**Mean**	**SD**	**F**	***p***
Caffeine Pre	35.286	16.973	39.357	15.619	0.436	0.515
Caffeine Post	26.357	16.284	38.214	14.766	4.073	0.054
Sucrose Pre	58.786	11.943	61.929	10.095	0.565	0.459
Sucrose Post	52.929	18.512	60.500	9.053	1.890	0.181
Saccharin Pre	51.429	15.500	55.357	7.938	0.712	0.406
Saccharin Post	47.429	18.793	53.360	9.740	1.098	0.304

Table 3. Intensity ratings for caffeine non-consumers and matched caffeine consumers.

	Caffeine Non-Consumers		Caffeine Consumers			
Intensity Ratings	**Mean**	**SD**	**Mean**	**SD**	**F**	***p***
Caffeine Pre	29.429	20.470	35.929	27.280	0.508	0.482
Caffeine Post	53.786	31.499	34.929	24.656	3.111	0.090
Sucrose Pre	41.786	24.974	28.071	12.982	3.324	0.080
Sucrose Post *	52.000	30.894	32.929	14.334	4.390	0.046
Saccharin Pre	35.071	23.206	34.214	16.348	0.013	0.911
Saccharin Post *	52.357	26.401	31.000	13.278	7.312	0.012

* Significant difference between caffeine consumers and caffeine non-consumers.

3. Results

3.1. Demographic

There were no significant differences in age ($F(1, 26) = 3.193$, $p = 0.086$), BMI ($F(1, 26) = 0.001$, $p = 0.972$) or gender ($F(1, 26) < 0.001$, $p = 1.000$). There were also no significant differences in taste threshold ($F(1, 26) = 0.169$, $p = 0.684$) or in the odor threshold for the right nostril ($F(1, 26) = 2.935$, $p = 0.099$) or for the odor threshold for the left nostril ($F(1, 26) = 1.252$, $p = 0.273$).

3.2. Psychophysical Data

A MANOVA was performed to examine between group differences of hedonic and intensity ratings (Tables 2 and 3). Caffeine non-consumers demonstrated significantly higher ratings for post-scan intensity ratings for sucrose ($F(1, 26) = 4.390$, $p = 0.046$) and saccharin ($F(1, 26) = 7.312$, $p = 0.012$) when compared to the post-scan intensity ratings for caffeine consumers.

There were no significant differences between caffeine consumers and non-consumers in pleasantness ratings of caffeine ($F(1, 26) = 1.3686$, $p = 0.253$), saccharin ($F(1, 26) = 0.094$, $p = 0.762$), or sucrose ($F(1, 26) = 0.392$ $p = 0.537$). There were also no significant differences between sucrose intensity ratings before and after stimuli were presented during the scan ($F(1, 26) = 0.442$, $p = 0.512$). There were significant differences between intensity ratings before and after the scan for caffeine ($F(1, 26) = 10.173$, $p = 0.004$) and saccharin ($F(1, 26) = 6.558$, $p = 0.016$). For caffeine consumers, neither saccharin intensity ratings ($F(1, 13) = 0.568$, $p = 0.464$) nor caffeine intensity ratings

(F (1, 13) = 0.077, p = 0.786) were significantly different before and after taste was presented during the scan. For caffeine non-consumers, caffeine intensity ratings (F (1, 13) = 11.833, p = 0.004) and saccharin intensity ratings (F (1, 13) = 6.551, p = 0.024), were significantly different before and after the taste was presented. Intensity ratings for caffeine non-consumers were significantly higher after the stimuli were presented during the scan.

3.3. Functional Neuroimaging

During the hedonic evaluation of caffeine, caffeine non-consumers had significantly greater neuronal activation in the right cuneus, right precuneus, left anterior cingulate, medial frontal gyrus, and left superior frontal gyrus (See Table 4 and Figure 2).

Table 4. Regions of significantly greater activity in caffeine non-consumers compared to caffeine consumers while judging the pleasantness of caffeine.

		Talaraich Coordinates				
Region	**Hem.**	**X**	**Y**	**Z**	**Regr. Coef.**	**Voxels in Cluster**
Cuneus	R	8	−85	26	1.76	38
Precuneus	R	16	−74	26	0.33	
Medial Frontal Gyrus	L	−1	47	41	0.757	28
Medial Frontal Gyrus	R	0	45	39	0.608	
Superior Frontal Gyrus	L	−1	54	34	0.65	
Anterior Cingulate	L	−7	38	22	0.52	

Hem.: Hemisphere; R: right; L: left; Regr. Coef.: Regression coefficient; Minimum cluster = 21 voxels, p = 0.015.

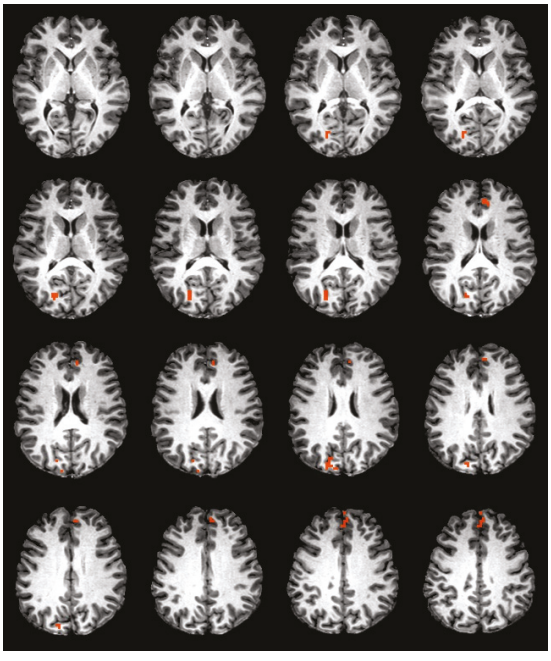

Figure 2. Brain activation during the hedonic evaluation of caffeine. Orange indicates areas where caffeine non-consumers had significantly greater activation in comparison to caffeine consumers.

During the hedonic evaluation of saccharin, caffeine non-consumers had significantly lower neuronal activation than caffeine consumers in the middle temporal gyrus, inferior temporal gyrus,

middle occipital gyrus, right fusiform gyrus, right lingual gyrus, and right cuneus (See Table 5 and Figure 3).

Table 5. Regions of significantly greater activity in caffeine consumers compared to caffeine non-consumers while judging the pleasantness of saccharin.

		Talairach Coordinates				
Region	**Hem.**	**X**	**Y**	**Z**	**Regr. Coef.**	**Voxels in Cluster**
Middle Temporal Gyrus	L	−55	−64	5	−0.87	50
Inferior Temporal Gyrus	L	−44	−69	−1	−0.617	
Middle Occipital Gyrus	L	−44	−62	−3	−0.396	
Middle Temporal Gyrus	R	59	−46	−7	−0.647	28
Inferior Temporal Gyrus	R	63	−48	−7	−0.39	
Fusiform Gyrus	R	46	−38	−7	−0.289	
Middle Occipital Gyrus	R	29	−88	5	−1.12	27
Lingual Gyrus	R	27	−89	−2	−0.849	
Cuneus	R	23	−91	−1	−0.712	

Hem.: Hemisphere; R: right; L: left; Regr. Coef.: Regression coefficient; Minimum cluster = 21 voxels, $p = 0.015$.

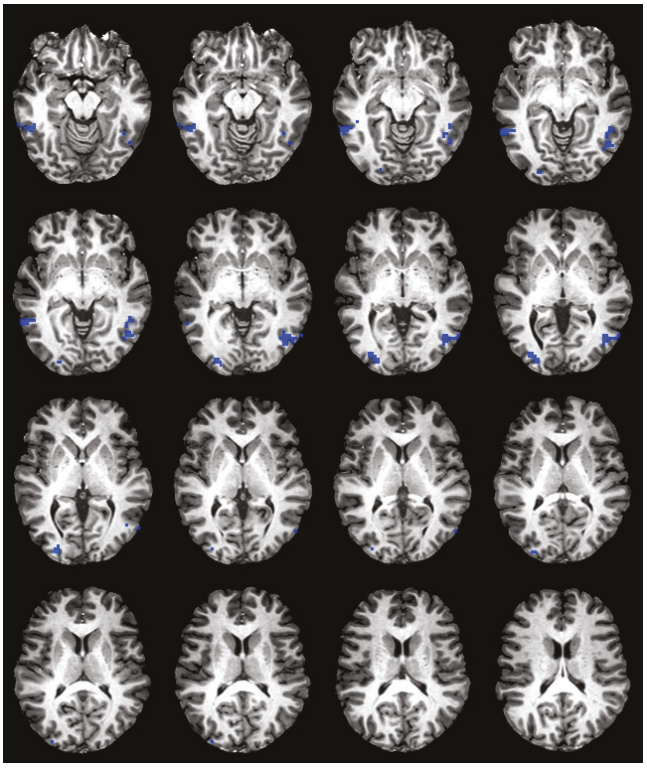

Figure 3. Brain activation during the hedonic evaluation of saccharin. Blue indicates areas where caffeine consumers had significantly greater activation in comparison to caffeine non-consumers.

During the hedonic evaluation of sucrose, caffeine non-consumers had significantly greater neuronal activation in the anterior cingulate, medial frontal gyrus, right superior frontal gyrus, OFC BA 10, posterior cingulate, cingulate gyrus, and precuneus (See Table 6 and Figure 4).

Table 6. Regions of significantly greater activity in caffeine non-consumers compared to caffeine consumers while judging the pleasantness of sucrose.

		Talairach Coordinates				
Region	**Hem.**	**X**	**Y**	**Z**	**Regr. Coef.**	**Voxels in Cluster**
Anterior Cingulate	R	2	41	−1	1.26	153
Medial Frontal Gyrus	R	2	62	20	1.03	
Anterior Cingulate	L	−2	42	−1	0.937	
OFC BA10	R	5	62	14	0.63	
Superior Frontal Gyrus	R	10	59	21	0.535	
Medial Frontal Gyrus	L	−10	40	14	0.446	
OFC BA10	L	−10	43	12	0.421	
Posterior Cingulate	L	−1	−46	14	1.27	90
Posterior Cingulate	R	2	47	13	1.24	
Cingulate Gyrus	R	2	−49	27	0.862	
Cingulate Gyrus	L	0	−50	29	0.64	
Precuneus	R	2	−49	32	0.583	
Precuneus	L	−7	−58	29	0.537	

Hem.: Hemisphere; R: right; L: left; Regr. Coef.: Regression coefficient; Minimum cluster = 21 voxels, $p = 0.015$.

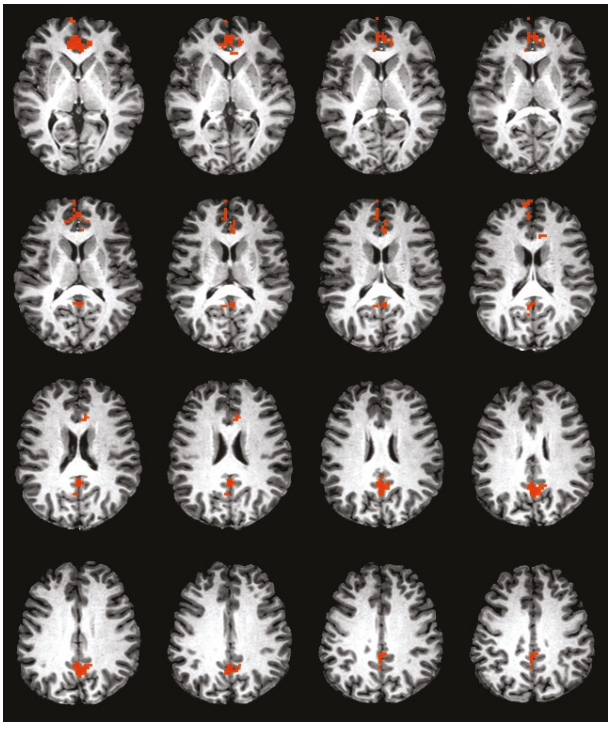

Figure 4. Brain activation during the hedonic evaluation of sucrose. Orange indicates areas where caffeine non-consumers had significantly greater activation in comparison to caffeine consumers.

4. Discussion

How one perceives taste stimuli has been shown to influence food choice and repeated consumption of a tastant may lead to altered taste preferences [32–35]. There are neuroimaging data to suggest that the human brain responds differently as a result of habitual consumption [17,18]. However, to our knowledge, there is no human research investigating brain response during hedonic evaluation of taste in caffeine consumers and non-consumers. In this study, we examined brain response in self-reported caffeine consumers and caffeine non-consumers during an fMRI scan to

investigate whether regular consumption of caffeine is associated with differential activation of areas related to memory, reward, and information processing. Imaging data from the present study indicate that caffeine consumers and caffeine non-consumers have significantly different neuronal activations in areas related to memory, reward, and information processing when processing individual taste stimuli. Each participant was exposed to 0.3 mL/sec of each tastant for 16 repetitions resulting in a total consumption of <5 mL, suggesting that these differences in activation occurred as a result from processing the taste alone, rather than the possible physiological effects of ingestion. When rating caffeine and sucrose, caffeine non-consumers had significantly greater activation in areas related to memory, reward, and information processing. During hedonic evaluation of saccharin, caffeine consumers had significantly greater activation in areas related to information processing. Overall, our results indicate differential neuronal activations between both groups during the processing of all three tastes. These results suggest differences in overall cognitive expenditure between the two groups, differing based on which taste was presented.

4.1. Psychophysical Data

Caffeine consumers and caffeine non-consumers demonstrated differences in taste perception. Post-scan intensity ratings of sucrose and saccharin were significantly higher in caffeine non-consumers compared to caffeine consumers. Further, caffeine non-consumer ratings of caffeine and sucrose intensity significantly increased from before to after stimulus presentation within an fMRI scan. The latter phenomena were not present in caffeine consumers.

Psychophysical results suggest that caffeine non-consumers perceived sucrose and saccharin as being more intense than caffeine consumers after the scan. Also, caffeine and saccharin intensity ratings significantly increased after stimuli were presented during the scan for caffeine non-consumers. A plausible explanation is that the sweet taste of sucrose and saccharin may have been potentiated by caffeine [20]. The increase of perceived intensity of caffeine and saccharin after the scans in non-consumers suggests a stronger reaction to bitter taste, which is present in caffeine and in saccharin as an aftertaste. There is evidence that the perceived intensity of caffeine's bitterness may be associated with whether caffeine is regularly consumed and the expression of bitter receptors, PAV-TAS2R38 [5,36]. It is plausible that both a genetic predisposition and caffeine consumption habits contributed to caffeine non-consumers perceiving all three tastes more intensely in comparison to caffeine consumers.

4.2. Reward Processing Areas

During the hedonic evaluation of caffeine and sucrose, caffeine non-consumers demonstrated greater activation in areas associated with reward processing.

During the hedonic evaluation of sucrose, caffeine non-consumers demonstrated significantly greater activation in both hemispheres of OFC BA 10, an area associated with encoding the incentive value of a stimulus during a decision-making task [37–39]. The OFC has been activated in response to abstract internal goals, such as rewards and punishments, while other tasks are being performed [37–39]. The OFC has been reported to be responsive to the reward value of tastes, as it associates other stimuli with tastes to produce representations of expected reward value [37,40]. A reward stimulus has been found to induce increased activation in OFC BA 10 when already activated by working memory processing [41]. Further, the OFC is activated by monetary rewards and punishment, with more activation reported following a punishment outcome [38].

During the hedonic evaluation of caffeine and sucrose, activation in the anterior cingulate cortex (ACC) was significantly greater in caffeine non-consumers. During the hedonic evaluation of caffeine, only activation in the left anterior cingulate cortex was found to be significantly greater in caffeine non-consumers in comparison to caffeine consumers. Lateralization in the ACC has been found during error processing and conflict monitoring, where correct inhibitions only occurred in the right ACC [42]. Further, observational fear learning has been found to only be activated in the right, but not the left ACC [43]. The distinction that right ACC activation only occurred during the hedonic evaluation of

sucrose and not during the hedonic evaluation of caffeine suggests that sucrose may have been a more intense experience for caffeine non-consumers. Psychophysical data supports this assertion, as caffeine non-consumers provided significantly higher intensity ratings for sucrose post-scan when compared to caffeine consumers (Table 3).

Overall, the ACC has been associated with an overall neural circuit that uses past action-reward history to learn action value in order to guide voluntary choice behavior [44]. This process requires referencing a history of outcomes regarding a given choice [44]. Further, previous studies suggest that reward processing in the ACC may also guide choice behavior, as it relates actions to their consequences [45]. This suggests that ACC has an essential role in learning and using extended action-outcome histories to make voluntary choices.

It is important to emphasize that activity in the OFC is representative not merely of a reward per se, but of a detailed and information rich representation of reward [46]. Similarly, the ACC references past-action reward history and is not a direct reflection of the reward value [44,45]. Therefore, the results are not necessarily indicative of caffeine non-consumers finding tastes to be more or less rewarding than caffeine consumers. A more plausible explanation may be that greater activation in the OFC and ACC found in caffeine non-consumers suggests a greater cognitive expenditure to use past reward history and process the representation of a reward, in order to make a voluntary choice, which in this case, was the hedonic rating.

4.3. Memory Processing Areas

During the hedonic evaluation of caffeine, caffeine non-consumers demonstrated significantly greater activation in right precuneus. During the hedonic evaluation of sucrose, caffeine non-consumers demonstrated significantly greater activation in both the left and right side of the precuneus. The right precuneus has been previously linked to autobiographical memory retrieval [47]. It is of particular interest that this area was activated during the hedonic evaluation of caffeine, an experience that would not be common in caffeine non-consumers. The precuneus is an area previously associated with episodic memory retrieval, the ability to recall a previously experienced stimulus [48]. Continuous theta burst stimulation (cTBS) over the precuneus in a picture memory task was associated with a decrease in source memory errors and improvement in context retrieval, suggesting that the precuneus is integral to a memory encoding and retrieval network [48]. During a source and item-recognition memory task, the left precuneus was activated during memory retrieval [49].

During the evaluation of sucrose, caffeine non-consumers also demonstrated greater activation in both the left and right of the posterior cingulate and cingulate gyrus. The posterior cingulate cortex has been associated with memory retrieval, namely autobiographical memory retrieval [50]. The posterior cingulate cortex also subserves evaluative functions such as monitoring sensory events and behavioral actions in the service of spatial orientation and memory [51].

These results support the hypothesis that caffeine non-consumers demonstrate greater cognitive expenditure in memory processing areas. We speculate that greater activations in the caffeine non-consumers while evaluating caffeine and sucrose could indicate a greater source memory retrieval expenditure. It is possible that caffeine non-consumers may have had less exposure to these tastes due to their dietary choices, and therefore, require greater cognitive effort to process them. Further, while sucrose is ubiquitous in all types of food, it is possible that experiencing caffeine's bitter taste is a new experience for caffeine non-consumers, not only in experiencing caffeine's flavor profile, but also its subsequent impact on other tastes.

4.4. Information Processing

Activation in information processing pathways was observed during hedonic evaluation of all three tastants. Activation in the right superior frontal gyrus (SFG) was significantly higher during the hedonic evaluation of sucrose. The right SFG has been linked to functioning in cognitive control, such that greater activation was linked to more efficient response inhibition, less motor urgency, as well

as greater self-regulation [52,53]. The left SFG was significantly higher during the hedonic evaluation of caffeine in caffeine non-consumers. The superior frontal gyrus, particularly the left SFG, has been associated with performing higher cognitive functions associated with working memory retrieval, especially in relation to task-related behavioral goals [54].

Both sides of the medial frontal gyrus were significantly activated in caffeine non-consumers during the hedonic evaluation of caffeine and sucrose, but not in the saccharin condition. Previous studies have linked activation in the left dorsolateral prefrontal cortex to processing and rating multimodal flavor stimuli [55]. Further, this is an area where the consequences of actions directly affect cognition in the preparation for and selection of response [55]. Results suggest a greater cognitive effort during the hedonic evaluation of caffeine and sucrose for caffeine non-consumers and during saccharin for caffeine consumers in information processing pathways coinciding with results previously stated. Due to the variability in between group activation within information processing areas, it is difficult to make a conclusive decision whether or not caffeine non-consumers activate a larger network than consumers in order to perform the hedonic evaluation task. While there was primarily more activation within the overall study in caffeine non-consumers, caffeine consumers demonstrated greater activation during the saccharin condition. We speculate that greater activation for caffeine consumers during saccharin evaluation may have occurred because saccharin evokes both sweet and bitter taste [56]. In addition to the stimulation of both sweet and bitter receptors, additional expenditure of cognitive effort may be required to hedonically evaluate this taste experience.

4.5. Further Considerations

There are limitations to this study. We did not investigate the potential differences in response between caffeine consumers who regularly consume caffeinated beverages with a higher sugar content and caffeine consumers who more regularly consume more bitter tasting beverages. Future studies may differentiate between the impact of taste processing for habitual consumers that drink primarily bitter tasting beverages (i.e., black coffee and tea) or items greater in sugar content (i.e., energy drinks). Further, in the caffeine consumers group, there were varying levels of caffeine consumption. Future studies may choose to expand on this paradigm, considering the effect of varying types and levels of caffeine consumption on taste perception.

We did not specify whether the taste stimuli were administered to the left or right side of the tongue. While we could not locate literature detailing a lateralization in processing of sweet and bitter taste alone, previous studies have reported laterization when discriminating tastes and rating taste quality [57,58]. Stevenson, Miller, and McGrillen [58] reported that when administering sour, sweet, salty, bitter, and umami solutions, discrimination among tastes was better when stimuli were applied to the right tongue tip and participants were better at taste quality judgements when tastants were applied to the left tongue tip [58]. All stimuli in the present study were administered to the tip of the tongue and whether the stimuli were more exposed to the left or the right side on trials was not specified. However, future studies could elaborate on this paradigm by taking this lateralization of gustatory processing into account.

The effects of caffeine consumption on taste perception are of considerable interest. Following a report that adenosine can enhance sweet taste in mice through its actions on A2B receptors in the taste bud, a recent report of a human psychophysical study suggested that caffeine, which is an adenosine-receptor antagonist, may decrease the perceived intensity of sweet taste through its action on adenosine receptors in sweet-sensitive taste cells [19,59]. Early studies of the effects of caffeine on taste had reported that in aqueous solutions of two component mixtures, caffeine decreased the sweetness of sucrose; and that when applied directly to the tongue with filter paper, caffeine enhanced the intensity of quinine HCl, NaCl, and a number of nonnutritive sweeteners, particularly those with bitter components (e.g., saccharin), but not the nutritive sweeteners sucrose and fructose [20,60]. The acute ingestion of caffeine has been reported to reduce the intensity of saccharin but not other taste stimuli, and that raising caffeine levels in the saliva for a period of three weeks had no measurable

effects on reported intensity of caffeine, denatonium benzoate or NaCl [61,62]. Differences in the effects of caffeine on sweetness intensity may be related to the stimuli, their concentrations, the route of administration or other methodological differences in these studies [19,20,60–62]. The current study focused on the effects of habitual caffeine consumption on fMRI of central brain response and found differential activation between caffeine consumers and non-consumers during hedonic evaluation of sucrose, caffeine, and saccharin stimuli. Further research on both intensity and hedonics of bitter and sweet stimuli, including natural as well as artificial sweeteners, in caffeine consumers and non-consumers will be of great interest to better understand the nature of caffeine's influence on taste perception.

5. Conclusions

In summary, we administered three tastants, caffeine, sucrose, and saccharin, to investigate differences in neuronal activation between those who were self-reported caffeine consumers and caffeine non-consumers. We found differences in intensity ratings between groups. We also found differences in activation patterns during a hedonic evaluation task. Our results suggest that there is greater activation for caffeine non-consumers while processing caffeine and sucrose and greater activation for caffeine consumers while rating saccharin. The results support differential memory, reward, and information processing of taste between those who habitually consume caffeine and those who do not. These results suggest that further research into the link between caffeine consumption and taste perception is warranted.

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nutrients

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Article

Sweet and Umami Taste Perception Differs with Habitual Exercise in Males

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Abstract: Taste is influenced by several factors. However, whether habitual exercise level is associated with differences in taste perception has received little investigation. The aim of this study was to determine if habitual exercise is associated with differences in taste perception in men. Active (n = 16) and inactive (n = 14) males, between ages 18–55, underwent two days of sensory testing, using prototypical taste stimuli of high and low concentrations for sweet, salt, bitter, sour, umami, and carbohydrate (maltodextrin). Mean perceived intensity and hedonic ratings were recorded. Eating behaviour was assessed by the three factor eating questionnaire and food intake by EPIC food frequency questionnaire (FFQ). There were moderate to large differences between the two groups in perceived intensity for sweet taste at the high concentration and umami taste at both high and low concentrations, with active males recording a higher perceived intensity ($p < 0.05$ for all). The active group also recorded a greater dislike for umami low and carbohydrate low concentration ($p < 0.01$). Salt, bitter and sour perception did not significantly differ between the two groups. FFQ analysis showed no difference in % energy from macronutrients between the groups. Eating behaviour traits correlated with sweet taste intensity and umami taste liking, independent of activity status. Results indicated that sweet and umami taste perception differ in active compared to inactive males. Habitual exercise level should be considered in taste perception research and in product development. Whether differences in taste perception could be one factor influencing food intake and thus energy balance with habitual exercise warrants further investigation.

Keywords: taste perception; umami; carbohydrate; sweet; salt; bitter; physical activity; intensity; liking

1. Introduction

The sense of taste allows us to identify and distinguish between sweet, sour, salty, bitter, and umami qualities [1], perceived on the tongue in the absence of odour. In addition, carbohydrate has recently been described as a taste [2] exemplified by maltodextrin. Taste sensitivity differs between individuals for different taste qualities [3,4]. There is considerable variation in the degree of taste perception, and a wide range of factors, including genetics [3,5], age [6], sleep [7] body mass index [8], anxiety level and neurotransmitters [9], hormonal factors [10], and habitual diet [11], among others, have been associated with differences in taste perception between individuals. Physical activity could potentially influence several of the modifiable factors associated with differences in taste perception. Although the outcomes are variable, some studies have reported alterations in taste perception during

and after a single bout of exercise (see References [12,13] for reviews). There is also some limited evidence that habitual exercise may be associated with differences in taste perception [14]. In a study of female swimmers and inactive females, swimmers were found to perceive high-sucrose stimuli as sweeter [14]. However, little other research to date has investigated taste perception and habitual exercise.

Characterising factors influencing food intake in active and inactive individuals is important to gain a greater understanding of the role of physical activity in energy balance [13,15,16]. Sedentary individuals have been proposed to be at a greater risk of overeating due to a lack of physiological regulation of appetite [17] and several aspects of appetite and food intake regulation have been shown to vary depending on habitual physical activity level [18–20]. Evidence from both cross-sectional and longitudinal studies suggests physical activity is associated with improved short-term appetite control [19]. Moreover, hedonic responses for high- or low-fat and sweet or savoury foods have been shown to differ between habitual exercisers and inactive individuals [20]. The underlying factors and mechanisms associated with differences in appetite control and food intake with physical activity, however, remain to be fully elucidated.

Understanding whether taste perception differs depending on physical activity level is important, as differences in taste perception could influence food choice or eating behaviour [3,4,21] and may be related to weight status [22,23]. Alterations in taste perception have been linked to weight gain, with a recent longitudinal study demonstrating attenuated sweet and salty taste perception was associated with weight gain in college-aged males [24]. Moreover, taste perception has been proposed as a factor that may influence athletes' food choices [25]. Determining whether taste perception differs in active and inactive individuals could, therefore, provide greater insight into factors influencing food choice and energy balance. For example, a reduced sensation of sweet or salty taste could potentially render inactive individuals more susceptible to weight gain.

The present study aimed to compare taste perception (taste intensity and liking) between active and inactive males for the five 'basic' taste qualities of sweet, sour, salty, bitter, and umami tastes, as well as the more recently proposed carbohydrate taste.

2. Materials and Methods

2.1. Study Design

Participants in this between-groups cross-sectional design study undertook two separate test mornings one week apart. Ethical approval for the study was provided by the University College Dublin School of Public Health, Physiotherapy and Sports Science undergraduate research ethics committee. The primary outcome measure was taste perception (intensity) and secondary outcomes were liking and identification of taste, anthropometry and body composition, eating behaviour, and habitual dietary intake.

2.2. Participants

Thirty men were studied (n = 14 inactive and n = 16 active) between the ages of 19 and 51 years. Inclusion criteria were: Male, aged 18–55 years, nondiabetic, no medical conditions and not taking medication known to influence taste perception, willing to consume study taste solutions, and nonsmokers. Participants were classified through a screening questionnaire based on their self-reported physical activity patterns over the last 6 months as either inactive (undertaking $\leq$1 structured exercise session per week and not engaged in strenuous work) or active (undertaking $\geq$4 structured exercise sessions per week). Individuals who did not fit either category were excluded. One exercise session was defined as at least 40 min of moderate to high intensity activity. Participants were also asked to record the typical intensity, frequency, and duration of each activity per week. These criteria were used as identical to previous studies showing differences in appetite control in active versus inactive individuals [18,26]. We have previously shown these categories to differ in

objectively measured physical activity [27]. Sample size calculations were conducted in G*Power [28] using data from a recent study assessing sweet intensity in adults [29], as taste intensity was the primary outcome measure, differences with physical activity in females were previously shown in relation to sweet taste [14], and the most similar literature that provided quantitative data to allow sample size calculation related to sweet taste. To detect a 10-point difference in sweet taste intensity ratings between the two groups in the present study with a power of 80% and a significance level of 5%, 14 individuals were required per group.

2.3. Recruitment and Setting

Recruitment was conducted through the distribution of recruitment flyers and emails throughout the university campus. Participants who were eligible to participate based on information provided in the screening questionnaire were invited to participate in the testing sessions. The testing sessions took place in the sensory evaluation suite at UCD's Institute of Food and Health. Participants were recruited from the 1 January 2018 until the 15 March 2018.

2.4. Body Composition Measurement and Taste Perception Assessment Day Protocol

On arrival, all participants provided written informed consent. On both test days, participants attended the laboratory in the morning, having avoided strong-flavoured foods/drinks, such as spicy foods and coffee, for 12 h and strenuous exercise for 24 h, and were instructed to wear light clothing for body composition measurements. Participants' height measurements were first taken using a stadiometer, followed by weight and body composition using a Tanita body composition analyser (BC-420MA, Tanita Ltd., Yiewsley, UK), which uses bioelectrical impedance (BIA) to assess body composition. Participants were then familiarised with the generalised labelled magnitude scale to assess perceived intensity (gLMS) [30]. The gLMS is a validated scale for assessing taste intensity, according to the standard protocol outlined previously by Green, Schaffer, and Gilmore [31] and Green et al. [32]. A generalised degree of liking scale (gDOL) with labels of 'neutral' and 'strongest liking/disliking of any kind' was used to assess liking of the stimuli.

2.4.1. Taste Stimuli

Food-grade, prototypical taste stimuli in water were prepared as follows: Sucrose (sweet) (27 mmol/L and 243 mmol/L), citric acid (sour) (1 mmol/L and 9 mmol/L), sodium chloride (salt) (33 mmol/L and 300 mmol/L), quinine (bitter) (0.056 mmol/L and 0.498 mmol/L), monosodium glutamate; MSG (umami) (0.51 g/L and 4.566 g/L) [24], and maltodextrin (carbohydrate) (dextrose equivalent 4.0–7.0, Sigma Aldrich, Arklow, Ireland) (35.5 g/L and 112.4 g/L) [2]. These concentrations were selected to provide 'low' and 'high' concentrations to allow comparison to recent research [2,24].

2.4.2. Taste Perception Rating

Participants undertook two identical taste sessions spaced one week apart. Previous work has indicated that at least two taste intensity ratings are necessary to achieve reliable estimates of individual taste responsiveness when using the gLMS [33]. Both sessions took place in the mornings, and participants followed identical instructions prior to each visit. The taste stimuli were the same in each session and mean results from the two ratings for each concentration of each taste were the primary outcome used in analyses. Results from the individual test days were also explored to examine the reliability of results at the individual test session. At each session, participants tasted 12 samples (high and low concentrations of the six tastes), served at room temperature in 20 mL medicine cups in a sip-and-spit manner in a randomised block design, presented blinded, using 3-digit randomised codes. The tests were administered on computers located in each sensory booth, using RedJade software (RedJade Software Solutions, LLC, Boulder, CO, USA). Participants were requested to identify the taste from a specified list ('sweet', 'salt', 'sour', 'bitter', 'umami', 'carbohydrate' or 'unsure') and then to rate the perceived intensity and then liking of the stimuli on a gLMS and gDOL respectively presented on

screen. A 30 s break was enforced with the software between each solution, with a 2-min break after every 4 samples, with water for rinsing provided between each sample.

2.4.3. Eating Behaviour and Habitual Diet

At the end of the taste protocol on the second test day, participants completed a paper-based version of the three factor eating questionnaire (TFEQ) [34] and European Prospective Investigation into Cancer and Nutrition (EPIC) food frequency questionnaire (FFQ) [35].

2.5. Statistical Analysis

Statistical analysis was performed using PASW Statistics 24.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 7.0 for Mac (GraphPad Software, San Diego, CA, USA). To determine if the data of the two groups was normally distributed, the Shapiro–Wilk test was used. For normally distributed data, a parametric independent *t*-test was used; otherwise, the Mann–Whitney U test was used. Pearson's correlation or Spearman's rank correlation coefficient were used to determine relationships between variables where appropriate. Effect size (ES: Cohen's d or r where appropriate) was also assessed. Multiple regression analysis was undertaken to identify the effects of confounding variables such as age and body composition on taste perception. There were no missing data for any outcomes, except for the TFEQ and FFQ for two participants in the active group. Only complete data for the TFEQ was used in analyses (n = 14 in both groups). Statistical significance was considered at $p < 0.05$ and data are reported as mean (SD) unless otherwise stated.

3. Results

3.1. Subject Characteristics

Descriptive data for the active compared to inactive groups are shown in Table 1. Age, height, weight and body mass index (BMI) did not significantly differ between the two groups; however, the active group had a lower body fat percentage.

Table 1. Subject characteristics of active (n = 16) and inactive (n = 14) groups.

	Active	Inactive	*p*-Value	Effect Size (*d*)
Age (years) Median (IQR)	21 (21.0–22.5)	21 (20.5–25.5)	0.79	r = 0.05 [1]
Height (cm) Mean (SD)	179.83 (4.91)	181.09 (5.12)	0.50	0.25
Weight (kg) Mean (SD)	79.03 (8.02)	81.49 (10.96)	0.49	0.26
BMI (kg/m^2) Mean (SD)	24.41 (1.93)	24.80 (3.14)	0.68	0.15
Body Fat (%) Mean (SD)	12.33 (4.30)	18.87 (6.45)	<0.01	1.19

[1] As data were not normally distributed, r is used for effect size.

3.2. Taste Identification

Although the study was not designed to assess identification of tastes, we explored whether it differed between the two visits and two groups, as it could potentially influence the results. Overall, taste identification did not differ significantly for any taste and concentration between the first and second session, suggesting no learning effect for taste identification occurred. Mean percentage of tastes correctly identified was greater at the high concentrations than at the lower concentrations for both groups ($p < 0.05$) but did not differ between the two groups ($p > 0.05$). When the individual tastes were compared at the two visits, a greater percentage of the active group correctly identified the umami taste compared to the inactive group ($p = 0.03$). However, there were no significant differences in the identification of all tastes between the two groups for all other tastes and concentrations at the two visits.

3.3. Taste Intensity

Perceived intensity in the active compared to inactive groups for the six tastes studied are shown in Figure 1. There was a large difference (ES: $d = 1.63$, $p < 0.05$) in perceived intensity for the high-concentration sweet (sucrose) taste between the two groups, with the active group recording a significantly higher intensity rating compared to the inactive group (Figure 1A). Significantly higher intensity ratings were also observed in the active group for the umami (MSG) taste (Figure 1C), with a large difference between the two groups for the low concentration (ES: $d = 1.18$, $p < 0.01$) and moderate difference at the high concentration (ES: $r = 0.38$, $p < 0.05$). Perceived intensity did not significantly differ between the two groups for the low concentration of sucrose, nor for either high or low concentrations of citric acid, quinine, sodium chloride, and maltodextrin ($p > 0.05$ for all; Figure 1). For the latter comparisons, effect sizes were small for all ($d < 0.30$), except for the low concentration of quinine ($d = 0.60$) and low concentration of maltodextrin ($d = 0.66$).

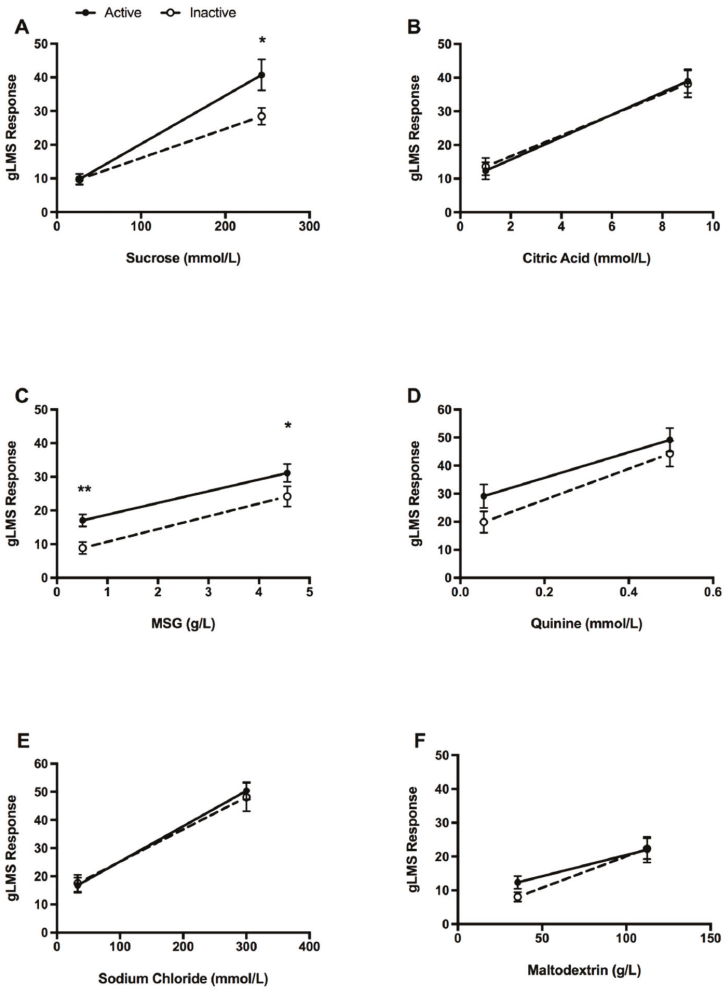

Figure 1. Differences (mean (SE)) in perceived intensity responses on a generalised labelled magnitude scale (gLMS) for high and low concentrations of: (**A**) Sweet, (**B**) sour, (**C**) umami, (**D**) bitter, (**E**) salt, and (**F**) carbohydrate (maltodextrin) taste intensity ratings with physical activity. Solid dark line indicates active group, dashed line indicates inactive group. * $p < 0.05$, ** $p < 0.01$. MSG, monosodium glutamate.

3.4. Hedonic Response

Hedonic ratings in the active compared to inactive group for the six tastes studied are shown in Table 2. Apart from the sweet taste, the majority of tastes had negative ratings, indicating varying levels of dislike on the gDOL. There was a large difference between the two groups in liking of the umami low concentration with the active group recording a dislike of the taste, compared to a mean response of a weak liking in the inactive group. Similarly, the active group recorded a dislike for the low-concentration carbohydrate taste, compared to a mean response of a weak liking in the inactive group (Table 2). Although there were no statistically significant differences in hedonic ratings between the two groups for the other solutions, moderate effect sizes were observed for the bitter taste, with a trend towards a greater dislike of the bitter taste in active individuals.

Table 2. Hedonic ratings of tastes assessed using a generalised degree of liking scale (gDOL) [1] in active (n = 16) compared to inactive (n = 14) males.

	Active Mean (SD)	Inactive Mean (SD)	p-Value	Effect Size (d)
Sucrose				
High	38.91 (27.68)	34.75 (28.66)	0.69	0.15
Low	1.44 (10.98)	5.18 (21.85)	0.42	0.22
MSG [2]				
High	−17.06 (31.66)	−16.79 (29.36)	0.98	<0.01
Low	−13.09 (20.31)	2.82 (11.50)	<0.01	0.96
Citric acid				
High	−3.72 (34.10)	0.36 (33.31)	0.74	0.12
Low	−5.34 (18.19)	2.07 (22.59)	0.33	0.36
Quinine				
High	−57.50 (20.97)	−38.79 (28.06)	0.05	0.76
Low	−34.00 (22.86)	−17.89 (29.02)	0.08	0.62
Sodium chloride				
High	−33.16 (43.22)	−29.57 (35.17)	0.50	0.09
Low	−18.72 (16.21)	−11.96 (23.87)	0.73	0.33
Maltodextrin				
High	−7.16 (25.5)	−3.32 (22.31)	0.67	0.16
Low	−12.28 (12.76)	0.21 (14.29)	<0.01	0.93

[1] The labels of the scale were 'neutral' and 'strongest liking/disliking of any kind'. [2] MSG, monosodium glutamate.

3.5. Reproducibility of Taste Intensity Comparisons between Groups at Individual Test Days

Interestingly, carbohydrate perceived intensity was higher at the first visit in the active group ($p < 0.05$) but did not differ at the second visit and therefore was not significantly different when mean ratings were compared. By contrast, umami high concentration intensity ratings did not significantly differ statistically between groups at the individual test days but differed when mean ratings were compared. However, most differences that were observed in mean ratings were also evident at both individual test days, with significant differences or similar trends observed ($p < 0.1$) for high-concentration sweet and low-concentration umami. Moreover, similar to mean ratings, perceived intensity for low-concentration sweet, and both concentrations of sour, bitter, and salty were not different between the two groups at the separate test visits.

3.6. Habitual Dietary Intake

There was no difference observed in the percentage of energy from macronutrients, or carbohydrate between the active and inactive group (Table 3), except for fructose, which was higher in the inactive group ($p = 0.04$), and fibre, which trended towards being higher in the inactive group ($p = 0.05$).

Table 3. Mean energy intake and percentage of energy from macronutrients for active and inactive men (FFQ data).

	Active (n = 13) [1] Mean (SD)	**Inactive (n = 13) [1] Mean (SD)**	***p*-Value (2-Tailed)**
Energy Intake kcal/day	2290.7 (841.6)	2018.7 (826.7)	0.41
Macronutrient Intake, % of energy			
Fat	36.4 (3.7)	37.7 (6.6)	0.53
Protein	21.5 (3.6)	19.9 (2.8)	0.22
Carbohydrate	43.8 (4.6)	42.0 (8.8)	0.50
Sugar	18.1 (3.5)	18.7 (4.6)	0.73
Sucrose	6.5 (2.3)	6.7 (1.9)	0.78
Fructose	2.5 (1.0)	3.4 (1.2)	0.04
Galactose	0.1 (0.1)	0.1 (0.1)	0.59
Maltose	0.5 (0.1)	0.5 (0.3)	0.77
Lactose	5.6 (2.0)	4.1 (2.7)	0.11
Starch	25.0 (3.8)	22.5 (6.9)	0.25
Fibre	2.9 (0.8)	3.6 (1.0)	0.05

[1] FFQ (food frequency questionnaire) data were available for n = 26 individuals (n = 13 per group). Data were missing for two participants and data for two individuals were removed due to energy misreporting (energy intake >2 SD above or below the mean energy intake were removed as per Low et al. [2].

3.7. Regression Analysis Including Age, BMI, and Body Composition

3.7.1. Taste Intensity

For the sweet taste, physical activity status was the only variable associated with perceived intensity at the high concentration (model adjusted R^2: 0.13; $ß = -0.39$, $p = 0.03$). Age, BMI or percentage of body fat were not independently associated with sweet taste intensity or when included in models for either concentration ($p > 0.1$ for all).

For the umami taste at both concentrations, physical activity status showed the strongest association with perceived intensity. BMI, body fat or age were not associated with differences in umami perceived intensity ($p > 0.1$).

For the sour taste, at the low concentration, both age and body composition were significantly associated with perceived intensity ($p < 0.01$) but not independently in the same model. Moreover, there were no associations between sour taste at the high concentration and age, BMI, percentage of body fat or physical activity status. Bitter and salty tastes also showed no significant associations with these variables at either the high or low concentrations ($p > 0.1$ for all). For the carbohydrate taste, BMI and activity status together in the same model significantly predicted perceived intensity at the low concentration (model adjusted R^2: 0.14; $p < 0.05$; activity status $ß = -0.30$, $p = 0.099$; BMI $ß = -0.31$, $p = 0.08$).

3.7.2. Liking

Sweet and sour taste liking were not associated with physical activity status, age or body composition ($p > 0.1$). However, activity status was associated with liking of the low-concentration umami ($ß = 0.44$, $p = 0.02$), high-concentration bitter ($ß = 0.37$, $p < 0.05$), and low-concentration carbohydrate ($ß = 0.43$, $p = 0.02$) tastes. Liking of the low-concentration salty taste ($ß = 0.45$, $p = 0.01$) and of the high-concentration umami taste ($ß = 0.45$, $p = 0.01$) were both associated with age. Liking of the low-concentration carbohydrate solution was the only variable associated with percentage of body fat ($ß = 0.45$, $p = 0.01$) and, together with activity status in the same model, was associated with 20% of the variance in liking for carbohydrate at the low concentration (model adjusted R^2: 0.20; $p < 0.05$; activity status $ß = 0.27$, $p = 0.18$; body fat $ß = 0.31$, $p = 0.13$).

3.8. Regression Analysis with Eating Behaviour

3.8.1. Hunger

Sweet taste perceived intensity for the high concentration was positively associated with the trait hunger ($ß = 0.39$, $p = 0.04$), (i.e., a higher hunger score was associated with greater perceived intensity). This remained significant when included in the same model as activity status. Together, activity status and hunger were associated with 27% of the variance in perceived intensity for sweet taste at the high concentration (model adjusted R^2: 0.27; $p < 0.01$). In addition, hunger was associated with perceived intensity for the high-concentration bitter taste ($ß = 0.44$, $p = 0.02$) and liking for the high-concentration salt taste ($ß = 0.43$, $p = 0.02$).

3.8.2. Disinhibition

Disinhibition was also associated with liking of the high-concentration salt taste ($ß = 0.49$, $p < 0.01$), but not with any other variables.

3.8.3. Restraint

Perceived intensity for the high-concentration bitter taste ($ß = -0.41$, $p = 0.03$), and liking of the low- ($ß = -0.46$, $p = 0.02$) and high- ($ß = -0.43$, $p = 0.02$) concentration umami taste were associated with dietary restraint. As activity status (active or inactive) was also significantly associated with liking for umami low concentration (model adjusted $R^2 = 0.19$, $ß = 0.439$, $p < 0.015$), they were included in the same model. Together, activity status and restraint accounted for 38% of the variance in umami low concentration liking (model adjusted R^2 0·38, $p < 0{\cdot}001$; activity: $ß = 0{\cdot}46$, $p < 0{\cdot}01$; restraint: $ß = -0.47$, $p < 0{\cdot}001$). Dietary restraint was also inversely associated with liking of the high-concentration carbohydrate taste ($ß = -0.41$, $p = 0.03$).

4. Discussion

The present findings demonstrate that taste perception intensity differed between active and inactive males. In this cohort, active males reported a greater perceived intensity for both sweet and umami tastes. Given previous evidence of associations between taste perception and food choice [3,4,21], and weight gain [24], these findings may have implications for understanding factors influencing the control of food intake and energy balance with habitual exercise.

Although limited research has investigated associations of habitual exercise with taste perception, our finding of a greater perceived intensity of sweet taste in active males is comparable to a previous study in females. Crystal, Frye, and Kanarek [14] found female swimmers perceived a high-concentration sucrose solution as sweeter compared to inactive females' using visual analogue scales (VAS). In response to acute exercise, increases, no change, and decreases in acuity of taste and rated preference for tastes have been previously reported, with results appearing to depend on differences in length and intensity of the exercise session and the taste [13]. Regarding sweet taste specifically, Westerterp-Plantenga et al. [36] observed an increase in perceived intensity of taste using VAS for a low-concentration sucrose solution (but not high-concentration) following 2 h of moderate intensity cycling. By contrast, others have reported no change in sweet taste intensity for a sucrose solution, but an increase in intensity of sour taste following 10 minutes of cycling to generate a 'light sweat' [37]. However, assessing differences with longer-term interventions and with habitual exercise is also essential, as the repeated effects of regular exercise on physiological and psychological processes of appetite control do not always mimic the acute effects of exercise. Participants in the present study were instructed to avoid strenuous exercise for 24 h before the test sessions to avoid influence of acute exercise on results.

Perceived intensity of umami taste also differed between the active and inactive groups. Perceived intensity of both low and high concentrations of MSG was rated as significantly higher in active males. In a previous study, along with the other 'basic' tastes, Horio and Kawamura [38] assessed umami

threshold and liking using six different concentration solutions of MSG after moderate-intensity cycling and found no difference compared to pre-exercise in healthy university students. Generally, the effects of both acute and chronic exercise on umami taste perception, however, have not been extensively studied previously.

Several factors could contribute to the differences in sweet and umami taste perception we observed with habitual exercise. Some previous studies have shown habitual diet to be associated with taste perception. For example, sweet taste intensity has been shown to negatively correlate with total energy and carbohydrate and sweet food intake [11], although this was not demonstrated elsewhere [39]. By contrast, higher carbohydrate taste intensity has been positively associated with greater energy and starch intakes, assessed by either FFQ or food diary [2]. In the present study, however, we did not observe differences in the percentage of energy consumed from starch, sugar or other carbohydrate forms, apart from fructose, which was higher in the inactive group, although the limitations of FFQ are recognised.

Eating behaviour traits could also contribute to differences in taste perception between individuals. Dietary restraint and disinhibition have been identified as factors that may influence relationships between adiposity and taste sensitivities to 6-n-propylthiouracil [40]. Therefore, we investigated whether eating behaviours could influence associations between habitual exercise and taste perception. The trait hunger and physical activity were both independently associated with sweet taste intensity perception at the high concentration in the same model. Hunger and restraint were both also associated with perceived intensity of the high-concentration bitter taste (quinine); however, eating behaviours were not related to other perceived intensity ratings. These findings suggest eating behaviour traits may be linked to taste perception of some tastes; however, the traits hunger, restraint or disinhibition do not explain the differences in taste perception observed with habitual exercise.

Although we did not assess hormonal status, hormonal differences could be another mechanism contributing to the differences in taste perception we observed in active compared to inactive males. It is interesting that perception of sweet and umami were the two tastes that differed between the active and inactive groups, indicating the differences in perception were specific mainly to these tastes and not an overall effect on taste function. Both sweet and umami share the same class of taste receptor in the mouth, which initiate a G protein-coupled signalling cascade [10,41,42]. There is now strong evidence that in addition to intestinal signalling, glucagon-like peptide-1 (GLP-1) signalling also occurs within the taste bud [43], and evidence from animal studies indicates GLP-1 signalling has an important role in the modulation of both sweet and umami taste [42]. In the present study, a sip-and-spit technique was used for the tasting of solutions, suggesting any differences in the taste responses observed are influenced by orososensory mechanisms and not a post-oral response to nutrients. Other hormones modified by regular exercise or body composition could also potentially contribute to the differences we observed (see Reference [10] for a review). For example, leptin can inhibit the response to sweet taste [10,44]. An increased circulating leptin (due to a greater body fat percentage) in less active individuals could be one potential mechanism contributing to a reduced intensity of sweet taste. However, BMI or percentage body fat did not moderate the relationship between activity status and sweet taste perception in the present study. Endocannabinoids [45] and glucose levels [46] also influence sweet taste responses and are altered with physical activity [47]. Characterising multiple hormonal factors is warranted in future studies, as it may provide mechanistic insight into differences in taste perception with habitual exercise.

Regarding liking, sweet was the only taste to be positively rated by both groups, adding support that sweet tastes are liked by most individuals [48]. We also observed that in addition to a greater perceived intensity in the active group, active males also had a lower (negative) hedonic rating for the low-concentration umami taste, suggesting the greater perceived intensity could have contributed to a dislike for the taste. In contrast, liking of the sweet and umami high concentration tastes did not differ between the active and inactive groups, despite differences in perceived intensity. Others have also observed no difference in hedonic rating, despite changes in taste perception of

simple solutions, including sucrose and quinine sulphate, with acute exercise [36]. One explanation could be the form in which the taste stimuli are provided. For standardisation, all samples here were provided to participants in solutions made up with water. However, when compared to more complex food and beverage matrices, possible differences are likely [49]. The implications of differences in perceived taste intensity with habitual exercise for liking of different foods and food choice warrant further investigation.

Various methodological aspects of the present study should be considered. Participants included males only to eliminate the possible interference of the menstrual cycle on taste perception, and to add to previous research in females [14]. Further research in females is warranted. As body composition has a role in some hedonic aspects of appetite control [15,20], body composition (fat and fat free mass) should also be further considered. Links between taste and body weight or BMI have been previously studied [8,22,23]; however, fewer studies [50] have examined fat or fat free mass. In the present study, BMI did not differ between active and inactive groups, while percent body fat differed significantly. These and other potential modifying variables were included in regression models but did not modify the relationship between physical activity status and taste perception. For logistical reasons, body composition was assessed using BIA. Relationships between taste and body composition have previously been reported using BIA [51]. However, studies have shown variable findings regarding the accuracy of BIA [52,53]. Future studies should explore relationships between taste perception and body composition (fat and free mass) on an individual level using more accurate measures. Measurement of waist circumference would also be relevant. In addition, objective measurement of physical activity should be considered in future studies, to further elucidate relationships with aspects such as energy expenditure and sedentary behaviour. Finally, a significant strength of the study is the use of six tastes, low and high concentrations and two taste sessions, allowing a comprehensive assessment of potential differences in taste perception with habitual exercise. Therefore, the findings can be considered to provide reliable estimates of individual taste responsiveness in active versus inactive males.

In conclusion, these data show sweet and umami taste perception differ in habitual exercisers compared to inactive individuals. There is evidence elsewhere that habitually active individuals have improved energy compensation for energy density of foods [19]. Alterations in taste perception could be one potential mechanism contributing to the regulation of energy balance with exercise. While causal inferences cannot be drawn due to the cross-sectional nature of this study, the findings have implications for researchers and for product development—indicating habitual exercise level should be considered in studies examining taste perception and for consumer selection for product development. Further studies are needed to examine longitudinal responses to exercise intervention and to further explore the underlying mechanisms and implications for food intake.

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Modeling Associations between Chemosensation, Liking for Fats and Sweets, Dietary Behaviors and Body Mass Index in Chronic Smokers

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Abstract: Chronic smokers have a greater risk for altered chemosensation, unhealthy dietary patterns, and excessive adiposity. In an observational study of chronic smokers, we modeled relationships between chemosensation, fat/carbohydrate liking, smoking-associated dietary behaviors, and body mass index (BMI). Also tested in the model was liking for sweet electronic cigarette juice (e-juice). Smokers (n = 135, 37 ± 11 years) were measured for: Taste genetics (intensity of 6-n-propylthiouracil—PROP); taste (NaCl and quinine intensities) and olfactory (odor identification) function; liking for cherry e-juice; and weight/height to calculate BMI. Smokers survey-reported their food liking and use of smoking for appetite/weight control. Structural equation models tested direct and indirect relationships between chemosensation, fat/carbohydrate liking, dietary behaviors, and BMI. In good-fitting models, taste intensity was linked to BMI variation through fat/carbohydrate liking (greater PROP intensity→greater NaCl intensity→greater food liking→higher BMI). Olfactory function tended to predict sweet e-juice liking, which, in turn, partially mediated the food liking and BMI association. The path between smoking-associated dietary behaviors and BMI was direct and independent of chemosensation or liking. These findings indicate that taste associates with BMI in chronic smokers through liking of fats/carbohydrates. Future research should determine if vaping sweet e-juice could improve diet quality and adiposity for smokers.

Keywords: sweet liking; fat liking; e-cigarettes; body mass index; dietary behaviors; smell; taste; tobacco; cigarettes; chronic smoking

1. Introduction

Cigarette smoking and obesity increase disease susceptibility and all-cause mortality risk. Although U.S. cigarette smoking rates have declined, 37.8 million adults (15.5%) were current smokers in 2016 [1]. Conversely, U.S. rates of obesity have increased, with 39.8% of adults affected in 2015–2016 [2]. Chronic smoking with obesity is a complex interplay between unhealthy behaviors and biological factors that fuel both conditions and require unique attention for health promotion efforts [3]. The smoking-adiposity relationship, however, is not linear. Nicotine, a parasympa-thomimetic alkaloid in tobacco, produces an anorectic effect [4] and elevates metabolic rate [5], elucidating findings of lower body weight among smokers [6,7]. Yet two population-based studies showed that long-term smokers had a greater risk of overweight [8] and obesity [9], which may result from unhealthy dietary patterns [10–12]. A population-based survey showed that heavy smokers consumed a more pro-inflammatory diet (energy-dense, rich in saturated fats, added sugars, and refined carbohydrates) than did nonsmokers [10], fueling greater adiposity [13]. There also are parallels in brain reward

circuitry in response to nicotine addiction and highly palatable fats and sweets, which support weight gain [14]. Of interest in the present study are associations between dietary behaviors and adiposity in chronic smokers, and whether these associations are influenced by chemosensory function, which may be altered by routine exposure to cigarette smoking.

There have been inconsistent associations between smoking and taste function in the literature, with most studies reporting effects on taste thresholds. Suprathreshold function, however, may have more applicability in efforts to understand links between taste and dietary behaviors. For example, a small trial showed that obese smokers reported less sweetness and creaminess from sugar/fat mixtures than did non-smokers and normal weight smokers [15]. Relative to non-smokers, chronic smokers from our laboratory-based study reported higher taste intensity from concentrated NaCl [16]. Variation in ability to taste the bitterness of phenylthiocarbamide (PTC) and propylthiouracil (PROP), phenotypes of genetic variation in taste, also has been studied in smokers. Cigarette smokers have been hypothesized as more likely to be PTC/PROP nontasters [17]. Smokers who are phenotypically nontasters show less aversion to the bitter taste of nicotine [18,19], may have greater nicotine dependence [20], and may be more likely to smoke based on sensory cues than smokers who are PTC/PROP tasters [21]. However, our laboratory-based study [16] and a crowdsourced cohort study [22] did not find greater frequencies of PROP nontasters among chronic smokers. Importantly, there is racial/ethnic variability in PTC/PROP tasting [23] and in cigarette smoking [1], convoluting the ability to study their intersection and effects on dietary behaviors. The olfactory function also may be influenced by cigarette smoking. A meta-analysis revealed significantly higher odds of olfactory dysfunction among current smokers [24]. Similarly, our laboratory-based study showed higher rates of hyposmia among chronic smokers from the nationally-representative 2012–2014 NHANES [16].

Of interest is how alterations in taste and smell function in chronic smokers might influence dietary behaviors and risk of excessive adiposity. Both senses play unique roles in dietary behaviors [25]. Taste function has been most commonly studied through the effects of PTC/PROP on taste and oral sensations [26], food preference [27], dietary patterns, and body weight [28]. Variation in taste function beyond PROP also has been linked to food preferences and ingestive behaviors, including salty taste [29]. Smell function plays a priming role in dietary behavior [25], cueing appetite and cephalic phase responses [30]. As reviewed, an individual's dietary and weight response to smell impairment varies partially by self-awareness and response to the impairment [31]. Some studies have reported that smell impairment has been linked to differences in food preferences [32], while other studies have reported no substantial differences in food preferences [33] or dietary choices [34].

Obese smokers [3], especially women [35], have reported using cigarette smoking for weight control. Electronic cigarettes (e-cigarettes) are also marketed [36] and perceived as safer for weight/appetite control than tobacco cigarettes [37,38]. E-cigarettes allow the inhalation of vaporized vegetable glycerol- or propylene glycol-containing fluids (e-liquids/e-juices) that vary in nicotine concentrations and flavorings. Sweet e-juice flavors are the most popular [39], increasing the liking [40], reward and reinforcement values of e-cigarettes [41], and ability to enjoy vaping flavors that mimic sweets without ingesting calories [38]. These factors may have contributed to the substantial increase in e-cigarette usage, with global sales totaling $3.5 billion USD in 2015 [42] and may surpass $20 billion USD by 2025 [43].

Because of growing interest in overlaps between cigarette smoking, sensory cues, and dietary behaviors [14], as well as interest in e-cigarettes for appetite/weight control, we aimed to describe associations between taste and olfactory functions, food liking, smoking-associated dietary behaviors, e-juice flavor liking, and body mass index (BMI) in chronic smokers exposed to e-cigarettes. We hypothesized that chemosensory function would influence food liking, which, in turn, would influence food/beverage liking and smoking-associated dietary behaviors, which, in turn, would be associated with BMI. We used structural equation modeling to describe simultaneous associations between taste and smell function, food and sweet e-juice liking, smoking-associated dietary behaviors, and BMI among chronic smokers.

2. Materials and Methods

2.1. Participants

Purposive convenience sampling was used to recruit chronic smokers, ages 18 to 55 years, who resided in Hartford County, Connecticut. Potential participants answered newspaper and radio advertisements from May 2014 to December 2016. A telephone screening ensured that all initial exclusion and inclusion criteria were satisfied. The criteria for exclusion were: (1) Unstable medical or psychiatric disorders, including uncontrolled hypertension (blood pressure $\geq$160/100 mm Hg); (2) pregnancy; (3) awareness of hypersensitivity to nicotine or propylene glycol; (4) medical history of myocardial infarction(s) or cerebrovascular accident(s). The criterion for inclusion was the current use of a minimum of ten cigarettes daily. The study was approved by the Institutional Review Board (IRB) at the University of Connecticut Health Center. Participants provided informed and written consent and were compensated $20 upon completion of baseline assessments in the initial visit. Data described in the present paper were obtained from 135 participants (65 males) who completed the baseline visit.

2.2. Study Procedures and Measures

Eligible participants were invited for baseline laboratory testing, conducted between 10:00AM and 2:00PM. All subjects were requested to refrain from cigarette smoking for at least three hours prior to testing. After completion of the consenting process, all subjects underwent a physical examination. Current smoking status was confirmed by a breath carbon monoxide (CO) test using a Bedfont Micro+ TM Smokerlyzer handheld CO monitor (Bedfont Scientific Ltd, Harrietsham, Kent, UK). Potential participants were instructed to first inhale, hold their breath for 15 s, and then exhale slowly into the mouthpiece, aiming to empty lungs completely. CO levels are heightened in current smokers; cut-off values of $\leq$12 ppm can identify smokers who have refrained from smoking for at least 8 h [44]. In addition, because alcohol consumption has been linked to cigarette smoking behaviors, smokers were briefly assessed for patterns of heavy and binge alcohol consumption via a timeline follow-back method (TLFB) [45]. Heavy drinking was defined as the consumption of $\geq$8 drinks per week over the preceding three months while binge drinking was considered to be the consumption of $\geq$4 drinks during a single occasion for females or $\geq$6 drinks for males.

After providing consent and completion of a physical examination, participants completed the following procedures in a single visit to a hospital-based clinical research center:

2.2.1. Taste and Smell Function

For the taste testing, participants were oriented, with a specific script, to the general Labeled Magnitude Scale (gLMS) and verbalized intensity ratings. The gLMS generalizes the LMS [46] to all sensations [47]. Shown in the vertical orientation on a 100-point scale, it ranges from "no sensation" (0) to "strongest sensation of any kind" (100) and includes intermediate labels at "barely detectable" (1.4), "weak" (6), "moderate" (17), "strong" (35), and "very strong" (53) [47]. Participants practiced rating the intensity of brightness of three remembered stimuli (well-lit room, dimly lit restaurant, brightest light ever seen) and then reported the intensity of 1000 Hz tone series presented in 12 dB steps from 50–98 dB. Then, participants rinsed their mouth with bottled water and then sampled tastants, served at room temperature and in plastic medicine cups. Participants reported the intensity of each tastant (1 mM quinine hydrochloride (QHCl; Sigma-Aldrich, St. Louis, MO, USA) and 1 M sodium chloride (NaCl; Morton Salt, Chicago, IL, USA), which were first drawn across the tongue apex with a medical cotton swab and then, in addition to 0.32 M NaCl, were sampled with the whole mouth per protocol via the National Health and Nutrition Examination Survey (NHANES) 2012–2014 guidelines [48]. Following this, 1 mM and 3.2 mM propylthiouracil (PROP; Tokyo Chemical Industry Co., Ltd., Portland, OR, USA) were sampled with the whole mouth. The 1 mM and 3.2 mM PROP intensities were averaged for use in the analyses. Because chronic smokers reported higher intensities from 1 M NaCl than did nonsmokers [16], only 0.32 M NaCl and averaged PROP intensities were tested

in the analyses that follow. Using an algorithm previously reported [16], categorized PROP taster statuses (supertasters, medium tasters, non-tasters) were explored via chi-square to assess differences by demographics.

Smell function was measured using a 16-item odor identification task, with odors generated by an olfactometer (Osmic Enterprises, Inc., Cincinnati, OH, USA). These odor items included food (cherry, strawberry, lemon, onion, coffee, cinnamon, chocolate, grape, vanilla), warning (gasoline, smoke, menthol); and household (soap, leather, baby powder, rose) odors. Participants were instructed to lean toward the olfactometer nozzle, sniff the generated odor, and, in a forced-choice procedure, refer to four choices (one correct option and three distractors shown as pictures and word-labels), and pick the best choice. Possible scores on the odor identification task ranged from 0 to 16 correct responses for classification of: Anosmia/severe hyposmia (0–7 correct), hyposmia (8–12 correct), and normosmia ($\geq$13 correct).

2.2.2. Liking for Saturated Fats/Carbohydrates

The intensities of liking/disliking of 40 foods and beverages and 11 non-foods (physical activities, smoking products, pleasurable/unpleasurable items) were measured on a validated liking survey [49,50]. The liking survey included a bidirectional, 100-point horizontal scale labeled with five faces and verbal descriptors, which ranged from "neither like nor dislike" (0) to "strongest disliking/liking of any kind" ($\pm$100), with survey items shown as a picture and verbal descriptor. Before starting the survey, the participants were oriented to the scale (verbally and in print) with examples that represented the intensity of disliking (running out of money, paper cut), liking (winning the lottery, succeeding) and neutral (doing a routine chore). The scores on 19 of the 40 individual foods/beverage items that contribute to excessive adiposity [51] were averaged together to comprise a reliable saturated fat/carbohydrate liking index (Cronbach's α = 0.8): Sweets and sugary beverages (doughnuts, cookies/cake/pie, chocolate, soda/sweet drinks, coffee drinks/Frappuccino®/Coolatta®, sports drinks); high fat foods (breakfast sausage/bacon, butter/margarine, beef steak, fried chicken, whole milk, ham/pork, mayonnaise); and carbohydrates (french fries, whole wheat bread, high-fiber bar, bagel/rolls, spaghetti/pasta, high-fiber cereal). This conceptual food group was used in the analysis.

2.2.3. Liking for E-juice Flavors

The e-cigarettes (Joyetech eGo-C, Shenzhen Joyetech Co., Ltd., Shajing Town, Baoan District, ShenZhen, China) were filled with e-juices (Americanliquidscore.com) comprised of a base (50% vegetable glycerin-50% propylene glycol) and 18mg/mL of nicotine alone (flavorless) or with a flavoring (tobacco, chocolate, cherry, or menthol). Participants rinsed the mouth with bottled water then blindly vaped each e-juice for one minute, presented in random order. Subjects rated the flavor-nicotine combinations for sweetness, bitterness/sourness, irritation, and level of liking/disliking on the hedonic gLMS [47]. Of the flavors tested cherry e-juice was rated highest in sweetness [52]. In the models explored here, all e-liquid flavors were tested, but only the cherry flavor showed a significant association with the variables of interest.

2.2.4. Smoking-Associated Dietary Behaviors

Five items from the 68-item Wisconsin Inventory of Smoking Dependence Motives (WISDM) [53] asked participants to report tendencies to use smoking for appetite and weight control on a seven-point Likert scale (1 = "not true of me at all" to 7 = "extremely true of me"). The index of the five summed items (range 5 to 35) had a Cronbach's α = 0.85.

2.2.5. Body Mass Index

A registered nurse obtained the weight and height from each participant at baseline visit. Body mass index (BMI) was calculated as: (weight (kg) /height (meters)^2). BMI was classified as:

Underweight (>18.5), normal weight (18.5–24.99), overweight (25.0–29.99), and obese (>30). In order to create more even distributions in BMI categories, underweight participants (n = 3) were included in the normal weight category.

2.3. Statistical Analysis

Statistical analyses were conducted using Statistical Package for the Social Sciences (SPSS) version 25.0 (IBM, Armonk, New York, NY, USA), R version 3.5.0 (*R* Foundation for Statistical Programming, Vienna, Austria), and AMOS version 25.0 (IBM, Armonk, New York, NY, USA) with statistical significance criterion set at $p \leq 0.05$. Demographic descriptors that have been previously linked with food liking or sweet preference [54], smoking-associated dietary behaviors [55], and BMI [56], were tested for differences in variables of interest (taste and smell function, food liking, sweet e-juice liking, smoking-associated dietary behaviors, and BMI). In these analyses, race/ethnicity was treated as two groups (non-Hispanic/Latino Caucasians vs. African Americans/Hispanics/Latinos/ multi-racial), which is consistent with previous research practices [57].

Independent sample *t*-tests were used to assess differences in BMI by age (younger/older than 38 years, by median split), heavy/binge drinking (yes/no), race/ethnicity (non-Hispanic/Latino Caucasian vs. African American/Hispanic/Latino/multiracial), income (≤$40,000 vs. >$40,000 annual household income), educational attainment (≤high school education/equivalent vs. some college), and marital status (single/divorced/widowed vs. married/cohabitating). One-way analysis of covariance (ANCOVA) was used to test differences in BMI by PROP taster status (non-taster, medium taster, supertaster), controlling for demographic and dietary behaviors.

The frequency distributions of BMI, the smoking-associated dietary behaviors index, and the sweet e-juice liking variable were evaluated and square root transformed. Pearson correlation analysis was used to test relationships between chemosensory function, fat/carbohydrate liking, sweet e-juice liking, smoking-associated dietary behaviors, and BMI.

Structural equation modeling (SEM) was used to test the idea that chemosensory function would influence food liking (controlling for race/ethnicity and age), which, in turn, would influence sweet e-juice liking and smoking-associated dietary behaviors (controlling for sex), which, in turn, would be associated with BMI [29,58]. A multiple imputation procedure was performed using the MICE package in R, based on restricted maximum likelihood estimation [59], to fill in missing data (<5%).

Univariate and multivariate outliers among the model variables were identified by the standardized residual (≥2.5) and by the Mahalanobis distance criteria [60]. Sensitivity analyses were conducted on original and transformed variables to assess differences in model statistics and significance [61]. Potential confounders were also included in the theoretical model, including age, breath CO readings (ppm), income, sex, race/ethnicity, and binge/heavy alcohol consumption. Confounders that were found to be non-significant were excluded from the final models. Tested associations were not found to differ significantly between the conceptual and final models, with or without the non-significant confounding variables. Measures of global fit, including χ^2, Tucker-Lewis Index (TLI), Comparative Fit Index (CFI), and the Root Mean Square Error of Approximation (RMSEA), were chosen a priori. The criteria for adequate model fit included a non-significant χ^2 ($p > 0.05$), TLI > 0.87, CFI > 0.92. and RMSEA < 0.05 [60]. Non-significant paths ($p > 0.1$) were trimmed from the model and the re-specified model tested, with fit parameters evaluated before being provisionally accepted [62].

3. Results

The sample has been described previously [16]. In brief, most were non-Hispanic/Latino Caucasians (68.1%) and 51.9% were female; this sex distribution was similar to the general distribution of the nationwide population of females (51.3%) in 2017 [63]. By race/ethnicity, our sample had fewer non-Hispanic/Latino Caucasians (68.1% vs. 74%) and Hispanics/Latinos (7.4% vs. 8.2%) and more non-Hispanic/Latino African Americans (23% vs. 7.1%) and multi-racial participants (1.5% vs. 1.3%)

than the U.S. racial/ethnic distributions [63]. By BMI, our sample had significantly heavier African American/Hispanic/Latino/multiracial participants compared to non-Hispanic/Latino Caucasians (30.9 ± 6.8 vs. 27.8 ± 6.5, respectively, $p = 0.01$). Females and males did not vary by age or BMI. Compared to the general U.S. population in 2017 the smokers in our sample were less educated (42% vs. 40% with a high school diploma/GED or less), more were unemployed or retired (39% vs. 30.5% unemployed/retired), and fewer were married/cohabitating (36% vs. 56.1%) [63].

3.1. Body Mass Index (BMI)

Mean BMI was 28.8 ± 6.74. More of our smokers were obese when compared with the 2017 U.S. Behavioral Risk Factor Surveillance System nationally-representative sample [63] (36% vs. 31.3%, respectively) while fewer were normal weight/underweight (31% vs. 33.8%, respectively). Table 1 shows bivariate correlations between age, chemosensory function, fat/carbohydrate liking, sweet e-juice liking, smoking-associated dietary behaviors, and BMI.

Table 1. Bivariate correlations among variables used in structural equation models in chronic smokers ($n = 135$) [1].

Variable Number	Variable	1	2	3	4	5	6	7	8
1	**Age**	1							
2	**PROP intensity**	0.05	1						
3	**0.32 NaCl intensity**	0.09	**0.32** [c]	1					
4	**Fat/carb liking**	**0.29** [c]	**0.18** [a]	**0.22** [a]	1				
5	**SDBI**	0.11	0.08	0.01	−0.11	1			
6	**Olfaction**	−0.12	0.06	−0.06	**−0.18** [a]	0.03	1		
7	**Sweet E-J liking**	0.12	−0.01	0.03	**0.26** [c]	−0.02	**−0.19** [a]	1	
8	**BMI**	0.17	0.07	−0.07	**0.24** [b]	**0.22** [a]	−0.02	**0.27** [c]	1

[1] The bolded correlation coefficients were statistically significant, where PROP intensity = perceived taste intensity of 6-n-propylthiouracil, a probe for genetic variation in taste, SDBI = Smoking Dietary Behavior Index [53], Sweet E-J Liking = Sweet E-juice Liking, and BMI = Body Mass Index. [a] correlations were significant at $p \leq 0.05$; [b] $p \leq 0.01$; and [c] $p \leq 0.005$.

3.2. Taste Function

The intensities of whole mouth PROP, NaCl, and quinine have been reported previously [16]. The mean intensity for the averaged 1 mM and 3.2 mM PROP solutions was 40.6±26.5 (between "strong" and "very strong" intensity), which tended to be higher in females ($t = 1.75$, $p = 0.08$). Those with heightened taste intensity of PROP were significantly more likely to be non-Caucasian (51.4 ± 28.6 vs. 35.5 ± 23.9, $t = 3.38$, $p = 0.001$) and report lower annual household income (44.5 ± 27.1 vs. 34.6 ± 25, $t = 2.13$, $p = 0.04$), but no significant differences were observed by binge/heavy alcohol consumption, age, or BMI category. The mean 0.32 M NaCl intensity was 38.3 ± 23 ("strong" intensity), which did not differ significantly by sex, age, race, income category, binge/heavy drinking, nor BMI category.

PROP taster statuses were observed to be 21% nontasters, 58% medium tasters, and 21% supertasters. Hispanic/Latino/African American/multiracial participants were significantly more likely to be supertasters (32.6%) compared to non-Hispanic/Latino Caucasians (15.6%), while significantly fewer, respectively, were PROP nontasters (11.6% vs. 25.6%; $\chi^2(2)=6.72$, $p = 0.04$).

3.3. Olfactory Function

The mean number of correctly identified odors was 12.8 ± 1.9. As reported previously, 40.7% were classified with hyposmia or anosmia/severe hyposmia [16]. Smell function was significantly poorer in those who reported a history of binge or heavy alcohol consumption (12.5 ± 1.9 vs. 13.4 ± 2, respectively, $t = 2.69$, $p = 0.008$), consistent with previous literature [64]. Smell function did not vary significantly by age, race/ethnicity, across males and females, or by BMI category.

3.4. Liking for Saturated Fats/Carbohydrates

Liking for saturated fat/carbohydrate foods and beverages was variable, ranging from −39.6 to +90.9, and averaging 31.4 ± 22. Older smokers reported greater liking of these foods than did younger counterparts when assessed by categorized median age of the sample (37.3 ± 21.6 vs. 24.9 ± 20.7, $t = 3.38$, $p = 0.001$). Food liking also varied by race, with greater liking ratings reported by Hispanic/Latino/African American/multiracial participants compared to non-Hispanic/Latino whites (40.7 ± 22.6 vs. 27 ± 20.4, respectively, $t = 3.52$, $p = 0.001$). Food liking also varied by BMI category ($F(2, 129) = 4.95$, $p = 0.008$), with obese smokers reporting the greatest overall liking ratings (38.80 ± 3.23 SEM) than normal weight/underweight (29.0 ± 3.37) or overweight (25.21 ± 3.0) smokers. There were non-significant differences in food liking between men and women or between binge/heavy alcohol consumption versus not (p's > 0.05).

3.5. Liking for E-juice Flavors

Cherry e-liquid liking averaged 16.8 ± 32.6 and was highest in obese smokers (27.9 ± 32.7 vs. 16.5 ± 27.9 vs. 4.6 ± 33.2 reported in obese, overweight, and normal weight/underweight participants, respectively, ($F(2, 127) = 6.58$, $p = 0.002$). Non-whites reported a significantly higher liking for cherry e-juice than did non-Hispanic/Latino white counterparts (24.8 ± 35.74.5 vs. 13.1 ± 30.6, respectively, $t = 2.31$, $p = 0.02$). There were no significant differences in cherry e-juice liking by income, sex, age, or binge/heavy alcohol consumption.

3.6. Smoking-Associated Dietary Behaviors

The mean score of the smoking-associated dietary behaviors was 15.2 ± 8.4. Consistent with existing literature, female chronic smokers reported a greater tendency to use cigarettes for appetite/weight management than did males (18.1 ± 8.9 vs. 12 ± 6.5, $t = 4.59$, $p < 0.001$). Self-reported tendencies to use smoking for appetite/weight control did not differ by race/ethnicity, age, income, or binge/heavy alcohol consumption (p's > 0.05). Because associations between PROP bitter phenotype and BMI may be influenced by dietary behaviors [65], we tested this association in a one-way analysis of covariance with smoking associated dietary behaviors and sex as covariates. BMI did not vary significantly by PROP taster groups ($F(2, 131) = 0.093$, $p = 0.91$).

3.7. Structural Equation Modeling of Chemosensation, Liking, Behaviors, and BMI

The SEM simultaneously tested the direct and indirect associations between chemosensation, liking for saturated fats/carbohydrates, liking for sweet e-juice, smoking-associated dietary behaviors, and BMI of the hypothesized conceptual model (Figure 1). The model had excellent global fit parameters ($\chi^2 = 25.6$, df = 27, $p = 0.54$; CFI = 1.00; TLI = 1.03; RMSEA = 0.000, 90% C.I. 0.000–0.063), but was over-fit (TLI >1). In the model, PROP intensity and olfactory function were not directly associated with fat/carbohydrate liking (p's > 0.1). Instead, NaCl taste intensity was associated with food liking ($\beta = 0.16$, $p = 0.053$), which, in turn, tended to associate with BMI ($\beta = 0.16$, $p = 0.069$). Additionally, fat/carbohydrate liking did not significantly predict smoking-associated dietary behaviors (p's > 0.1). Olfactory function demonstrated tended to inversely associate with sweet e-juice liking ($\beta = -0.14$, $p = 0.093$). Finally, sex, race/ethnicity, and age did not associate significantly with BMI (p's > 0.1) and were removed as covariates from the conceptual model.

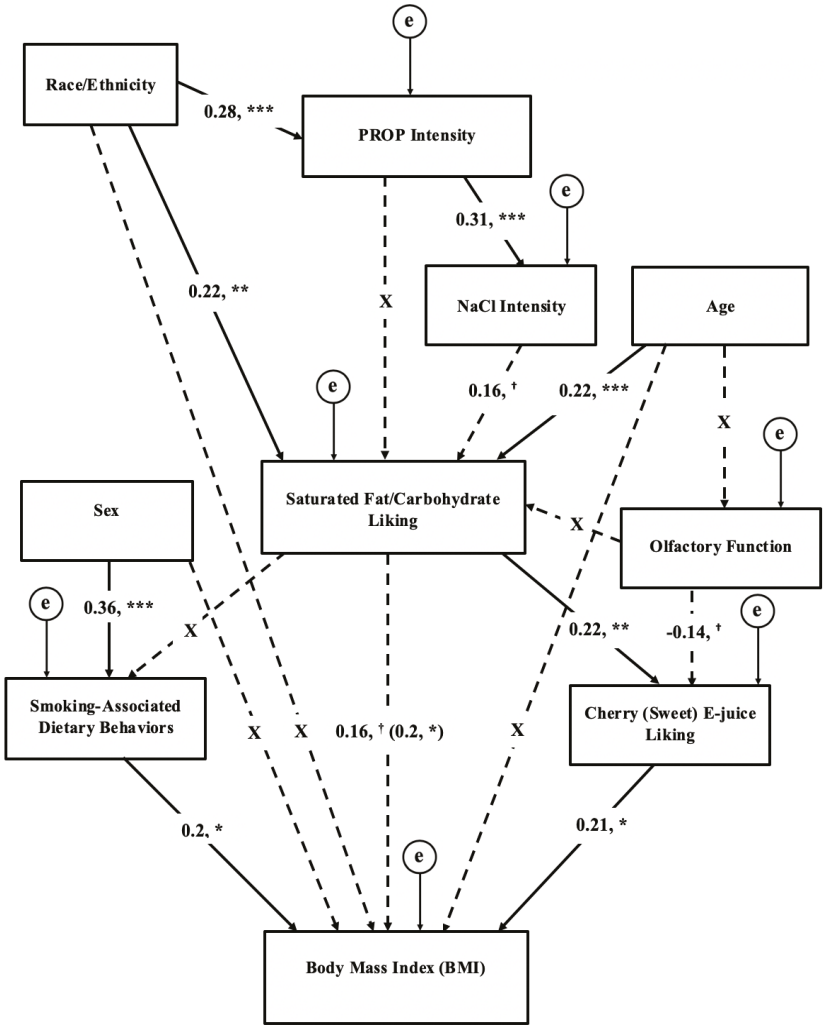

Figure 1. Conceptual hypothesis-based model of associations between chemosensation, liking, smoking-associated dietary behaviors, and BMI in chronic adult smokers. The numerical values labeled on the arrow lines represent standardized beta coefficients. Errors (represented by the encircled letter "e") are required computationally, but are not of theoretical interest. The coefficient in parenthesis represents the associations before cherry e-juice liking was added to the model. indicating that cherry e-juice mediated the dietary preference-BMI relationship (indirect effect coefficient = 0.05, $p < 0.05$). Dashed lines with "X" coefficients indicate non-significant associations. The model was adequately fit (CFI = 1.00, TLI = 1.03, Chi-square = 25.6, df = 27, $p = 0.54$, RMSEA = 0.00, 90% C.I. 0.000–0.063). *** indicates that $p \leq 0.005$; ** $p \leq 0.01$; *$p \leq 0.05$; † $p \leq 0.1$. (PROP= 6-n-propylthiouracil).

Based on findings from the tested conceptual model and supporting bivariate analyses, a re-specified model with all non-significant pathways ($p > 0.1$) trimmed was tested in SEM (Figure 2). The global fit remained excellent and showed an improvement in the TLI, which showed a good fitting model ($\chi^2 = 34$, df = 34, $p = 0.47$; CFI = 1.00; TLI = 1.00; RMSEA = 0.000, 90% C.I. 0.000–0.063). In the final model, PROP intensity was related to 0.32 NaCl taste intensity, which was linked to food

liking. Furthermore, food liking predicted liking for sweet e-juice and BMI, but not smoking-associated dietary behaviors. In the final model, olfactory function tended to inversely associate with sweet e-juice liking ($\beta = -0.14$, $p = 0.089$), which, in turn, partially mediated the association between food liking and BMI. Smoking-associated dietary behaviors were also found to predict BMI, but separately from chemosensory or liking variables.

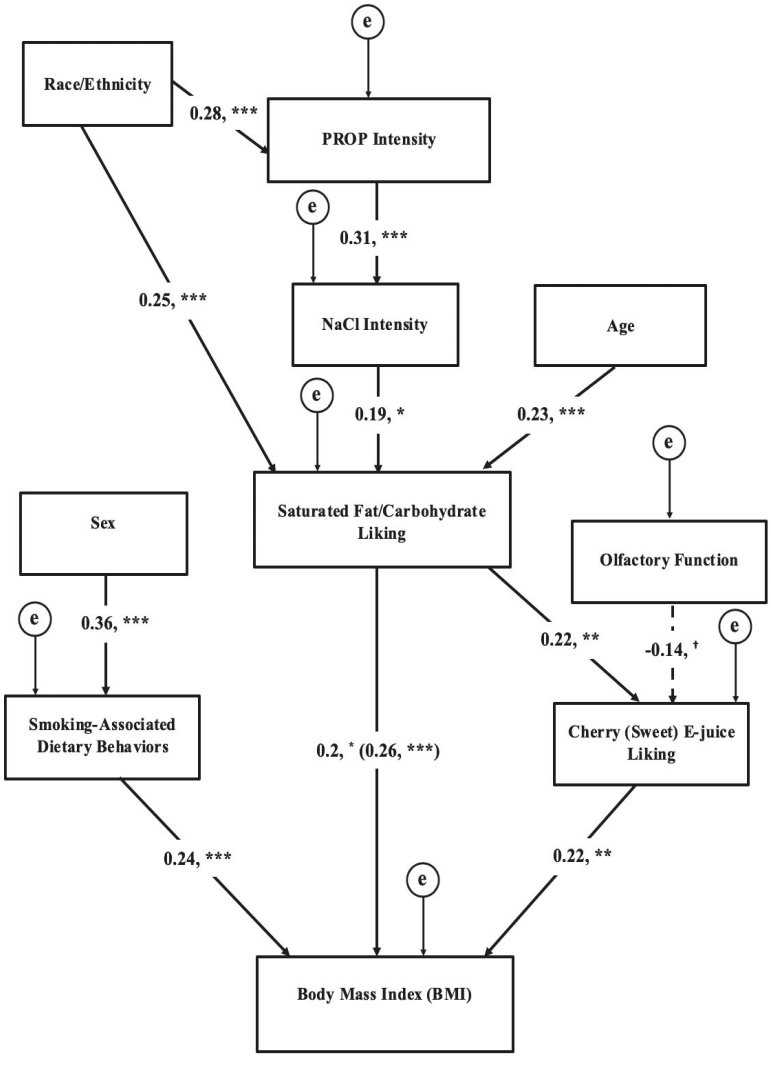

Figure 2. Structural equation model testing direct and indirect associations between taste, liking, smoking-associated dietary behaviors, and BMI in chronic adult smokers. Errors (represented by the encircled letter "e") are required computationally, but are not of theoretical interest. The coefficient in parenthesis represents the associations before cherry e-juice liking was added to the model indicating cherry e-juice partially mediated the dietary preference-BMI relationship (indirect effect coefficient = 0.05, $p < 0.05$). With all non-significant pathways removed, the model remained an adequate fit (CFI = 1.00, TLI = 1.00, Chi-square = 34, df = 34, $p = 0.47$, RMSEA = 0.00, 90% C.I. 0.000–0.063). *** indicates that $p \leq 0.005$; ** $p \leq 0.01$; * $p \leq 0.05$; † $p \leq 0.1$. (PROP = 6-n-propylthiouracil).

4. Discussion

In this observational study we sought to model associations between chemosensory function, food and beverage liking, smoking-associated dietary behaviors, and body mass index (BMI) among a sample of chronic smokers. Furthermore, we modeled the interplay between liking for a vaped sweet e-cigarette juice flavor, liking for foods and beverages, and BMI. The best fitting model had measures of taste intensity that were associated with variability in fat and carbohydrate liking, which, in turn, was associated with variability in BMI. Ability to taste PROP bitterness did not show a direct association with fat and carbohydrate liking, and instead was associated through the intensity of NaCl. Greater fat/carbohydrate liking was associated with greater BMI, an association partially mediated by greater liking of the vaped sweet e-juice, suggesting that liking for sweetness, even in an e-juice, was a primary determinant of BMI in this sample. Olfactory function, measured by an odor identification task, failed to associate significantly with the liking variables. Finally, reported use of smoking to control appetite and weight showed a separate pathway of association from either chemosensory or liking variables.

The sample of chronic smokers showed sufficient variability to test these models of association, including demographic, chemosensory, food liking, dietary behavior, and BMI characteristics. The study sample was gender-balanced and diverse in race/ethnicity, education, household income, and employment consistent with characteristics of smokers in the U.S. [1]; these demographic variables have associated with chemosensory function [66] and dietary behaviors [67,68]. In addition, our sample of chronic smokers captured a range of BMI from underweight to obese, with a higher frequency of obesity than that reported for adults in the U.S. [63], but consistent with greater odds of obesity in chronic smokers [9].

The observed variation in perceived PROP bitterness across the sample was similar to what we have seen previously (e.g. [69]) and that reported by other laboratory-based studies (e.g. [70]). The frequency of PROP nontasters, medium, and supertasters in our sample [16] was comparable to theoretical rates (i.e., 25% nontasters, 50% medium tasters, 25% supertasters). Greater PROP bitterness is linked with heightened ability to perceive oral sensations and tastes from fat, which may be explained in part by a higher density of fungiform papillae found on the tongue of PROP tasters compared to nontasters [71]. Among nonsmokers, this has furthermore been associated with a lower liking for fats/sweets among PROP tasters compared to nontasters [72–74]. Although we observed a positive bivariate association between PROP bitterness and fat/carbohydrate liking, as previously observed in young adults with poor dietary behaviors [75], perceived PROP bitterness did not contribute directly to fat/carbohydrate liking in the full structural equation model.

Instead, we observed that PROP taste intensity was indirectly associated with the liking of fats/carbohydrates through the intensity of NaCl. The PROP-NaCl association is consistent with the previous reports [29], but the ability of 0.32 M NaCl to associate with food liking among chronic smokers is a new finding. Chronic smoking likely diminishes the ability of PROP to serve as a marker for differences in oral sensation to associate with dietary behaviors and health outcomes [26]. Most studies of taste phenotype, diet, and health are conducted with non-smokers and study groups who are homogenous in race/ethnicity. As expected among chronic smokers, our study sample was diverse in race/ethnicity, which also could have contributed to a lack of direct association between PROP bitterness perception and food liking. We found more PROP supertasters among the Hispanic/Latino/African American/multiracial smokers and more nontasters among non-Hispanic/Latino Caucasians. This is consistent with the global distribution of *TAS2R38* receptors gene mediating the ability to taste PROP and PTC bitterness [76]. Thus, there likely was a race/ethnic interaction between PROP tasting and food liking as reported previously [77].

We also tested the ability of smell function to influence liking variables and BMI. In the bivariate association, greater functioning associated with a lower liking of fat/carbohydrate foods and beverages, as well as a lower liking of sweet e-juice. However, in the final model, we could not detect variability in reported food liking based on performance on an odor identification task. However, our sample

showed an overrepresentation of hyposmia and lower frequency of less severe dysfunction (severe hyposmia, anosmia) compared to the distributions reported in the nationally-representative U.S. NHANES sample [66]. This may have resulted in insufficient variability in olfactory function to associate with differences in food liking and dietary behaviors. In addition, although chronic smoking increases the risk of olfactory dysfunction [24], our study sample was younger than the age associated with declines in olfactory function [66].

The smokers in the current study varied in liking for less healthy foods (fat/carbohydrates) and greater liking was associated with greater BMI, which remained significant after controlling for age and race/ethnicity. These findings suggest that not all chronic smokers have unhealthy eating behaviors. A positive association between a liking for fat/carbohydrate foods and BMI is consistent with findings from our laboratory [78–80] and others [81,82]. This relationship may be pronounced in heavy smokers; nicotine, as the predominant addictive constituent and reinforcing property in tobacco products, is the primary driver of repeated cigarette smoking secondary to the neurological rewarding effects [83], especially with chronic nicotine exposure [84].

In our model, we found that some of the association between food liking and BMI was partially mediated by liking for the sweetest (cherry) nicotine-containing e-juice flavor. As expected, food liking was found to influence the liking for the sweet e-juice flavor, which, in turn, associated with BMI. The physiological effects of nicotine on appetite and weight [4,5], delivered through e-cigarettes (which are considered safer than tobacco cigarettes [85]), could provide a tool for chronic smokers with greater BMI to achieve and maintain a healthy weight. Although e-juice flavors are perceived retronasally [86], one with a greater preference for sweets may be more drawn to e-juice flavors that simulate sweet flavors [87], which may explain why the other vaped nicotine-containing e-juice flavors (chocolate, unflavored, menthol, tobacco) was unable to mediate the relationship between food liking and BMI. Such an association was only found in cherry, which was reported as the sweetest e-juice flavor choice amongst participants. Sweet flavors appear to elicit a stronger response in the nucleus accumbens (the predominant reward center) than non-sweet flavors [88]. With nicotine-containing e-cigarette sweet flavors, a supra-addictive response in the reward center of the brain was observed via fMRI. Thus, sweet e-juice flavors may be the predominant driver of the reinforcing effects of nicotine in e-cigarettes as a result of heightened neurological responses in the reward center [88]. There also may be a genetic susceptibility to the reward of sweets among smokers [89]. Furthermore, artificial sweeteners are commonly included in the ingredients of e-juice flavors [87]. Consumption of these sweeteners is high in the U.S., especially among individuals with obesity [90]. A greater level of scientific evidence is needed [91] to address the active debate on whether or not these sweeteners support weight management [92] or fuel the risk of obesity and associated chronic disease [93]. Finally, our model also demonstrated an inverse tendency between olfactory function and sweet e-juice preference. With the insufficient representation of olfactory impairment among our smokers, we were unable to fully test the olfactory function, sweet liking, and sweet e-juice relationships.

Smoking-associated dietary behaviors were associated with BMI in our statistical model without interacting with any of the liking variables. These findings are consistent with prior reports that suggest a separation of pathways between liking and wanting in the brain and that these circumstances for a stimulus, consequently, do not co-depend [94]. Of note, variability of self-reported smoking-associated dietary behaviors in our sample showed variability similarly to what has been previously reported [95]. The greater obesity and overweight amongst our sample may explain the elevated levels of using smoking to control appetite and weight in our sample, as overweight and obese individuals are more likely to use smoking for appetite/weight control [3]. Our findings were consistent with differences observed in smoking-associated dietary behaviors between males and females, with females reporting higher mean tendencies to smoke for appetite and weight control [95].

This current study is not without limitations. The observational cross-sectional nature of this study cannot be used to draw cause-and-effect relationships. In addition, the range of e-cigarette flavors tested was rather narrow. Furthermore, because our study sought to assess associations between

chemosensation, diet behavior and BMI in smokers, we did not compare our findings to non-smokers, which limits the generalization of our findings. An additional limitation is that we did not attempt to measure dietary intake directly (e.g., food frequency questionnaire, biomarker). However, measuring usual dietary behaviors by asking what is liked/disliked is a novel and feasible alternative to intake reporting which is often biased [96]. Reported food liking correlates with reported intake [69,97] and biomarkers of dietary intake and/or adiposity young adults [69,78], and adults [50,79]. Liking survey responses can be formed into an index of diet quality (similar to the Healthy Eating Index) that explains the variability in adiposity or cardiovascular disease risk factors [49,69,78]. Additionally, body composition was not analyzed beyond the calculation of BMI, which may have resulted in an overestimation of obese and overweight classifications among the sample [98]. However, chronic smokers have been reported to have a less healthy lifestyle than nonsmokers and lighter smokers [12]. Because BMI has been found to correlate highly among more sedentary individuals [98], however, the risk for overestimating overweight and obesity in our sample is not likely to be significant. Finally, a laboratory procedure may not faithfully reflect true preferences and behaviors with respect to vaping, eating, and BMI.

5. Conclusions

This observational study supports the idea that variation in taste perception associates with variation in fat/carbohydrate liking. Food liking, in turn, was associated with some of the variations in BMI amongst chronic smokers. Moreover, liking for sweet e-juice flavors partially mediated the association between food liking and BMI. The associations between taste, food liking, and BMI were separate from the associations between reported use of smoking to control appetite/weight and BMI. Dual chronic smoking with obesity presents a greater risk of further chronic conditions and diseases than either health risk alone. Chronic smokers can lose weight comparable to nonsmokers in a weight loss intervention [99]. The present study provides observational findings that sweet e-cigarettes may attenuate some of the association between greater liking of sweets and high-fat foods/beverages and greater BMI. Prospective studies are needed to test whether chronic smokers with obesity would benefit from the availability of sweet e-cigarette e-juice flavors in order to satisfy their liking for less healthy foods and assist in weight control.

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Effects of Varying the Color, Aroma, Bitter, and Sweet Levels of a Grapefruit-Like Model Beverage on the Sensory Properties and Liking of the Consumer

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Abstract: Color, aroma, sweet, and bitter tastes contribute to the sensory perception of grapefruit juice. Consumers differ about liking grapefruit. A reason is the bitter taste that characterize the fruit. The objective was to determine the effect of varying the color (red or yellow), aroma (two levels), bitterness (three levels), and sweetness (three levels) of a grapefruit-like model beverage, on consumers' liking and perception of its sensory properties. The sensory profiles of thirty-six grapefruit-like beverages, created on the basis of a factorial design, has been described. Consumers rated their liking of color, aroma, and flavor of the twelve most diverse beverages. Bitter and sweet levels of the beverages had a significant effect on the flavor and aftertaste attributes. Aroma concentration had a significant effect on the majority of the sensory attributes. Color had a significant effect on perception of some of the aroma attributes, as well as the grapefruit's flavor intensity. Consumers liked the red beverages more than the yellow ones, and those with low aroma over the high aroma intensity. Consumers preferred the low bitter/high sweet beverages. Pungent and grapefruit aroma were found to be negative drivers for liking of the aroma. Sweet and citrus flavors were found to be positive drivers and sour and bitter flavors were found to be negative drivers of flavor-preferences (or liking) of the tested beverages.

Keywords: grapefruit; sensory; consumer; bitter; naringin; sweet; aroma; color; hedonic

1. Introduction

The sensory properties of grapefruit (*Citrus X paradisi*) are distinctive characterizing components and play a key role in reasons why consumers choose or not choose to consume the fruit and its products, e.g., juice. Grapefruit is a rich source of vitamin C, and health-promoting citrus flavonoids and limonoids and has beneficial antioxidant and anti-inflammatory properties [1,2]. Its appearance, aroma, flavor, and mouthfeel properties contribute to the sensory perception of the fruit.

Consumers differ widely in opinions on liking or disliking grapefruit and part of this individual preference is attributed to liking or disliking of the bitter taste that characterizes the fruit. Excessive bitterness of the juice was considered to be an important problem in commercial grapefruit juice production [3]. Naringin and limonin are mainly responsible for the bitter taste commonly associated with grapefruit [4]. The consumption of fresh grapefruit, and grapefruit products has been declining [5] and plant breeders are working on ways to select for desirable sensory traits. A better understanding of the impact of the different sensory modalities contributing to the sensory perception of grapefruit products (e.g., juice) might assist product developers to optimize formulations and

improve uptake of the products among consumers, thereby, maintaining or enhancing profitability for the role-players, along the grapefruit value chain.

Flavor perception is complex, due to the simultaneous stimulation of a number of senses. It is the result of processes that respond to sensory signals, from the activation of multiple sensory modalities, including smell (retronasal olfaction), mouthfeel (somatosensation), as well as taste (gustation), and to some extent also sight. When different senses are stimulated, concurrently, and perceptually interact with each other, the perceived flavor is the result of the cross-modal sensory interaction [6]. Cross-modal interactions can change the intensity and perceived character of individual tastes and aromas, and even the overall flavor [7].

The present study aimed broadly, to determine the relations between the stimulus components of a model beverage (formulated to be similar to grapefruit juice) and their effects on the perceived sensory properties and hedonic responses. We factorially combined, in the same acidified neutral base, each of three possible levels of bitter naringin (low, medium, and high) with each of three levels of sweet sucrose (low, medium, and high), two levels of grapefruit aroma (low and high) and two color variants (red and yellow). We hypothesized that perceived bitterness of the model grapefruit-like beverage will drive consumers dislike for the beverage but that bitterness perception will be a function of cross-modal color–taste, aroma–taste, and sweet–bitter taste interactions.

The color of the natural juice extracted from the grapefruit depends on the variety used and ranges from greenish-yellow to pale yellow, pink, and light red [8]. We hypothesized that a rose red grapefruit-like beverage would be perceived as sweeter than a pale yellow option. Previously, it was reported [9] that a red color decreased the perception of bitter taste intensity of a caffeine + water solution, with the yellow and green color having had no effect. The color of food and drinks impacts subsequent perception of taste, flavor, and overall sensory perception. It has been reported in several studies that the color of a solution greatly impacts the ability to identify its flavor and also affects the liking responses [7].

We hypothesized that a beverage with high, compared to a low grapefruit aroma, would suppress the bitter taste perception and enhance the taste of sweetness [10]. A new study [10] reported that lemon extract, sucrose, and citric acid, when presented separately and also together, affected the perception of sweet, sour, and citrus flavors. The aroma of the products can influence the perception of basic tastes and vice versa [11–15].

It is well-known that sucrose and other sweet tasting compounds can suppress bitterness. This is practically applied when bitter tasting coffee or tea is sweetened with sugar. Here the expectation was that sweetness would suppress bitterness but an enhancement effect on volatile aroma and flavor compounds was also expected. When sucrose was added to the fruit juices, not only were the perceived level of bitterness and sourness reduced and the sweet taste intensity increased, but the sweet aroma intensity rating also changed [16].

2. Materials and Methods

2.1. Preparation of the Grapefruit-Like Beverages

Thirty-six grapefruit-like beverages (Table 1) were manufactured, following a factorial design with deflavored, clarified, deionized, and acidified apple juice, as base, with an addition of naringin (three bitter levels), sucrose (three sweet levels), a grapefruit aroma compound mixture (two intensity levels) consisting of caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal, aldehyde C10 (decanal), and two colorants (red or yellow). The addition of naringin was intended to reflect a low-level, in-between, and a high-level, based on the typical content in the grapefruit juice (218–340 mg/kg) [17]. The low level of sweetness was based on the industry minimum requirement for export purposes, with incrementally higher levels added to reflect medium and high sweetness. These aroma compound mixture and levels used were selected in consultation with a flavorant supplier. The typical grapefruit juice color was copied using artificial colorants. The red color was a 0.001% solution blend of 30% sunset yellow

and 70% ponceau red. The yellow color consisted of 0.0125% quinoline yellow. Standard preparation and mixing procedures were used for all added stimuli to ensure uniformity. The grapefruit-like beverages were filled in 250 mL plastic bottles, with lids, for easy handling and uniformity, and were kept frozen at −18 °C, until use. The beverages were defrosted overnight at an ambient temperature and kept at 14 °C, until served. A summary of the physico-chemical characterization of the 36 grapefruit-like beverages is presented in the Supplementary Material (Table S1).

Table 1. Factorial design for the 36 grapefruit-like beverages.

Number	Code [1]	Bitter Level Naringin mg/kg	Sweet Level Sucrose Brix	Aroma[2] Level mg/kg	Color[3]
1	LMHR	158 low	10 medium	10 high	Red
2	***MMHR***	***315 medium***	***10 medium***	***10 high***	***Red***
3	HMHR	473 high	10 medium	10 high	Red
4	***LHHR***	***158 low***	***12 high***	***10 high***	***Red***
5	MHHR	315 medium	12 high	10 high	Red
6	HHHR	473 high	12 high	10 high	Red
7	LLHR	158 low	8 low	10 high	Red
8	MLHR	315 medium	8 low	10 high	Red
9	***HLHR***	***473 high***	***8 low***	***10 high***	***Red***
10	LMLR	158 low	10 medium	2.5 low	Red
11	***MMLR***	***315 medium***	***10 medium***	***2.5 low***	***Red***
12	HMLR	473 high	10 medium	2.5 low	Red
13	***LHLR***	***158 low***	***12 high***	***2.5 low***	***Red***
14	MHLR	315 medium	12 high	2.5 low	Red
15	HHLR	473 high	12 high	2.5 low	Red
16	LLLR	158 low	8 low	2.5 low	Red
17	MLLR	315 medium	8 low	2.5 low	Red
18	***HLLR***	***473 high***	***8 low***	***2.5 low***	***Red***
19	LMHY	158 low	10 medium	10 high	Yellow
20	***MMHY***	***315 medium***	***10 medium***	***10 high***	***Yellow***
21	HMHY	473 high	10 medium	10 high	Yellow
22	***LHHY***	***158 low***	***12 high***	***10 high***	***Yellow***
23	MHHY	315 medium	12 high	10 high	Yellow
24	HHHY	473 high	12 high	10 high	Yellow
25	LLHY	158 low	8 low	10 high	Yellow
26	MLHY	315 medium	8 low	10 high	Yellow
27	***HLHY***	***473 high***	***8 low***	***10 high***	***Yellow***
28	LMLY	158 low	10 medium	2.5 low	Yellow
29	***MMLY***	***315 medium***	***10 medium***	***2.5 low***	***Yellow***
30	HMLY	473 high	10 medium	2.5 low	Yellow
31	***LHLY***	***158 low***	***12 high***	***2.5 low***	***Yellow***
32	MHLY	315 medium	12 high	2.5 low	Yellow
33	HHLY	473 high	12 high	2.5 low	Yellow
34	LLLY	158 low	8 low	2.5 low	Yellow
35	MLLY	315 medium	8 low	2.5 low	Yellow
36	***HLLY***	***473 high***	***8 low***	***2.5 low***	***Yellow***

[1] Code: 1st letter = bitter level (High, Medium, or Low); 2nd letter = sweet level (High, Medium, or Low); 3rd letter = aroma level (High or Low); 4th letter = color (Red or Yellow). Samples in bold italics were used for consumer evaluation. [2] Aroma blend = Caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), aldehyde C10 (decanal). [3] Red color = 0.001% solution (30% Sunset yellow and 70% Ponceau red); Yellow color = 0.0125% Quinoline yellow.

2.2. Descriptive Sensory Analysis

The sensory profiles of the beverages were described by a sixteen-member trained sensory panel with one to two years of descriptive sensory analysis experience. The specific training for attribute and methodology development for the evaluation of the beverages consisted of two sessions of 2 h each, using the generic descriptive analysis method [18]. A total of 21 attributes were generated to characterize the aroma, flavor, and aftertaste of the grapefruit-like beverages (Table 2). Beverage samples (±30 mL) were served at ±14 °C, in 125 ml polystyrene cups with plastic lids, and marked with randomly selected three-digit numbers. Samples were evaluated in duplicates, 12 beverages per 2 h session per day and a total of six sessions. The presentation order of samples per day for the different panelists followed a Williams Latin square design. Reference standards were available during training and evaluation sessions.

Table 2. Definitions of attributes used for describing the aroma, flavor, and aftertaste of the grapefruit-like beverages.

Attribute	Definition (References Indicated Where Applicable)
Aroma	
Overall aroma intensity	The aroma of the beverage upon taking the first few sniffs
Citrus aroma	The aroma associated with the general impression of citrus fruits
Grapefruit aroma	The aroma of fresh grapefruit
Chemical aroma	A very general term associated with many different types of compounds, such as solvents and cleaning compounds
Deteriorated/rotten aroma	Aroma associated with rotten, deteriorated, and decayed fruit/material
Muddy/moldy aroma	Aromatic characteristic of damp soil, wet foliage, or slightly undercooked boiled potato
Fruity aroma	Aroma associated with a mixture of non-specific fruits (apples, pears, melons, and guava)
Green/grassy aroma	Aromatic characteristic of freshly cut leaves, grass, or green vegetables (green beans)
Peely/peel oil aroma	Aroma associated with grapefruit peel or skin; Ref: Grapefruit oil extracted from grapefruit
Soapy aroma	Aroma associated with unscented soap
Pungent aroma	Aroma causing a sharp sensation of the nasal mucous membranes; Ref: vinegar
Woody/spicy aroma	Aroma associated with dry, fresh-cut wood; balsamic or bark-like; Ref: 10 ppm alpha-humulone in water
Sweet aroma	Aroma associated with high sugar content vegetables;Ref: Freshly boiled sweet corn
Flavor	
Overall flavor	The intensity of the flavor that is released from the beverage upon taking the first sip
Sour taste	Basic taste on tongue stimulated by acids; Ref: citric acid in water
Sweet taste	Taste on the tongue stimulated by sugars;Ref: 5% sugar (sucrose) in water
Bitter taste	Taste on tongue stimulated by bitter solutions;Ref: 473 mg/kg naringin in water
Astringent flavor	The chemical feeling factor on the tongue or surface of the oral cavity described as puckering/dry and associated with tannins;Ref: Strong black tea
Citrus flavor	Flavor associated with the general impression of citrus fruits;Ref: Cut lemon fruit and lime cordial
Grapefruit flavor	The flavor of fresh grapefruit; Ref: Cut red and white grapefruit flesh
Bitter aftertaste	Bitter taste remaining in the mouth after swallowing the beverage

Panel performance was monitored to test reproducibility and consistency of the panel ratings using PanelCheck 1.3.2 (www.panelcheck.com; Nofima, Ås, Norway).

The attributes were evaluated on a structured horizontal line scale (10 cm) with descriptors at the scale ends ranging from 'not intense' (at the left end of the scale, 0 cm) to 'very intense' (at the right end of the scale, 10 cm). Data was captured using Compusense® five release 4.6 software (Compusense Inc., Guelph, ON, Canada).

2.3. Consumer Evaluation

Ninety six young South African female consumers aged 18–24 years were recruited by trained fieldworkers. Each consumer completed an online screening survey and were invited to participate if in a self-reported good state of health, and if not limited by any food intolerance(s) and/or allergies. Participants were briefed and gave written consent before evaluating the beverages. Participants were requested not to eat, drink (except for water) or smoke for at least 1 h prior to the session.

The consumers (n = 90) evaluated liking of the color, aroma and flavor of the 12 most diverse beverages (selected on the basis of composition) (Table 1) using the Simplified Labeled Affective Magnitude (SLAM) scale [19], a 10 cm line scale labelled with descriptors 'greatest imaginable dislike' (at 0 cm), and 'greatest imaginable like' (at 10 cm). Sample preparation and presentation was the same as for the trained panel. The 12 samples were evaluated in one session and the order of presentation to different consumers followed a Williams design.

Data was captured using Compusense® five release 4.6 software (Compusense Inc., Guelph, ON, Canada).

Ethical approval for this study was obtained from the Faculty of Natural and Agricultural Sciences Ethics Committee at the University of Pretoria (EC 130827-088).

2.4. Statistical Analysis

An analysis of variance (ANOVA) model fitted using PROC GLM in SAS v9.4 (SAS Institute Inc., Cary, NC, USA) was used to determine the main effects of the panelists, the bitter level, the sweet level, the aroma level, and the color type, together with the respective two-way interactions on the sensory attributes of the beverages. Tukey's HSD test (p = 0.05) was used to compare beverages that differed in an attribute. Principal component analysis (PCA) using XLSTAT 2014 (Addinsoft, Paris, France) was applied to the correlation matrix of the sensory panel mean ratings, for all attributes of all grapefruit-like beverages.

Consumer liking of the color, aroma, and flavor of the 12 most diverse beverages was analyzed by a three-way ANOVA model, including the effects of color, aroma level, and tastants (bitter and sweet levels in three combination). Means were compared using Fisher's least significant difference test at $p < 0.05$. Data were analyzed using GenStat® (VSN International Ltd., Hertfordshire, UK). Consumer liking ratings (y) for color, aroma, and flavor of beverages were modeled as a function of the descriptive sensory attributes (x), using three separate partial least squares (PLS) regression models. Preliminary models were run with all sensory attributes and their squared terms. Variable importance (VIP), which measures how important a variable is in terms of modeling the liking attributes, was used to select a smaller number of linear and squared terms for the final model. The VIP values summarize the overall contribution of each X-variable to the PLS model, summed over all components, and weighted according to the Y variation, accounted for by each component. Only those linear terms with a VIP greater than 0.8, as well as the five squared terms with the highest contribution, were retained. The PLS models were used to determine the positive and negative drivers of color, aroma, and flavor liking, and also to predict consumer liking of the 24 samples that were profiled by the descriptive sensory panel, but not evaluated by the consumers. The SIMCA-P package (Umetrics, Umea, Sweden) was used for the PLS modeling.

3. Results

3.1. Descriptive Sensory Profiles of the Grapefruit-Like Beverages

Table 3 presents a summary of the main effects (color, aroma, bitter, and sweet) and two-way interaction ANOVA effects (provided in Supplementary Tables S2, S3 and S4) on sensory attributes of grapefruit-like beverages, as evaluated by the trained sensory panel. Means for each of the samples represent the average of duplicate ratings by 16 panelists. Color of the grapefruit-like beverages had a significant effect on perception of some aroma and flavor properties. The overall aroma and grapefruit, deteriorated/rotten, muddy/mouldy, fruity and sweet aroma, and grapefruit flavor of the red colored beverages were perceived as significantly ($p < 0.05$) more intense than the yellow colored beverages.

The level of aroma added had a significant effect on the majority of the sensory attributes, namely, overall aroma intensity and citrus, grapefruit, chemical, muddy/moldy, fruity, green/grassy, peely/peel oil, soapy, pungent, woody/spicy, and sweet aroma, with the lowest intensities perceived in the beverages with the low aroma level added. Aroma level had a significant effect on the bitter, astringent, and citrus flavor, and the bitter aftertaste perception, with the highest bitter and astringent flavor and bitter aftertaste being perceived in the beverages with a low aroma level and the highest citrus flavor being perceived in the beverages with a high aroma level.

Varying the naringin content (bitter level) of the beverages did not have any significant effect on any of the aroma attributes. It did, however, have a significant effect on the intensities of overall flavor and the astringent flavor, with the highest values observed for beverages with medium and high naringin concentrations. The naringin level had a significant effect on the intensities of sweet, sour, bitter, and grapefruit flavor, and the bitter aftertaste perception. The highest sweetness, but lowest sourness and grapefruit flavors were perceived in the beverages with low and medium naringin concentrations. Intensity of bitter flavor and bitter aftertaste followed the level of bitter compound addition.

Sweetness level contributed by sucrose had a significant effect on the perception of the many sensory properties of the grapefruit-like beverages. Significantly higher soapy aroma was perceived in the beverages with low and medium levels of sucrose, compared to a high sucrose addition. Sucrose level in the beverages had a significant effect on sour, sweet, bitter, astringent, and grapefruit flavor, and the bitter aftertaste intensities. Sour, bitter, astringent, and bitter aftertaste intensities decreased as the sweet level increased, while sweetness increased. A less intense grapefruit flavor was perceived in the high sweet level beverages, compared to the low and medium sweet levels.

Table 3. Summary of the significance of varying the main effects of color hue, aroma, bitter, and sweet levels of a model grapefruit-like beverage, on the mean values [1] (±SEM) for sensory attributes as evaluated by a trained sensory panel (n = 16).

Attributes	Color [2]			Aroma [3] mg/kg			Bitter (Naringin mg/kg)				Sweet (Sucrose mg/kg)			
	Red	Yellow		2.5	10		158 Low	315 Medium	473 High		8 Brix	10 Brix	12 Brix	
Overall aroma intensity	6.06a (0.05)	5.83b (0.05)	**	5.52b (0.05)	6.37a (0.05)	***	5.87a [2] (0.06)	6.04a (0.06)	5.93a (0.06)	NS	5.96a (0.06)	5.91a (0.06)	5.96a (0.06)	NS
Citrus aroma	4.54a (0.05)	4.55a (0.05)	NS	4.23b (0.05)	4.86a (0.05)	***	4.51a (0.06)	4.58a (0.06)	4.55a (0.06)	NS	4.51a (0.06)	4.58a (0.06)	4.55a (0.06)	NS
Grapefruit aroma	4.40a (0.05)	4.15b (0.05)	**	4.06b (0.05)	4.49a (0.05)	***	4.28a (0.06)	4.23a (0.06)	4.32a (0.06)	NS	4.23a (0.06)	4.35a (0.06)	4.25a (0.06)	NS
Chemical aroma	4.09a (0.06)	4.03a (0.06)	NS	3.86b (0.06)	4.25a (0.06)	***	3.99a (0.07)	4.13a (0.07)	4.06a (0.07)	NS	4.06a (0.07)	4.03a (0.07)	4.09a (0.07)	NS
Deteriorated/rotten aroma	2.14a (0.04)	2.00b (0.04)	**	2.09a (0.04)	2.05a (0.04)	NS	2.08a (0.05)	2.04a (0.05)	2.09a (0.05)	NS	2.05a (0.05)	2.04a (0.05)	2.12a (0.05)	NS
Muddy/moldy aroma	2.20a (0.03)	2.09b (0.03)	**	2.09b (0.03)	2.20a (0.03)	**	2.12a (0.04)	2.13a (0.04)	2.18a (0.04)	NS	2.18a (0.04)	2.13a (0.04)	2.13a (0.04)	NS
Fruity aroma	3.93a (0.05)	3.78b (0.05)	*	3.71b (0.05)	4.00a (0.05)	***	3.89a (0.06)	3.83a (0.06)	3.85a (0.06)	NS	3.83a (0.06)	3.90a (0.06)	3.83a (0.06)	NS
Green/grassy aroma	3.13a (0.04)	3.14a (0.04)	NS	2.91b (0.04)	3.36a (0.04)	***	3.12a (0.05)	3.09a (0.05)	3.19a (0.05)	NS	3.12a (0.05)	3.18a (0.05)	3.10a (0.05)	NS
Peely/peel oil aroma	3.53a (0.05)	3.47a (0.05)	NS	3.20b (0.05)	3.79a (0.05)	***	3.53a (0.06)	3.47a (0.06)	3.49a (0.06)	NS	3.49a (0.06)	3.55a (0.06)	3.46a (0.06)	NS
Soapy aroma	3.29a (0.05)	3.23a (0.05)	NS	3.16b (0.05)	3.36a (0.05)	**	3.27a (0.06)	3.20a (0.06)	3.31a (0.06)	NS	3.41a (0.06)	4.33a (0.06)	3.04b (0.06)	**
Pungent aroma	3.16a (0.05)	3.11a (0.05)	NS	2.84b (0.05)	3.43a (0.05)	***	3.10a (0.06)	3.14a (0.06)	3.18a (0.06)	NS	3.17a (0.06)	3.13a (0.06)	3.11a (0.06)	NS
Woody/spicy aroma	2.49a (0.04)	2.40a (0.04)	NS	2.33b (0.04)	2.57a (0.04)	***	2.43a (0.04)	2.44a (0.04)	2.47a (0.04)	NS	2.48a (0.04)	2.48a (0.04)	2.37a (0.04)	NS
Sweet aroma	3.79a (0.05)	3.62b (0.05)	*	3.56b (0.05)	3.86a (0.05)	***	3.73a (0.06)	3.69a (0.06)	3.70a (0.06)	NS	3.69a (0.06)	3.72a (0.06)	3.72a (0.06)	NS
Overall flavor intensity	6.39a (0.05)	6.34a (0.05)	NS	6.37a (0.05)	6.36a (0.05)	NS	6.14b (0.06)	6.51a (0.06)	6.46a (0.06)	***	6.41a (0.06)	6.29a (0.06)	6.40a (0.06)	NS
Sour flavor	5.09a (0.06)	5.17a (0.06)	NS	5.14a (0.06)	5.11a (0.06)	NS	4.90b (0.08)	5.08b (0.08)	5.40a (0.08)	***	5.93a (0.08)	5.07b (0.08)	4.38c (0.08)	***
Sweet flavor	4.48a (0.05)	4.43a (0.05)	NS	4.38a (0.05)	4.53a (0.05)	NS	4.64a (0.06)	4.51a (0.06)	4.21b (0.06)	***	3.04c (0.06)	4.50b (0.06)	5.82a (0.06)	***
Bitter flavor	4.56a (0.07)	4.48a (0.07)	NS	4.77a (0.07)	4.27b (0.07)	***	3.94c (0.08)	4.46b (0.08)	5.17a (0.08)	***	5.24a (0.08)	4.43b (0.08)	3.89c (0.08)	***
Astringent flavor	4.95a (0.06)	4.81a (0.06)	NS	4.97a (0.06)	4.79b (0.06)	*	4.62b (0.07)	4.91a (0.07)	5.12a (0.07)	***	5.35a (0.07)	4.88b (0.07)	4.41c (0.07)	***
Citrus flavor	4.47a (0.05)	4.43a (0.05)	NS	4.30b (0.05)	4.60a (0.05)	***	4.52a (0.06)	4.44a (0.06)	4.39a (0.06)	NS	4.43a (0.06)	4.45a (0.06)	4.48a (0.06)	NS
Grapefruit flavor	4.53a (0.05)	4.36b (0.05)	*	4.49a (0.05)	4.40a (0.05)	NS	4.19b (0.07)	4.41b (0.07)	4.73a (0.07)	***	4.53a (0.07)	4.54a (0.07)	4.26b (0.07)	**
Bitter aftertaste	4.32a (0.07)	4.26a (0.07)	NS	4.49a (0.07)	4.08b (0.07)	***	3.69c (0.08)	4.26b (0.08)	4.91a (0.08)	***	4.90a (0.08)	4.25b (0.08)	3.71c (0.08)	***

[1] Attribute intensity scale from 'not intense' (0) to 'very intense' (10); [2] Red = 0.001% solution (30% Sunset yellow and 70% Ponceau red); Yellow = 0.0125% Quinoline yellow. [3] Aroma blend {caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), and aldehyde C10 (decanal)}. abc—different letters indicate significantly different mean values across design variable levels. Means represent the average of duplicate ratings by 16 panelists; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.0001$; NS = not significantly different.

Very few two-way interactions were significant. The detailed tables for the significant interaction effects are presented in the Supplementary Material (Tables S2–S4). The bitter level x aroma level interaction effect (Table S2) was significant for the perception of the intensity of chemical aroma and overall flavor intensity, the bitter flavor, and the bitter aftertaste. A trend was observed in that the chemical aroma was more intensely perceived in the beverages with high aroma, although only significantly so in the low and high bitter samples and not in the medium bitter samples. The overall flavor intensity was significantly but slightly lower in the high aroma/medium bitter, compared to the low aroma/medium bitter sample. Aroma level did not affect the overall flavor perception at the low or high bitter levels. Bitter flavor and bitter aftertaste were notably less intense in the high aroma samples, compared to the low aroma samples, but only significantly so for the medium and high bitter level beverages.

The bitter level x color type interaction effect (Table S2) was significant for the bitter aftertaste intensity. However, bitter aftertaste was essentially driven more by the bitter level than the color type. The bitter level x sweet level interaction effect (Table S3) was significant only for the pungent aroma. A significantly lower pungent aroma was noted between the medium sweet and low sweet beverages, at the medium bitter level.

The aroma level x color interaction effect (Table S3) was significant only for bitter flavor intensity. At a low aroma level, no difference in bitter flavor intensity was found between the two colors. However, at the high aroma level, the yellow beverage was perceived as being significantly bitterer.

The sweet level x aroma level interaction effect (Table S4) was not significant for any of the sensory aroma attributes. A sweet level x color interaction effect (Table S4) was significant for the astringent and citrus flavor perception. While no significant differences were found between the red and yellow beverages at the medium sweet level, the red beverage was perceived as significantly more astringent at low sweet and high sweet levels. A similar effect was found for the citrus flavor, although the red beverages were found to have a more intense citrus flavor, only at the low sweet level.

The multivariate differentiation of the beverages is presented in Figure 1 as a PCA map over a two-dimensional space. The first and second principal components (F1 and F2) explained 37% and 35%, respectively, of the variance across the samples. F1 clearly separated beverages based on intensity of overall aroma, peely/peel oil aroma, citrus aroma, sweet aroma, and pungent aroma. Beverages that were more intense in terms of the mentioned attributes are located on the right of the plot. Note that all of these beverages have an H as third letter, therefore, they have a high aroma level. The beverages with lower intensities are located on the left of the plot and notably has L as the third letter, therefore, with a low level aroma. F2 separated the beverages based on 'taste' perception, i.e., naringin (bitter)-sucrose (sweet) levels. Beverages with high and medium bitter levels and low sweet are positioned at the top, and beverages with low bitter level and medium and high sweet levels, are at the bottom. Beverages at the top, namely HLLY and HLLR with a high level of naringin and MLHY with a medium level, were characterized by more intense astringency, sour, and bitter tastes, and with grapefruit and overall flavor intensities. Beverages (e.g., LHHR) with a low naringin level (at the bottom), were characterized by a more intense sweet taste. The attributes citrus flavor, chemical aroma, and muddy/moldy aroma in the middle of the plot, did not discriminate beverages on the first two PCs.

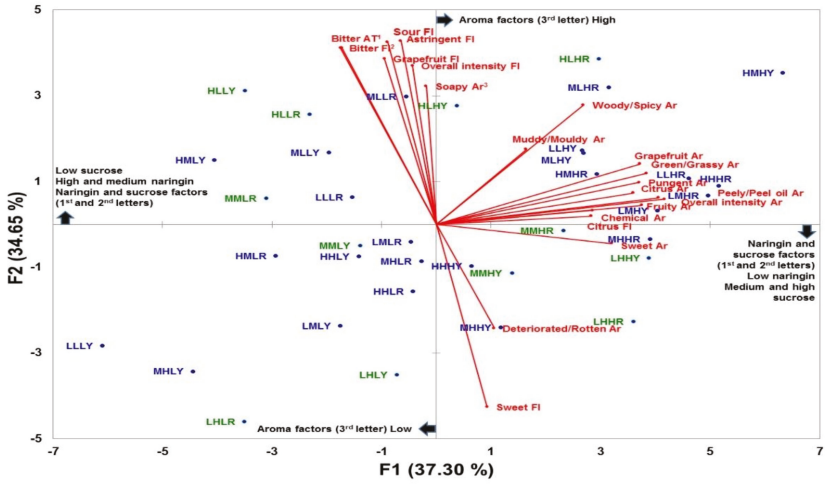

Figure 1. Principal Component Analysis (PCA) of the sensory profiles of the 36 grapefruit-like beverages. The vectors indicate the loadings for sensory attributes while the position of the sample codes indicate the score values. The four-letter codes indicate levels of naringin (1st letter: L = Low, M = Medium, or H = High), sucrose (2nd letter: L = Low, M = Medium, or H = High), aroma (3rd letter: L = Low, or H = High) and color (4th letter: R = red or Y = yellow). Sensory attributes [1]AT = Aftertaste, [2]Fl = Flavor, [3]Ar = Aroma. Beverages in green font were selected for the consumer tests.

3.2. Consumer Evaluation of the Grapefruit-Like Beverages

The effects of color, aroma level, and bitter/sweet levels of the grapefruit-like beverages on mean liking ratings for the color, aroma, and flavor, as evaluated by the consumers, are presented in Table 4. Two-way interaction effects were not significant.

Table 4. The effect of varying the color, aroma, and bitter/sweet gustatory flavorants on mean liking ratings [1] (±standard deviation) for color, aroma, and flavor of grapefruit-like beverages by (n = 90) consumers.

	Color [2]			Aroma [3] mg/kg [3]			Bitter-Sweet Naringin mg/kg /Sucrose Brix			
	Red	Yellow		2.5	10 mg/kg		158/12 Low/High	315/10 Medium/Medium	473/8 High/Low	
Liking of color	64b (30)	60a (30)	**	63a (30)	61a (31)	NS	62a [1] (30)	62a (31)	61a (30)	NS
Liking of aroma	51a (30)	51a (30)	NS	53a (29)	49b (31)	**	50ab (30)	53a (30)	49b (29)	*
Liking of flavor	45a (33)	45a (34)	NS	45a (34)	45a (34)	NS	55a (33)	46b (33)	34c (32)	***

[1] Simplified Labelled Affective Magnitude Scale (SLAM); 0 = greatest imaginable dislike, 100 = greatest imaginable liking. abc—different letters indicate significantly different mean values across the design variable levels. NS = not significant, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. [2] Red = 0.001% solution (30% Sunset yellow and 70% Ponceau red); Yellow = 0.0125% Quinoline yellow. [3] Aroma blend {caryophyllene, citral, nootkatone, aldehyde C8 (octanal), aldehyde C9 (nonanal), and aldehyde C10 (decanal)}.

The standardized PLS regression coefficients for attributes as part of the prediction models are presented in Table 5. PLS regression (PLSR) models were used to predict liking of the color, aroma, and flavor of the 36 beverages, including the beverages that were not evaluated by consumers (Table 6). Expected errors of prediction for the models were low, lying between ±1.288 for the aroma model to ±2.458 for the color model, and ±2.678 for the flavor model, with a 95% confidence interval, indicating reliable prediction estimations of the liking variables.

Table 5. Standardized partial least squares (PLS) regression coefficients for factors to summarize the relationship between predictors (X, consumer liking variables) and Y, sensory response variables. Only selected important variables (main effects and squared effects, noted as '2') from the refined models are shown.

Liking of the Color R^2 = 0.871		Liking of the Aroma R^2 = 0.970		Liking of the Flavor R^2 = 0.982	
Overall aroma intensity [2]	0.23	Fruity aroma [2]	0.08	Sweet aroma	0.16
Citrus aroma [2]	0.16	Citrus flavor	0.03	Chemical aroma [2]	0.15
Sweet aroma	0.16	Sweet flavor	0.03	Citrus flavor	0.12
Astringent flavor [2]	0.15	Astringent flavor	0.02	Deteriorated/rotten aroma	0.03
Green/grassy aroma [2]	0.10	Grapefruit flavor	0.02	Green/grassy aroma [2]	−0.01
Fruity aroma	0.00	Bitter aftertaste	−0.01	Greed/grassy aroma	−0.01
Overall aroma intensity	0.00	Bitter flavor	−0.01	Chemical aroma	−0.01
Grapefruit aroma	0.00	Sour flavor	−0.02	Woody/spicy aroma [2]	−0.04
Green/grassy aroma	−0.01	Overall flavor intensity	−0.04	Overall flavor intensity	−0.04
Astringent flavor	−0.04	Deteriorated/rotten aroma	−0.05	Bitter flavor	−0.07
Peely/peel oil aroma	−0.04	Soapy aroma	−0.05	Grapefruit flavor	−0.07
Pungent aroma	−0.05	Chemical aroma	−0.06	Woody/spicy aroma	−0.08
Citrus aroma	−0.06	Woody/spicy aroma	−0.06	Fruity aroma	−0.08
Citrus flavor	−0.07	Sweet aroma	−0.07	Muddy/moldy aroma	−0.10
Muddy/moldy aroma	−0.08	Fruity aroma	−0.07	Bitter flavor	−0.11
Woody/spicy aroma	−0.09	Peely/peel oil aroma	−0.08	Astringent flavor	−0.11
Soapy aroma	−0.13	Pungent aroma [2]	−0.08	Sour flavor	−0.12
Chemical aroma [2]	−0.18	Citrus aroma	−0.09	Bitter aftertaste	−0.12
Chemical aroma	−0.19	Grapefruit aroma	−0.09	Soapy aroma	−0.19
		Overall aroma intensity	−0.10		
		Green/grassy aroma	−0.11		
		Muddy/moldy aroma	−0.12		
		Bitter flavor	−0.14		
		Pungent aroma	−0.14		
		Sweet flavor [2]	−0.16		
		Sweet aroma [2]	−0.17		

Table 6. Partial least square regression (PLSR) model predicted liking ratings for color, aroma, and flavor of the grapefruit-like beverages.

		Color Liking		Aroma Liking		Flavor Liking	
Number [1]	**Code [2]**	**Observed [3]**	**Predicted**	**Observed**	**Predicted**	**Observed**	**Predicted**
2	***MMHR***	***61ab (31)***	***61***	***51ab (30)***	***51***	***44abc (31)***	***45***
4	***LHHR***	***64ab (29)***	***64***	***45b (30)***	***45***	***54a (32)***	***52***
9	***HLHR***	***63ab (33)***	***64***	***47ab (30)***	***47***	***35bc (33)***	***36***
11	***MMLR***	***66a (29)***	***64***	***57a (29)***	***56***	***48a (33)***	***48***
13	***LHLR***	***67a (30)***	***68***	***55ab (29)***	***55***	***55a (34)***	***55***
18	***HLLR***	***62ab (30)***	***61***	***51ab (29)***	***50***	***32c (29)***	***32***
20	***MMHY***	***62ab (32)***	***61***	***52ab (29)***	***52***	***48a (35)***	***48***
22	***LHHY***	***55b (32)***	***56***	***48ab (33)***	***48***	***54a (34)***	***54***
27	***HLHY***	***59ab (30)***	***60***	***49ab (31)***	***49***	***35bc (34)***	***34***
29	***MMLY***	***60ab (32)***	***60***	***54ab (31)***	***54***	***46ab (34)***	***47***
31	***LHLY***	***61ab (29)***	***62***	***52ab (29)***	***53***	***55a (33)***	***56***
36	***HLLY***	***61ab (28)***	***62***	***50ab (28)***	***51***	***35bc (32)***	***34***
1	LMHR		59		44		41
3	HMHR		62		49		41
5	MHHR		63		47		47
6	HHHR		60		46		45
7	LLHR		62		46		41
8	MLHR		60		47		38
10	LMLR		61		53		47
12	HMLR		64		54		45

Table 6. *Cont.*

		Color Liking	Aroma Liking	Flavor Liking
14	MHLR	61	52	50
15	HHLR	63	52	48
16	LLLR	62	53	43
17	MLLR	59	48	32
19	LMHY	59	49	44
21	HMHY	62	41	38
23	MHHY	62	52	57
24	HHHY	62	52	51
25	LLHY	60	48	36
26	MLHY	61	45	40
28	LMLY	63	55	56
30	HMLY	62	54	40
32	MHLY	66	55	56
33	HHLY	63	52	46
34	LLLY	68	57	53
35	MLLY	60	52	39

[1] Refer to Table 1 for number. [2] Code: 1st letter = bitter level (High, Medium, or Low); 2nd letter = sweet level (High, Medium, or Low); 3rd letter = aroma level (High or Low); 4th letter = color (Red or Yellow). Samples in bold italic were used for consumer evaluation. [3] Values are means (± standard deviation); Observed means in a column with different letters are significantly different ($p < 0.05$).

Liking of the color of the red grapefruit-like beverages were rated, on average, slightly higher than the yellow ones ($p < 0.05$) (Table 4). Whether the beverage was colored yellow or red, it did not affect the liking of the aroma or the flavor. Predicted mean liking of the color for the highest and lowest liked of the 36 beverages differed, however, only by a maximum of 12.2 scale units (Table 6). Notably the research found no significant sensory attribute drivers for liking of the color of the grapefruit-like beverages (Table 5).

Liking of the aroma of beverages with a low added-aroma level, was higher ($p < 0.05$) than for those with a high added-aroma level (Table 4). Aroma level did not have an effect on the liking of the color of the beverage. Aroma level also did not affect the liking of the flavor of the beverage. The predicted mean liking of the aroma for the highest and the lowest liked beverages, differed by 16.5 scale units (Table 6). Main effects and squared effects are indicated as '^{2}'. Positive attribute drivers for liking of the aroma of the grapefruit-like beverages were the square term of fruity aroma (noted fruity aroma^2), citrus flavor, and sweet flavor, while negative drivers were sweet aroma ^{2}, sweet flavor^2, and pungent aroma (Table 5).

As expected, the level of the gustatory flavorants, the naringin, and the sucrose, did not affect the liking of the color of the beverages (Table 4). Surprisingly the non-volatile taste level did have a significant effect ($p < 0.05$) on the liking of the aroma of the beverages. The aroma of the most bitter/least sweet beverages was liked significantly less than the other two taste combination levels. Not surprisingly, liking of the flavor of the beverages decreased significantly ($p < 001$) as the bitter level increased and the sweet level decreased. Predicted mean ratings for liking of the flavor, the highest liked and the lowest liked of the 36 beverages, differed by 27.5 scale units. Positive drivers for liking of the flavor of the grapefruit-like beverages were sweet taste, squared term for chemical aroma (noted as 'chemical aroma^2), and citrus flavor intensities, while the negative drivers were intensity of soapy aroma, bitter aftertaste, and sour taste (Table 6).

4. Discussion

The research studied the effect of varying the bitterness, sweetness, color, and aroma intensity of grapefruit-like beverages on the cross-modal perception of sensory properties and its effects on consumer liking. A model grapefruit-like beverage standard formulation was created and a sensory lexicon with a total of 21 attributes and definitions were generated to characterize the aroma, the flavor,

and the aftertaste of the grapefruit-like model beverage with variations in color, aroma, and gustatory flavorant levels.

Color hue of the grapefruit-like beverage affected the perception and description of the aroma and flavor sensory properties, as evaluated by the trained human panelists. Color of the beverages, and, in particular, the sample with the rose-red hue had a significant enhancing effect on perception of overall aroma intensity and grapefruit, deteriorated/rotten, muddy/moldy, fruity, and sweet aroma intensities. It also corresponded to the consumer liking—the red beverages were liked more than the yellow ones. The cross modal effect of the beverage color on aroma and flavor of the beverages, however, did not lead to significant differences in the liking of aroma or a liking of the flavor of the red and yellow beverages. The difference in methodology followed and the cognitive tasks employed by the two groups of panels might be the reason. When the group of consumers evaluated the liking of the color of the beverages, solely based on appearance, a slight but significant preference for the red-colored beverages was noted. This preference was solely driven by visual cues, since the consumers did not yet smell or taste the beverages. After smelling and tasting the beverages, it is likely that the opinion and preference might have changed, based on the cross-modal, color-aroma/flavor sensory interaction, as demonstrated by the results for the trained panel in this study. Considering that the consumers first evaluated the liking of the color, then the aroma (retronasally), and lastly the flavor (after consumption) of each sample, sequentially, it cannot be excluded that some form of learning, anticipation, and association might have occurred over the evaluation of the sequence of twelve samples, of which 50% were red and 50% were yellow.

A study [20] reported that the red color decreased the perception of the bitter taste sensitivity of a water solution. Coloring a clear bitter solution red, decreased the perception of the bitter taste, while the addition of yellow and green coloring had no such effect [9]. Other researchers [21] suggested that color-induced olfactory enhancement observed when odorous solutions are smelled orthonasally, might be the result of a conditioned olfactory percept caused by the color. Conditioned expectations predict that certain colors would be strongly associated with particular flavors, e.g., red with cherry, orange with orange, and green with lime [22]; yellow with lemon, blue with spearmint, and red with strawberry, raspberry, and cherry [23]. In South Africa, the location for the study, both yellow and red/pink grapefruit are marketed. The Star Ruby variety with a red color is the most planted (84%) grapefruit variety in South Africa, followed by the white variety Marsh (16%) (the juice of this type of grapes is pale yellow) [24]. In another study [25] it was found that the relationship of green and yellow colors in the lemon and lime-flavored sucrose solutions was altered; such color changes were found to have an impact on the perceived sweetness ratings. In another study, results showed that color–odor solution pairings were rated as having more intense odors with color cues than without, regardless of the color–odor pairing appropriateness [21]. This cross-modal effect presumably results from the color-cue setting up an expectation concerning the likely identity and intensity of a food or drink's taste or flavor [20]. No significant sensory attribute drivers for liking of the color of the grapefruit-like beverages was identified, since the trained sensory panel did not evaluate the appearance attributes.

Aroma level added to the model beverage had a significant enhancing effect, on the majority of the aroma and flavor sensory attributes. The enhancement of overall aroma and characteristic aroma qualities, including citrus flavor, as a function of the level of aroma added, was expected and confirmed. When consumers evaluated liking of the aroma of the beverages, solely based on orthonasal inspection, surprisingly the beverages with low aroma were slightly preferred over those with high aroma. It is possible that the higher aroma level was more distinctive and clearly reminiscent of grapefruit and possibly evoked a stronger cue for those disliking grapefruit. An interesting and unexpected finding was the apparent suppression of bitter and astringent gustatory sensations, due to a higher load of olfactory stimuli (high aroma level). Previous studies have found that aroma–taste interactions can result in complicated changes in the perceived flavor. The addition of an aroma can, e.g., elevate the bitter-detection threshold [26,27]. The perceived intensity of tastes in solutions was increased by volatile compounds, especially when there was a logical association between them, such as between

sweetness and fruitiness [28]. Apple and strawberry aromas evoked both sweetness and sourness. A study found that tasteless aromas, namely green tea and coffee, predominantly evoked bitterness, while the vanilla aroma predominantly evoked sweetness [29]. The grapefruit aroma consisted of a blend of caryophyllene, citral, nootkatone, and various aldehydes; octanal, nonanal, and decanal. No study could be found that specifically indicated that any of these compounds evoked bitterness. Nootkatone at the above threshold concentrations was reported as tasting bitter [30]. Consumption of a beverage results in the simultaneous perception of aroma and taste, coupled with tactile sensations, all of which contribute to an overall impression of flavor. Compounds that stimulate taste perception (e.g., naringin contributing a bitter taste) can increase the apparent intensity of aromas. In this study, the grapefruit flavor was enhanced by the naringin addition. The aroma compound (containing a citral component) of the grapefruit-like beverages had an enhancing effect on the citrus aroma intensity. An additive effect of the sweet components with citral or limonene volatiles having a 'citrus'-like aroma was reported by [31] but was not observed in this study. The suppression of bitterness in the high aroma beverages, however, did not affect the liking of the flavor, since there was no difference found in the liking of the flavor of beverages with low or high aroma levels. Positive drivers for liking of the aroma of the grapefruit-like beverages were fruity aroma^2, citrus flavor, and sweet flavor, while negative drivers were sweet aroma^2, sweet flavor^2, and pungent aroma.

The low bitter/high sweet beverages were preferred over the high bitter/low sweet samples. A study [32] reported that with an increase in the ratio of °Brix/acidity of reconstituted grapefruit juice, the consumer perception of sweetness increased and bitterness and aroma intensity decreased. Some bitterness in processed grapefruit products is acceptable for consumers, but excessive bitterness is one of the major consumer objections to such products [28,31]; this was confirmed in this study. The variation in sensitivity of the individual consumers to bitter compounds in grapefruit beverages could be explored further to identify whether subgroups might have different preferences. As expected, the contribution of varying concentrations of naringin affecting the bitterness of the grapefruit-like beverages did not have a significant effect on any of the aroma attributes. Similarly, [32] reported that consumers did not find any difference in aroma with increased levels of naringin in processed grapefruit juice. However, the concentration of bitterness of the grapefruit-like beverages had a significant effect on the flavor attributes (astringent, sweet, sour, bitter and grapefruit flavor, and the bitter aftertaste). A study [32] has also reported that an increase of limonin (also a bitter compound) in processed grapefruit juice, increased the perceived bitterness and tartness, while decreasing the sweetness.

In a previous study, an increase in the °Brix with sucrose, enhanced the taste of sweetness, and had a decreasing effect on the sour, bitter, astringent, and grapefruit flavors, and the bitter aftertaste. When sucrose was added to fruit juices, not only were the perceived levels of bitterness and sourness reduced (as was also found in this research) but the sweet aroma intensity rating also changed [16] (although this was not found here). Sucrose was also reported to mask the bitter taste of sinigrin, goitrin, and quinine [33]. In the complex beverage model, increasing sucrose did not have the often reported enhanced effect on the perceived fruity aroma. Increasing the sugar concentration of blueberry and cranberry fruit juices, increased their fruitiness (evaluated by sipping), even though no difference in aroma was perceived by sniffing alone [16]. Sucrose in the mouth significantly enhanced the "citrus" ratings, compared to when citral was inhaled alone [12]. Similarly, increases in the intensity of different 'fruity' aromas were perceived in a multichannel flavor delivery system [34], model dairy desserts [35], and custard desserts [36], when increasing the sweetness with sucrose. Sweet level also affected the soapy aroma of the grapefruit-like beverages. The reason for the effect on soapy aroma is unclear. It is possible that the aroma blend contributed a slight soapy aroma.

The effect of aroma level and color on the perceived sensory attributes, as observed in this study, are evidence of cross-modal sensory interactions. It was anticipated that the intensity and character of the aroma level of a grapefruit juice would increase the perception of the citrus flavor, a positive driver of grapefruit flavor liking and reduce the negative attributes, the bitter and astringent flavor, as well as

the bitter aftertaste. Positive drivers for liking of the flavor of grapefruit-like beverages were the sweet taste, the chemical aroma, and the citrus flavor intensities, while negative drivers were intensity of soapy aroma, bitter aftertaste, and sour taste.

5. Conclusions

This study indicated that aroma, bitterness, and sweetness levels, and also product color (hue) influences the perception of grapefruit-like beverages, as well as their hedonic value. A grapefruit-like beverage model was created and a lexicon to describe the sensory properties of the cross-modal interaction of stimulus components of the model beverage was developed. From the descriptive sensory profiles, prediction models for liking of the color, aroma, and flavor of grapefruit-like beverages were developed. In the next phase, the models should be applied to a wide range of grapefruit juice samples to determine validity and reliability in real juices. The models can then be optimized for application in grapefruit quality control and product development programs.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1. Table S1: Physico-chemical characterization (means ± standard deviation) of the 36 grapefruit-like beverages. Table S2: Summary of sensory attribute mean values1 [± standard error of means (SEM)] and significance of bitter x aroma and bitter x color two-way ANOVA interactions of the model grapefruit-like beverages as evaluated by a trained sensory panel (n = 16). Table S3: Summary of sensory attribute mean values1 [± standard error of means (SEM)] and significance of bitter x sweet and aroma x color two-way ANOVA interactions of the model grapefruit-like beverages as evaluated by a trained sensory panel (n = 16). Table S4: Summary of sensory attribute mean values1 [± standard error of means (SEM)] and significance of sweet x aroma and sweet x color two-way ANOVA interactions of the model grapefruit-like beverages as evaluated by a trained sensory panel (n = 16).

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The Influence of Water Composition on Flavor and Nutrient Extraction in Green and Black Tea

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Abstract: Tea is made from the processed leaves of the *Camellia sinensis* plant, which is a tropical and subtropical evergreen plant native to Asia. Behind water, tea is the most consumed beverage in the world. Factors that affect tea brewing include brewing temperature, vessel, and time, water-to-leaf ratio, and, in some reports, the composition of the water used. In this project, we tested if the water used to brew tea was sufficient to influence perceived flavor to the everyday tea drinker. Black and green tea were brewed with bottled, tap, and deionized water, with brewing temperature, vessel, time, and the water-to-leaf ratio matched. The samples were analyzed with a human consumer sensory panel, as well as instrumentally for color, turbidity, and Epigallocatechin Gallate (EGCG) content. Results showed that the type of water used to brew tea drastically affected sensory properties of green tea (and mildly also for black tea), which was likely driven by a much greater degree of extraction of bitter catechins in teas brewed with more purified bottled or deionized water. For the everyday tea drinker who drinks green tea for health, the capability to double the EGCG content in tea by simply brewing with bottled or deionized water represents a clear advantage. Conversely, those drinking tea for flavor may benefit from instead brewing tea with tap water.

Keywords: taste; sensory evaluation; tea; EGCG; hedonics

1. Introduction

1.1. Tea and Tea Processing

Tea is a beverage steeped in culture and history. Valued for its taste and caffeine content as well as its numerous health properties [1], tea has been consumed for centuries [2]. Behind water, tea is the most consumed beverage in the world [3]. The botanical name for the plant producing tea is *Camellia sinensis* (L.) Kuntze. There are many other plants used for extraction such as rooibos and chamomile, however these are not strictly teas. Instead, they are classified under the category of tisanes or herbal infusions. The main difference between various styles of tea is the level of oxidation of the leaf during processing. Green and white teas are unoxidized, oolongs vary in the levels of oxidation, and black tea leaves are fully oxidized. A cup of tea is made from processed fresh tea leaves. Biochemical changes that occur during processing help reduce the bitter taste of fresh tea leaves. Processing the tea leaves lowers water content to aid in shelf stability, deactivates enzymes, and adds sweetness and a myriad of colors to the cup. Physically the leaf transforms from a sturdy crisp leaf to limp and pliable during withering. Chemically, caffeine content increases, hydrolysis of hydrophobic carbohydrates begins, non-gallated catechins and aroma compounds form, and the levels of chlorophyll and various enzymes increase [4]. For black teas, after withering, the leaves are purposefully crushed to speed oxidation. This step is what gives black tea its defining quality, whereby enzymatic oxidation converts catechins into theaflavins and thearubigins. Polyphenols give black tea its reddish-brown coloration [5].

1.2. Tea Flavanols

The main polyphenols found in tea are flavonoids. Flavonoids are a group of bioactive compounds synthesized during plant metabolism. Flavonoids are found in fruits and vegetables, prominently in spinach, apples, and blueberries, as well as in beverages like tea and wine. Previous health-related research on tea has largely focused on the flavonoid group. Flavonoids contain two six-carbon rings linked by a three-carbon unit, which is also known as a chalcone structure [6]. Catechins (also referred to as flavanols) are bioactive compounds that are a subclass of flavonoids, and, in tea, are the main secondary metabolites. The main catechins in tea are: catechin, epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin-3-gallate, and gallocatechin. Catechin content in tea differs by tea type or style. Catechins in green tea are relatively stable since they do not go through oxidation during processing, and are what gives green tea its characteristic bitterness and astringency. In black tea, the catechins are largely oxidized to theaflavins and thearubigins [6], which reduces catechin content by around 85% compared to green tea [7], leaving the tea darker and less bitter.

1.3. Tea and Water

After tea leaves are harvested and processed, the final product is ready to consume. However, unlike many other beverages, the final processing step is left to the consumer. A high-quality tea that has gone through many labor-intensive steps can be ruined in an instant by improper brewing. Factors that alter the taste of the brewed cup are brewing temperature, time, vessel, the water-to-leaf ratio, and the water composition [8,9]. This study focuses on the water used to brew tea, specifically how water quality influences the sensory and chemical qualities of black and green tea. Taste is a key factor in consumer acceptance of water [10], however water is often not a top priory when making tea, despite its critical role as the vehicle for the infusion. References to the importance of water content in brewing tea can be found as early as 758AD, in The Classic of Tea by Lu Yu [11]. Lu Yu was an orphan during the Tang Dynasty, raised by an abbot in the Dragon Cloud Monastery. He authored an efficient 7000-character book detailing how to harvest, process, and brew tea, including what types of water are suitable for tea, as well as the proper tools and utensils. Lu Yu felt that tea made from mountains streams was ideal, river water was sufficient, and well water was inferior [3]. In a more recent book from Kuroda & Hara [12], tap water is recommended as the most suitable water for making tea, although specific recommendations are that water should be clear of odors and deficient in magnesium and calcium.

Previous work suggests that tap water can influence the amount of tea flavanols extracted in green tea compared to brewing green tea with purified water [13]. Tap water has a differing (inconsistent between regions, and over time) mineral balance. "Hard" water is high in minerals such as calcium and magnesium. Tea infusions are particularly affected by calcium, with previous studies showing that levels of theaflavins and caffeine extracted decrease with high levels of calcium [14]. Magnesium and calcium can also promote two undesirable outcomes of tea brewing: tea cream and scum formation. Tea cream is the precipitate matter that forms as the tea cools and is caused by the reaction between caffeine and tea flavanols, while tea scum is a surface film that forms on the tea infusion surface, which is composed of calcium, hydrogen carbonates, and other organic material. This film occurs due to calcium carbonate triggering oxidation of organic compounds [9]. It has also been demonstrated that catechin extraction can be increased in white tea by brewing with purified water [15].

1.4. Tea Flavor

Between 25% to 35% of the fresh tea leaf is composed of phenolic compounds with 80% of these being flavanols [16]. Both phenolic compounds and alkaloids such as caffeine contribute to the bitter taste in tea, though the catechins are thought to be the main contributors to bitterness [17]. Glucose, fructose, sucrose, and arabinose in tea account for its sweet taste. Free amino acids make up about 1% to 3% of the dry leaf, and, in green tea, may yield an umami characteristic [16]. Astringency, albeit

not a taste, is a common oral sensation in tea, thought to arise from its catechin content [18]. Despite tea being consumed for several thousand years, there are few consumer sensory studies of tea flavor, with researchers more often favoring evaluation by trained or expert panels. The goal of this project was to test if the water source used to brew tea (tap, bottled, or deionized) influenced flavor or liking from the everyday tea drinker, using both black and green tea. Tea samples were analyzed with a human consumer sensory panel as well as with a number of instrumental methods.

2. Materials and Methods

2.1. Mineral Analysis of Water Samples

Ithaca city tap water, Poland Spring™ bottled water (Nestle Waters, Paris, France), and deionized water used for the study were tested by the Community Science Institute, Inc (Ithaca, NY, USA), assaying calcium, iron, magnesium, sodium, and copper content. Methods followed those recommended by the Environmental Protection Agency (EPA). Briefly, Iron, Magnesium, and Sodium were measured spectrochemically (EPA protocol 200.2, Rv. 2.8) and with inductively coupled plasma-atomic emission spectrometry (EPA 200.7, Rv 4.4), while copper was measured using Inductively Coupled Plasma Mass Spectrometry (EPA 200.8/EPA200.8, Rv 5.4). Calcium and residual chlorine were measured colorimetrically, using an EDTA titration for calcium (SM 3500-Ca B), and a Lamotte test kit for chlorine (LaMotte DPD-1R, LaMotte Co., Maryland, USA).

2.2. Preparation of Tea Infusions

Two high-quality loose leaf teas known as Zhejiang green and Mao Feng black teas were purchased from In Pursuit of Tea (New York, NY, USA). Both teas are from the Zhejiang Province in China, which is a highly regarded tea region, with both produced on the same farm. Green teas were brewed in tap (GT) water, bottled (GB) water, and deionized (GD) water, with black similarly denoted as black tea in tap (BT), bottled (BB), and deionized (BD) water. For the green tea samples, 2.5 g of tea was weighed out into pre-warmed Gaiwan tea brewing vessels (Figure S1), with 125 mL of water at 80 °C added to the vessel. The green tea infusion was brewed for three minutes and then strained through a fine mesh strainer. Black tea samples were brewed at 100 °C for 5 min (more typical for black tea preparation), and strained. Samples were then either cooled to room temperature for instrumental analysis or served fresh in pre-heated cups for sensory analysis (see 2.6 below).

2.3. Colorimetry

Analysis of tea color was performed with a Hunter Lab UltraScan VIS colorimeter (Reston, VA, USA). L (light vs dark), a (red vs green), and b (yellow vs blue) values were recorded for each sample with each of the samples measured in triplicate.

2.4. Turbidity

The turbidity of each sample was measured in triplicate with use of a HACH 2100P portable Turbidity meter (Loveland, CO, USA), with measurements recorded in Nephelometric Turbidity Units (NTU). The samples were held at a 90° angle to the incident beam using single detection. Turbidity standards used were 0.1 NTU, 20 NTU, and 100 NTU.

2.5. Analysis of EGCG

Epigallocatechin Gallate (EGCG) in the tea infusions was measured using high performance liquid chromatography (HPLC), following the methods of Wang and Helliwell [13]. Samples were run using an Agilent 1100 HPLC system (Santa Clara, CA, USA) with a DAD detector. Separations were carried out using a Waters Cortecs (Milford, MA, USA) C18 (4.6 mm × 100 mm) column using an isocratic solvent system consisting of 90% 0.01% phosphoric acid in Millipore water (v/v) and 10% methanol with a flow rate of 0.6 mL/min. The column was held at a constant temperature of 30 °C. The DAD

detector was set to 210 nm. Sample injection volume was 10 µL. The total run time was 20 min. All samples were filtered just before being loaded onto the HPLC using a 0.22 µm Polyvinylidene Fluoride (PVDF) filter from Celltreat (Pepperell, MA, USA). Quantification was performed by the use of an external standard curve using purified EGCG purchased from Sigma Aldrich (St Louis, MO, USA). Identification of EGCG in tea samples was performed using retention time of the pure standards (10.26 min).

2.6. Sensory Evaluation

All human study procedures were approved by the Cornell University Institutional Review Board for Human Participants, with all methods performed in accordance with relevant guidelines and regulations. A total of 103 panelists were recruited from the local community, pre-screened for their tea drinking behavior, and all gave informed consent. All the participants in the study drank tea three to five times a week or more, and were both green and black tea drinkers. The panelist either habitually consumed tea with no milk or sugar added to it or stated no dislike of tea in this manner. Participants knew that the study involved tea but were unaware of the true objective of the research. The session took approximately 45 min, with panelists compensated for their time. The panelists answered questions about samples in individual booths, using Red Jade sensory evaluation software (Curion, Deerfield, IL, USA). The samples were delivered monadically, in a counterbalanced full-block design, but panelists either received 3 green tea samples or 3 black tea samples first. Each tea sample was evaluated for overall liking, appearance liking, and flavor liking with 9-point scales, and then used the generalized Labeled Magnitude Scale (gLMS) to test sweetness, bitterness, sourness, astringency, vegetal quality (for green tea only), and earthiness (for black tea only). All panelists were briefly trained on how to use the gLMS before beginning the tasting [19]. The color of the tea was also evaluated by panelists with a color matching sheet (Figure S2) from which they chose the closest match for each tea sample. Teas were freshly brewed every 30 min. A total of 10 g of tea was brewed with 500 mL of water, at 80 °C for green tea, and 100 °C for black tea. All infusions were kept warm in pre-heated, insulated carafes until the panelist was ready for the sample. Samples were served in pre-heated (80 °C) white ceramic Gung Fu cha teacups (see Figure 1 below) labeled with random 3-digit codes. After each sample, panelists were instructed to cleanse their palette with water and non-salted crackers to avoid fatigue as well as deter any lingering bitterness or astringency. At the end of the questionnaire, panelists were asked a series of demographic questions and for information on their tea drinking habits.

2.7. Statistical Analysis

Data were analyzed with repeated measure analyses of variance (ANOVA) and post-hoc Tukey's tests using Graphpad Prism 5.0 (Graphpad Software, La Jolla, CA, USA). Separate ANOVAs were used for green and black tea samples since such large differences in taste and chemical properties have been shown previously. Statistical significance was inferred at $p < 0.05$. Multivariate analysis was performed using XLSTAT (Addinsoft, Paris, France) whereby two separate Principal Components Analyses were run on sensory and instrumental data as well as these two datasets combined in a Multiple Factor Analysis.

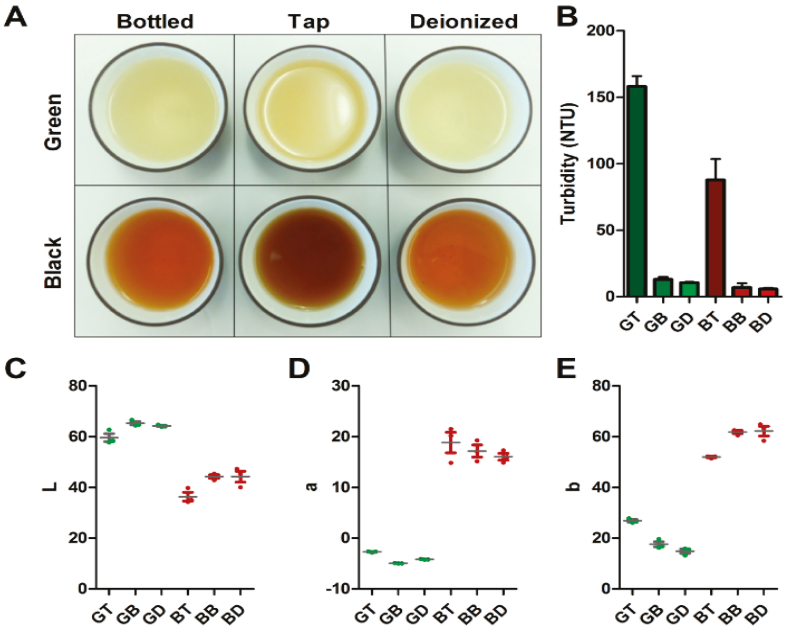

Figure 1. (**A**) Image of black and green tea samples brewed in tap, bottled, or deionized water. For both green and black tea, infusions appear darker and cloudier from tap wate compared to the teas brewed in DI or bottled water. (**B**)Turbidity measurements (NTU) for each tea infusion showing average of three replicates with SEM. (**C–E**) Colorimeter readings from tea infusions, L, a and b values displayed with individual readings as dots, lines denoting average, and SEM. Samples denoted as green tea brewed in tap (GT), bottled (GB), and deionized (GD) water, black tea brewed in tap (BT), bottled (BB), and deionized (BD) water. Green tea samples represented in green, black tea in dark red.

3. Results and Discussion

3.1. Water Analysis

Deionized, tap, and bottled water samples were tested for calcium, magnesium, copper, iron, residual chlorine, and sodium (Table 1). The amount of calcium, magnesium, and sodium in tap water was far greater than that in bottled or deionized water.

Table 1. Mineral analysis of the different water types in mg/L.

	Bottled	Tap	Deionized
Calcium	8.000	53.600	3.000
Iron	0.050	0.050	0.050
Magnesium	1.370	9.460	0.100
Sodium	10.600	20.900	0.100
Copper	0.002	0.176	0.002
Residual Chlorine	0.200	0.200	0.200

3.2. Turbidity and Color

Figure 1A shows the appearance of tea samples when brewed with three different water types. Teas brewed in tap water appear more cloudy and darker in color than teas brewed in bottled water or deionized (DI) water for both green and black teas. Turbidity measurements (Figure 1B) in green ($p < 0.001$) showed GT was more turbid than both GB (95% CI = 133.3 to 156.7) and GD (95% CI = 135.7

to 159.1), with no difference between GB and GD. In black tea, the turbidity of BT was also higher ($p < 0.001$) than both BB (95% CI = 57.66 to 103.9) and BD (95% CI = 58.81 to 105.1), with no difference between BB and BD. Adding high concentrations of calcium or magnesium in water can cause cloudiness and tea scum in tea infusion as well as possibly influencing tea's sensory properties [9,18] since both calcium and magnesium were higher in tap water used in this project. This was likely the cause of the observed turbidity increase.

Both green ($p = 0.016$) and black ($p = 0.023$) tea infusions significantly differ in lightness. Green tea brewed in tap water exhibited lower L values compared to the same tea brewed in bottled (95% CI = −9.992 to −1.288) or DI (95% CI = −8.952 to −0.2476) water, with BT similarly lower than BB (95% CI = −15.14 to −0.7051) or BD (95% CI = −15.13 to −0.6918). The a values for green ($p < 0.001$) but not black ($p = 0.425$) tea significantly differed between samples, with all pairs differing between green teas (95% CI for GT vs GB = 2.042 to 2.458; GT vs GD = 1.269 to 1.685; GB vs GD = −0.9814 to −0.5652). The b values for both green ($p < 0.001$) and black ($p = 0.001$) teas significantly varied between treatments, with tap water against the different sample. GT was higher compared to GB (95% CI = 5.661 to 12.80) and GD (95% CI = 8.401 to 15.540), with BT higher than BB (95% CI = −14.94 to −4.711) or BD (95% CI = −15.33 to −5.105).

3.3. EGCG Content

The amount of EGCG in black tea is customarily lower than that found in green tea, since the majority of the catechins in black tea are converted to theaflavins and thearubigins [5]. The small amount of EGCG in the black tea infusions did not vary with water type ($p = 0.250$, Figure 2C,D). Conversely, with green tea (natively much higher in EGCG), there was a significant difference between green tea infusions ($p < 0.001$) and with green tea brewed in bottled water (95% CI = −6350 to −3984) and in deionized water (95% CI = −5890 to −3524) having around double the amount of EGCG compared to green tea brewed in tap water (Figure 2A,B), despite being brewed from the same leaves, at the same strength, time and temperature, in identical vessels. Green teas brewed from bottled or deionized water achieved around the same level of EGCG extraction (95% CI = −723.0 to 1643). Such dramatically inferior EGCG extraction in tap water is important to green tea consumers, many of whom are consuming green tea due to a perceived consequence of health promotion [20]. EGCG is the most abundant catechin in green tea [21] as well as one of the most bitter tasting [22]. That green tea acceptance has been linked to bitter taste genes [23], and that bitterness in tea is largely a product of EGCG content [24], implies that extraction of bitter catechins in bottled or deionized water may lead to more healthy and yet less palatable tea infusions.

3.4. Sensory Testing of Tea Samples

There was no significant difference between panelists' overall ($p = 0.646$), or flavor ($p = 0.553$) liking of black tea samples (Figure 3A,C). Panelists did find significant differences in appearance liking between the samples (Figure 3B, $p = 0.0345$), which is likely a reflection of the color differences between the black tea infusions evident in Figure 1A. However, this trend was not strong enough to reflect differences between sample pairs in post-hoc Tukey's tests. Panelists also evaluated various flavor attributes of the black tea infusions. No differences were evident with water type between black tea infusion for astringency, bitterness, sourness, or sweetness (Figure 3D–F,H, all $p > 0.05$). However, panelists did find a difference in earthy flavor (Figure 3G, $p = 0.025$), specifically between that brewed in bottled water compared to black tap water (95% CI = −7.339 to −0.5252). While the panel perceived black tea brewed in tap water to be earthier, it had little effect on liking, which suggests that water may not be a critical factor in determining liking in black tea.

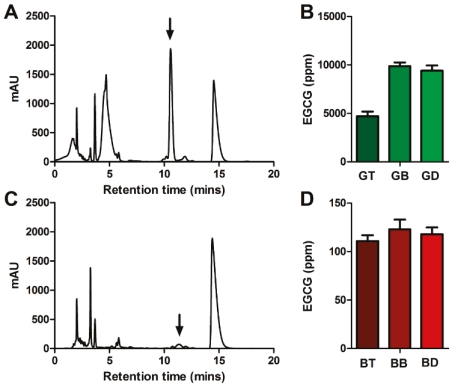

Figure 2. (**A**) Chromatogram illustrative of HPLC spectrum from green tea. EGCG peak at arrow. Y axis in milli-Absorbance Units. (**B**) Total EGCG content for green tea in ppm, brewed in tap (GT), bottled (GB), and deionized (GD water. Display shows mean of three readings plus SEM. (**C**) Chromatogram illustrative of HPLC spectrum from black tea. EGCG peak at arrow. (**D**) Total EGCG content for black tea in ppm. Samples denoted as black tea brewed in tap (BT), bottled (BB), and deionized (BD) water.

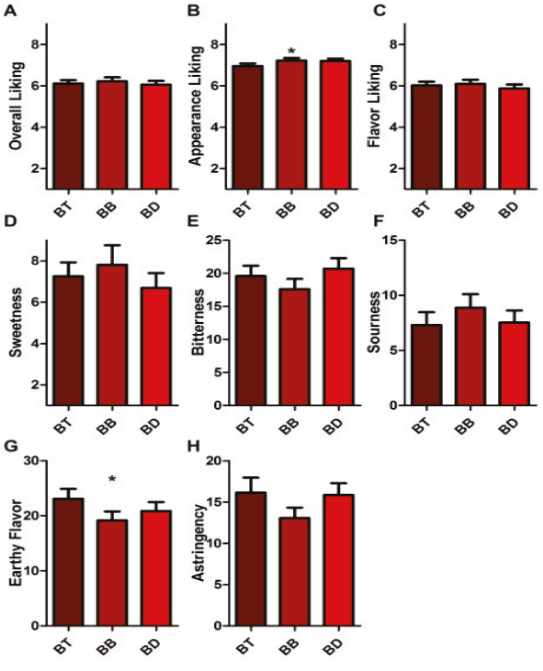

Figure 3. Consumer perception of black tea brewed in tap (BT), bottled (BB), and deionized (BD) water. (**A**) Overall liking of samples, from dislike extremely (1) to like extremely (9). (**B**) Appearance liking of samples, from dislike extremely (1) to like extremely (9). (**C**) Flavor liking of samples, from dislike extremely (1) to like extremely (9). (**D**) Perceived sweetness of samples, rated on gLMS, scale descriptors no sensation (0.0), barely detectable (1.4), weak (6.0), moderate (17.0), strong (34.7), very strong (52.5), and strongest imaginable sensation of any kind (100.0). (**E**) Bitterness, scale as in D. (**F**) Sourness, scale as in D. (**G**) Earthy flavor, scale as in D. (**H**) Astringency, scale as in D. Bars display mean rating of panel (n = 103) plus SEM. * indicates $p < 0.05$.

For green tea samples, the effects of water were clearer. Panelists rated their overall liking (Figure 4A, $p < 0.001$) of green tea samples as differing across water treatments, with the tap clearly higher than bottled water (95% CI = −1.138 to −0.2993), with tap vs. deionized water approaching significance (95% CI = −0.04054 to 0.7978). Interestingly, this reduction in liking seemed to be driven by the panel's liking of the sample's flavor (Figure 4C, $p = 0.001$), and not its appearance (Figure 4B, $p = 0.099$). In investigating changes to the green tea's flavor properties, panelist found no significant difference in astringency, sourness, or vegetal flavor (Figure 4F–H, all $p > 0.05$). However, the panel judged the green tea samples brewed with tap water to be far less bitter (Figure 4E, $p < 0.001$) than both the sample brewed with bottled (95% CI = 0.6244 to 6.502) or with deionized water (95% CI = −9.162 to −3.285). Since only around half the amount of EGCG was extracted in green tea brewed from tap water compared to the other samples, and EGCG is experienced as highly bitter, this would result in less bitter tea infusion when brewing with tap water. Since bitterness is closely linked to liking tea regardless of ethnicity or tea drinking habits [25,26], this likely drove the increase in liking of green tea brewed in tap water. The GT sample was also experienced as sweeter by the panel (Figure 4D, $p = 0.012$), which was likely due to mixture suppression [27,28] of sweetness in samples with more bitter catechins. EGCG has been noted to extract more efficiently from green tea with purer water [29] and with higher conductivity (thus higher impurity) water producing poorer catechin extraction [30]. Rossetti and colleagues [31] measured the detection threshold of EGCG (perceived to be bitter and astringent) to be 183 mg/L (at 37 °C). Despite the fact that bitterness may be somewhat depressed by temperature [32], the bitterness of green tea in our study would be clear in the samples' flavor profile. Thus, doubling the EGCG content of tea in bottled or deionized water (compared to tap) was likely the driving factor behind reduced liking of these samples in consumer testing. Since black tea has fewer catechins than green tea due to the oxidation process in manufacturing, the type of water used seems less important to the everyday tea drinker.

As well as instrumental measurement of color changes in tea samples, and assessment of appearance liking of samples, we were also interested in whether variation in color between samples was visible to the human eye. Panelists used a color matching chart for both black and green tea samples (see Figure S2), divided into eight color segments for green teas, and eight more for black teas. The panelists could clearly discern differences between samples of both black ($p < 0.001$, chi-square 39.91), and green tea samples ($p < 0.001$, chi-square 43.87), although this did not influence their liking of the samples overall, nor their liking of the appearance of the samples, which suggests flavor is more critical in determining liking of tea infusions than their appearance. It is clear, however, that consumer perception of beverages can be altered by their color and appearance [33], and, thus, some of the effects observed may have been due to the cross modal influence of the different colored tea samples.

Some work exists concerning the influence of various brewing conditions on the sensory properties of tea. Liu et al. [34] found optimal conditions for acceptance, at least in a small expert panel, were brewing for 5.7 min at 82 °C, with tea of around 1100 μm in particle size, in a 70 mL/g ratio of water to tea. From instant green tea preparations, increasing the calcium concentration in the brewing solution was found to weaken bitter taste in the mixture purportedly provided by EGCG [18], which is in good agreement with our observations. However, influences on the sweetness of infusions (attributed in part to theanine) were not seen in our work, possibly due to the around 4 mg/100 mg sucrose found in the group's instant tea preparations. A study of hot and cold-brewed tea infusions of varying strength by Lin et al. [2] proposed a linkage between higher EGCG and EGC (epigallocatechin) levels and lower sensory appeal, which was attributed to lower bitterness and astringency in these samples. In a small group of trained panelists, sensory differences were reported in green tea brewed with various water types [30], with mineral water found to produce tea with lower EGCG levels than tap water, purified water, or mountain spring water, as well as perceived bitterness mapping onto EGCG levels. However, samples from this report were liked more with higher bitterness (and EGCG) unlike our own results. A similar result was reported by Zhang et al. [15], whereby EGCG levels from green tea extractions varied with water quality. Sensory reports of taste quality were higher for the

high EGCG samples, though no report was made of panel size or makeup. Such differences are likely attributed to the difference in palate of a small group of experts from China versus a large panel of tea consumers in the US. Alternatively, those regularly consuming diets high in salty [35], sweet [36], or umami [37] stimuli have shown some reduced ability to perceive these stimuli possibly due to receptor regulation in taste [38]. Thus, it is possible that regular consumers of very bitter tea experience how they taste in a fundamentally different manner.

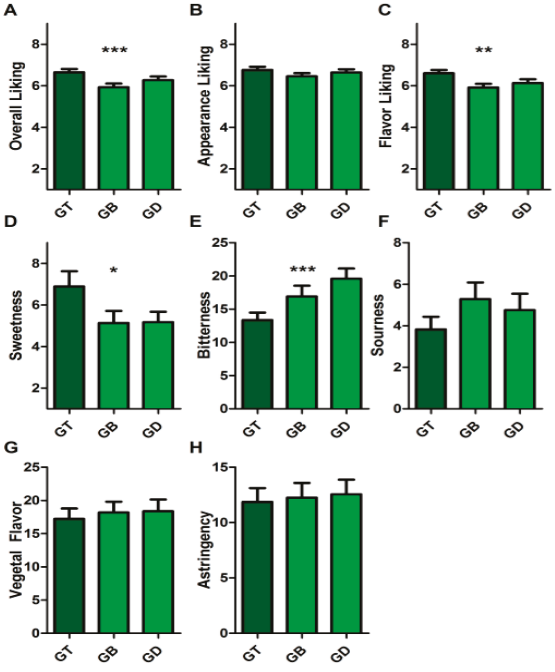

Figure 4. Consumer perception of green tea brewed in tap (GT), bottled (GB), and deionized (GD) water. (**A**) Overall liking of samples, from dislike extremely (1) to like extremely (9). (**B**) Appearance liking of samples, from dislike extremely (1) to like extremely (9). (**C**) Flavor liking of samples, from dislike extremely (1) to like extremely (9). (**D**) Perceived sweetness of samples, rated on gLMS, scale descriptors no sensation (0.0), barely detectable (1.4), weak (6.0), moderate (17.0), strong (34.7), very strong (52.5), and strongest imaginable sensation of any kind (100.0). (**E**) Bitterness, scale as in D. (**F**) Sourness, scale as in D. (**G**) Earthy flavor, scale as in D. (**H**) Astringency, scale as in D. Bars display mean rating of panel (n = 103) plus SEM. * indicates $p < 0.05$. ** indicates $p < 0.01$. *** indicates $p < 0.001$.

Following sensory testing, panelists participated in a survey of their attitudes toward tea. When asked their primary motivation for drinking black tea, only 7% of panelists responded due to healthful properties and, instead, favoring taste or flavor (84%), with a small number of respondents citing other reasons. However, when asked their primary motivation for drinking green tea, 26% cited its health benefits, with 67% for taste or flavor, and again a small number citing other reasons. This suggests the ability to almost double the EGCG content of green tea would be of great interest to many green tea consumers.

3.5. Multivariate Analysis

Further analysis of the data with Principal Components Analysis (PCA) and Multiple Factor Analysis (MFA) was performed. Scree plots revealed that data could be plotted well on two axes both in the case of sensory and instrumental data, with 88.5% and 98% of the variance accounted for by

the first two factors in the analysis, respectively. The sample of green tea brewed with tap water was located close to both dimensions of overall and flavor liking, which, in turn, were negatively correlated with bitterness (Figure 5A). In plots of instrumental results, samples pairs GD and GB as well as BD and BB plotted almost exactly on top of one another (Figure 5B). In the case of both black and green tea, the tap-brewed sample was the clear outlier. Samples GD and GB plotted closely to the axes represent phenolics, EGCG, and colorimetric L-value. MFA plots combining both sensory and instrumental data showed similar patterns (Figure 5C), with sample GT lying in the directions of overall and flavor liking, and anti-parallel to that of bitterness.

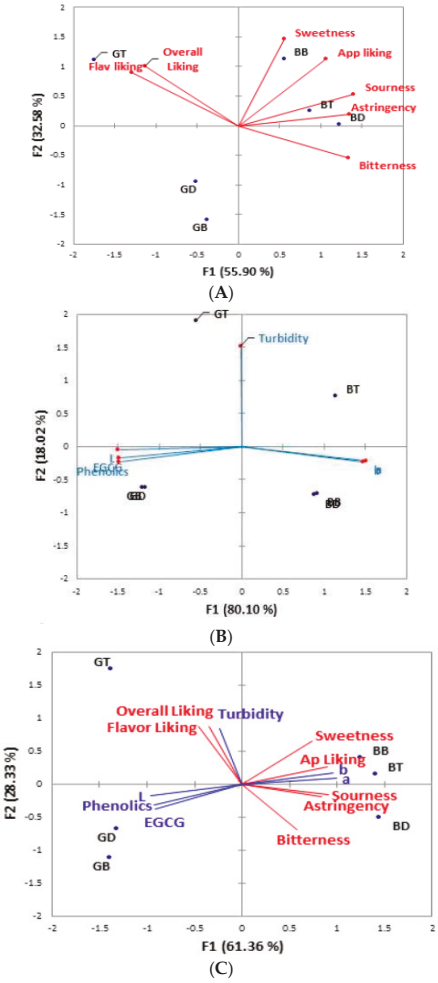

Figure 5. Multivariate analysis of tea samples. (**A**) Principal components analysis of sensory data. Samples shown in black, original axes in red, variance from new factors in parentheses. (**B**) Principal components analysis of instrumental data. Samples shown in black, original axes in blue, variance from new factors in parentheses. (**C**) Multiple factor analysis of sensory and instrumental data. Samples shown in black, sensory axes in red, instrumental axes in blue, variance from new factors in parentheses. Samples denoted as green tea brewed in tap (GT), bottled (GB), and deionized (GD) water, black tea brewed in tap (BT), bottled (BB), and deionized (BD) water.

4. Conclusions

Tea is the most consumed beverage besides water in the world. This project sought to get a better understanding of whether the type of water used to brew tea is of importance to the everyday tea drinker. Through the instrumental analysis of green and black tea brewed in tap, bottled, and deionized water, we demonstrated a difference in color, turbidity, and the amount of EGCG extracted from tea leaves depending on the water type. The high mineral content of the tap water used in this study led to inferior extraction of catechins in green tea, and thus, produced an infusion that was less bitter, and also perceived as sweeter than the same tea brewed in bottled or deionized water, with an accompanying higher degree of liking for green tea when brewed in this manner. For tea drinkers consuming green tea for either flavor or its health benefits, our results highlight that the type of water used to brew tea is clearly important, and suggests that those seeking greater health benefits should use a more purified water source to brew green tea, while those more concerned with flavor may prefer to use water from the tap.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/11/1/80/s1, Figure S1: Traditional Gaiwan brewing vessel, Figure S2: 8-option color matching diagram provided to consumer panel.

Author Contributions: Conceptualization, M.F. and R.D.; formal analysis, M.F., P.L., A.A., and R.D.; investigation, M.F. and P.L.; writing—original draft preparation, M.F. and R.D.; writing—review and editing, M.F., P.L., A.A., and R.D.; project administration, R.D.

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Conflicts of Interest: The authors declare no competing interests.

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Estimation of Olfactory Sensitivity Using a Bayesian Adaptive Method

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Abstract: The ability to smell is crucial for most species as it enables the detection of environmental threats like smoke, fosters social interactions, and contributes to the sensory evaluation of food and eating behavior. The high prevalence of smell disturbances throughout the life span calls for a continuous effort to improve tools for quick and reliable assessment of olfactory function. Odor-dispensing pens, called Sniffin' Sticks, are an established method to deliver olfactory stimuli during diagnostic evaluation. We tested the suitability of a Bayesian adaptive algorithm (QUEST) to estimate olfactory sensitivity using Sniffin' Sticks by comparing QUEST sensitivity thresholds with those obtained using a procedure based on an established standard staircase protocol. Thresholds were measured twice with both procedures in two sessions (Test and Retest). Overall, both procedures exhibited considerable overlap, with QUEST displaying slightly higher test-retest correlations, less variability between measurements, and reduced testing duration. Notably, participants were more frequently presented with the highest concentration during QUEST, which may foster adaptation and habituation effects. We conclude that further research is required to better understand and optimize the procedure for assessment of olfactory performance.

Keywords: smell sensitivity; olfaction; threshold; staircase; QUEST

1. Introduction

The appreciation of food involves all senses: sight, smell, taste, touch, and also hearing. While the sight of a cup of coffee may indicate its availability, it is typically its smell that makes it appealing and that triggers an appetite for most people. During consumption, the smell or aroma is perceived again retronasally and supported by its pleasant temperature and a bitter taste. These largely parallel sensations occur automatically and only raise awareness when one or more senses are disturbed. That said, the sense of smell has been shown to influence food choice and eating behavior [1], and its impairment has even been associated with a higher risk for diet-related diseases like diabetes [2]. Even more, olfactory stimuli can invoke emotional states, are linked to memory storage and retrieval, and as such also serve as important cues to rapid detection of potentially dangerous situations and threats (see e.g., [3,4]. Given that the estimated prevalence of smell impairment is 3.5% in the United States [5], continuous efforts are made toward an efficient and precise assessment of olfactory function.

The Sniffin' Sticks test suite (Burghart, Wedel, Germany; [6]), is an established tool in the assessment of olfactory function. It consists of three tests involving sets of impregnated felt-tip pens: odor detection threshold (T), odor discrimination (D), and odor identification (I). Each test produces numbers in the range from 1 to 16 (T) or from 0 to 16 (D and I) as a performance measure. Overall olfactory function is assessed by summing all three test results, resulting in the *TDI score*. Comparison of individual TDI scores to the comprehensive set of available normative data (e.g., [7,8]) facilitates the interpretation of test scores and allows to reliably diagnose olfactory impairment. Notably, threshold,

discrimination, and identification measure different facets of olfactory function [9]. The threshold, however, has been found to explain a larger portion of variability in TDI scores than the two other measures [10]. Moreover, the discrimination and identification tests follow relatively simple test protocols in which all stimuli are presented only once and in a predefined order. The threshold, in comparison, is of a more complex nature, and the method, therefore provides the largest potential for possible improvements. It follows a so-called adaptive method, specifically, a "transformed" one-up/two-down staircase procedure [11]. The procedure first assesses a starting concentration and then moves on to the "actual" threshold estimation, during which fixed step widths are used: for each incorrect answer, the stimulus concentration is increased by one step; and for two consecutive correct answers, the stimulus concentration is decreased by one step [6].

Since the one-up/two-down staircase was first conceived, several new approaches to threshold estimation, including Bayesian methods, have been published. Bayesian methods estimate parameters of the psychometric function (e.g., threshold or slope) using Bayesian inference: based on prior assumptions about the true parameter value, the stimulus concentration to be presented next is selected such that the expected information gain (about the parameter) is maximized. The first published Bayesian adaptive psychometric method is the QUEST procedure [12], which is still popular today. QUEST has two distinct properties that set it apart from the staircase described above. Firstly, it always considers the entire response history and is not solely based on the past one or two trials to select the optimal stimulus concentration to be presented next. Secondly, QUEST is not tied to a fixed step width, allowing it to traverse through a large range of concentrations more quickly.

In a clinical setting, at the otorhinolaryngologist's (ear-nose-throat, ENT) practice or at the bedside in the hospital, shorter testing times are always beneficial, as they reduce strain on patients and free up time for other parts of diagnostics and treatment. But also when working with healthy participants, e.g., in a psychophysical lab or in large cohort studies, reduced testing time spares resources and allows for a larger number of measurements in a given time.

QUEST has been shown to converge reliably and quickly in gustatory threshold estimations [13,14]. Inspired by these results we set out to design and test a QUEST-based procedure for olfactory threshold estimation and to compare its performance with that of the established staircase method.

2. Materials and Methods

2.1. Participants

36 participants (32 women; median age: 29.5 years, age range: 19–61 years) completed the study. The influence of gender on olfactory performance has been investigated in previous studies. The results typically showed no (e.g., [15], several hundred participants; [7], >3000 participants, no main effect) or only rather small gender differences with negligible diagnostic and real-world relevance (e.g., [8], >9000 participants). We therefore did not enforce a gender balance in our sample. Due to a technical error, the identification test data was not recorded for one participant (female, 26 years old). All participants were non-smokers and reported being healthy and not having suffered from an infectious rhinitis for at least two weeks before testing. The study conformed to the revised Declaration of Helsinki and was approved by the ethical board of the German Society of Psychology (DGPs).

2.2. Stimuli

Stimuli were so-called Sniffin' Sticks (Burghart, Wedel, Germany; [6]), felt-tip pens filled with an odorant. The Sniffin' Sticks test battery consists of three subtests: an odor threshold test, an odor detection test, and an odor identification test. The threshold test comprises 48 pens. There were 16 pens filled with different concentrations of 2-phenylethanol (rose-like smell) ranging from 4% to approx. 1.22×10^{-4}% (a geometric sequence with the common ratio of 2, so the first pen contained a 4% dilution, the second 2%, the third 1%, and so on), dissolved in 4% propylene glycol, an odorless solvent. Note that in this test, the 1st pen contained the highest, the 16th pen the lowest odorant

concentration. The remaining 32 pens contained 4% propylene glycol and served as blanks. The pens were arranged in triplets such that each triplet contained one pen with odorant and two blanks. The detection test comprised 48 pens that were filled with 16 different odorants at supra-threshold concentrations. The pens were arranged in triplets such that two pens contained the same and one pen a different odorant. The identification test comprised 16 pens filled with different odorants at supra-threshold concentrations.

2.3. Procedure

2.3.1. Experimental Sessions

Participants were invited for two experimental sessions – the Test and Retest session for the odor threshold. To ensure similar testing conditions across sessions, participants were instructed to refrain from eating and drinking anything but water 30 min before visiting the laboratory. Further, both sessions were scheduled at approximately the same time of day, and took place with a median inter-session interval of 3.0 days (SD = 2.6, range: 0.9–8.9 days); only four participants had an inter-session interval of more than 7.0 days. In each session, olfactory detection thresholds were determined using two distinct algorithms, staircase and QUEST, described below. The order of algorithms was balanced across participants and kept constant for Test and Retest within each participant. Additionally, odor discrimination and odor identification ability were measured at the end of one session following the standard Sniffin' Sticks protocol (Burghart, Wedel, Germany).

2.3.2. Stimulus Presentation

Testing took place in a well-ventilated testing room and was performed by the same experimenter, who refrained from using any fragrant products (e.g., soap, lotion, perfume, etc.) and wore odorless cotton gloves when presenting the stimuli. At the beginning of each test session, participants were blindfolded. To present a stimulus, the experimenter removed the cap from the pen, held the tip of the pen in front of the participant's nose, approx. 2 cm from the nostrils, and asked the participant to take a sniff. For the threshold test, participants were blindfolded and informed that the odorant may be presented in very low concentrations, and that only one of the three pens presented in each trial contained the odorant, while the others contained the solvent exclusively. The task was to "indicate which of the three pens smells different from the others", and participants had to provide a response even when unsure. Participants were familiarized with the odorant by presenting pen no. 1 (highest concentration) before testing commenced.

A similar procedure was used for the discrimination test: participants were blindfolded and presented with a triplet of pens containing clearly perceivable odorants. Each triplet consisted of two pens with the same and one pen with a different odorant. Again, participants were to indicate the pen that smelled different from the others. During threshold and discrimination testing, stimulus triplets were presented during each trial, which lasted approx. 30 s and included the presentation of three pens (approx. 3 s each) and a pause of 20 s. These tests yield a probability of ⅓ of guessing correctly.

For the identification task, the blindfold was removed and participants smelled one pen at a time. They were to identify the odor by pointing to the matching word on a response sheet with four written response options. The interval between pens was approx. 30 s. The probability of guessing correctly in this task was ¼.

2.3.3. Staircase

Following the standard protocol as detailed in the test manual; see also [16]), the order of presentation within the triplets varied from trial to trial. In the first trial, the odor pen was presented first, in the second trial, it was presented between two blanks, and in the third, after two blanks. After the third trial, this sequence was repeated.

We first determined the starting concentration. Beginning with the presentation of triplet no. 16 or 15 (balanced across participants), participants had to indicate which of the pens smelled different. Concentration was increased in steps of two (e.g., from pen 16 to 14) for each incorrect response. Once participants provided a correct response, the same triplet was presented again. If the response was incorrect, the concentration was increased again by two steps as before. However, if the triplet was correctly identified a second time, that dilution step served as the starting concentration.

Contrary to the standard protocol, where testing would then continue without interruption, our participants were granted a short break of approx. 1 min before the actual threshold estimation started with the presentation of the triplet containing the starting concentration. The threshold was determined in a one-up/two-down staircase procedure: odor concentration was increased by one step after each incorrect response (one-up), and decreased by one step after two consecutive correct responses at the same concentration (two-down). This kind of staircase targets a threshold of 70.71% correct responses ([11]; but cf. [17], who found small deviations from this value). That is, if presented repeatedly with a stimulus at threshold intensity, participants would be able to correctly identify it in about 71 out of 100 cases. The probability of providing *two consecutive* correct responses purely by guessing is ⅓ × ⅓ = ⅑. The procedure finished after seven reversal points were reached. The final threshold estimate was the mean of the last four reversal concentrations. This procedure is referred to simply as staircase throughout the this manuscript.

2.3.4. QUEST

QUEST requires to set parameters that describe the assumed psychometric function linking stimulus intensity and expected response behavior. We assumed a sigmoid psychometric function of the Weibull family, as proposed by [12] (albeit in a slightly different parametrization) and used for gustatory testing [13], with a slope $\beta = 3.5$, a lower asymptote $\gamma = 1/3$ (chance of a correct response just by guessing), and a parameter $\lambda = 0.01$ to account for lapses (response errors due to momentary fluctuation of attention):

$$\Psi(x) = \lambda\gamma + (1-\lambda)[1-(1-\gamma)\exp(-10^{\beta(x+T)})]$$

Here, the presented concentration is denoted as x, and the assumed threshold as T. This yielded a function extending from 0.33 to 0.99 in units of "proportion of correct responses". The granularity of the concentration grid was set to 0.01. All parameters of this function were constant, except for the threshold, which was the parameter of interest that was going to be estimated in the course of the procedure. The prior estimate of the threshold was a normal distribution with a standard deviation of 20, which was centered on the concentration of pen no. 7, which was used as the starting concentration. The algorithm was set to target the threshold at 80% correct responses, which is slightly higher than the threshold target in the staircase procedure, but had proven to produce good results both in pilot testing as well as in gustatory threshold estimation [13,14]. Unlike in the staircase procedure, where the order of pen presentation varied systematically from triplet to triplet, triplets were presented in random order during the QUEST procedure.

Notably, QUEST updates its knowledge on the expected threshold after each response and proposes the concentration to present in the next trial such that it maximizes the expected information gain about the "true" threshold. As the set of concentrations was discrete and limited to 16, QUEST might propose concentrations other than those contained in the test set. In this case, the software selects the triplet with the concentration closest to the one proposed. In contrast to the staircase, where the concentration was always decreased or increased by a single step after the starting concentration had been determined, the step width was not fixed in QUEST. For example, QUEST might step up three concentrations in one trial, step down two in the next, and present the exact same concentration again in the following trial. Whenever the same concentration had been presented on two consecutive trials, the concentration for the next trial was decreased if both responses were correct, and increased if

both responses were incorrect. QUEST might suggest to present concentrations outside of the range of available dilution steps. Therefore we set up the algorithm such that, whenever the presentation of a pen below 1 or above 16 was suggested, we would instead present pen no. 1 and 16, respectively. QUEST would be informed about the actually presented pen, and incorporate this information into the threshold estimate. Note, however, that final threshold estimates outside the concentration range could still occur occasionally, and needed to be dealt with accordingly; see the data cleaning paragraph in the next section for details.

The procedure ended after 20 trials. The final threshold estimate is the mean of the posterior probability density function of the threshold parameter. We will refer to this procedure as "QUEST".

2.3.5. Analysis

Odor Discrimination and Identification

The discrimination and identification tests comprised 16 trials each. For each test, the number of correct responses was summed up, resulting in a test score which can range from 0 to 16. Together with the staircase threshold, which yielded values from 1 to 16, the sum of all three test results formed a cumulative score: the TDI score.

Data Cleaning

When a participant reached one of the most extreme concentrations (i.e., pens no. 1 or 16) and provided a response that would, theoretically, require us to present a concentration outside the stimulus of set, the staircase procedure cannot be safely assumed to yield a reliable threshold estimate anymore. For example, if a participant fails to identify the highest concentration (pen no. 1), the staircase procedure would accordingly demand to present a hypothetical pen no. 0, which obviously does not exist. Since our sole termination criterion was "seven reversals", we would repeatedly present pen no. 1 until a correct identification allowed the procedure to move up to pen no. 2 again. The resulting threshold estimate, then, would systematically overestimate this participant's sensitivity. Therefore we set the threshold values of staircase runs where participants could not identify pen no. 1 at least once to $T = 1$ after the run was completed, following [7] (but cf. [16], who suggest to set the value to $T = 0$ instead). This was the case in five out of the 72 staircase threshold measurements (two during test, three during retest; five participants affected). Conversely, when a participant were to correctly identify the lowest concentration (pen no. 16), the staircase procedure would require the presentation of a hypothetical pen no. 17, in which case we would have assigned a threshold value of $T = 16$; however, this situation did not occur in the present study after the starting concentration had been determined.

For QUEST, pen no. 1 was not correctly identified at least once in 12 of the 72 measurements, concerning 11 participants; no participant reached and correctly identified pen no. 16. QUEST yielded final threshold estimates $T < 1$ in 11 measurements (8 during Test, 3 during Retest; 10 participants affected). Similarly to the data cleaning procedure for the staircase, we assigned threshold $T = 1$ in these cases. Notably, this again concerned 3 of the 5 participants for whom we had assigned $T = 1$ in a staircase experiment.

Test–Restest Reliability

To establish test–retest reliability, we first compared the means of Test and Retest thresholds for each procedure. Q–Q plots and Shapiro–Wilk tests revealed that thresholds were not normally distributed for the QUEST test session ($W = 0.90$, $p < 0.01$); we, therefore, compared the means using non-parametric Wilcoxon signed-rank tests. We then correlated Test and Retest threshold estimates via Spearman's rank correlation (Spearman's rho, denoted as ρ) to estimate the degree of monotonic relationship between measurements. Ordinary least squares (OLS) models were used to fit regression lines to provide a better understanding of the nature of the relationship between the threshold estimates

(i.e., whether test thresholds could predict retest thresholds). Q–Q plots and Shapiro–Wilk tests showed that the regression residuals were normally distributed (all $p > 0.05$) and thus satisfied an important requirement for OLS regression.

Although correlation and regression analyses are widely used to assess test–retest reliability and to compare methods, it has been argued that these measures may in fact be inappropriate (see e.g., [18–20]). Instead, analyses that focus on the *differences* between, not agreement of, measurements should be preferred. A possible approach is to calculate the mean difference $\bar{d}$ and standard deviation of the differences between two measurements to derive *limits of agreement*, $\bar{d} \pm 1.96 \times \mathrm{SD}$ [18]. These limits correspond to the 95% confidence interval. This means that in 95 out of 100 comparisons, the difference between two measurements can be expected to fall into this range. Narrower limits of agreement indicate a better agreement between two measurements. The related repeatability coefficient (RC) was simply $1.96 \times \mathrm{SD}$, and its interpretation was very similar to the limits of agreement: only 5% of absolute measurement differences will exceed this value, and a smaller RC indicates better agreement. (It should be noted that an alternative method for calculating the repeatability coefficient has been suggested, based on the within-participant standard deviation, s_w [20]. The results we obtained from these calculations were similar to those based on the standard deviation of the measurement differences. Because the latter are directly visualized in the Bland–Altman plot by the limits of agreement, i.e., mean difference $\pm 1.96 \times \mathrm{SD}$, we opted to only report these values.)

If the differences between two measurements are plotted over the mean of the measurements, and $\bar{d}$ and the limits of agreement are added as horizontal lines, the resulting plot is called a Bland–Altman plot (sometimes also referred to as Tukey mean difference plot). It can be used to quickly visually inspect how well measurements can be reproduced, specifically which systematic bias ($\bar{d} \neq 0$) and which variability or "spread" of measurement differences to expect. Accordingly, we assessed the RC, limits of agreement, and produced Bland–Altman plots for both methods, staircase and QUEST, to gain more insight into the repeatability (or lack thereof) of measurements for each method. The use of these analyses requires the measurement differences to be normally distributed, which we confirmed using Q–Q plots, and Shapiro–Wilk tests failed to reject the null hypothesis of normal distributions (all $p > 0.05$). Confidence intervals for the limits of agreement were calculated using the "exact paired" method [21].

Lastly, to test whether the duration of the inter-session interval might be a confounding factor in the threshold estimates, we also calculated the Spearman correlation between inter-session intervals and differences between Test and Retest thresholds.

Comparison between Procedures

To compare the threshold estimates across procedures, we averaged Test and Retest thresholds for each participant within a procedure, and, similarly to the analysis of reliability, compared the means with a Wilcoxon signed-rank test, followed by the calculation of Spearman's ρ and the fit of a regression line using an OLS model. The regression residuals were normally distributed, according to a Q–Q plot and a Shapiro-Wilk test ($W = 0.96$, $p = 0.26$), satisfying the normality assumption of errors on which OLS regression crtitically relies.

Additionally, we estimated the 95% limits of agreement from the differences between the within-participant session means for the two procedures, and generated Bland-Altman plots. The measurement differences were normally distributed, according to a Q-Q plot and a Shapiro-Wilk test ($W = 0.96$, $p = 0.30$). Like in the investigation of test-retest reliability, we assessed confidence intervals of the limits of agreement via the "exact paired" method [21].

Because the limits of agreement derived from session means might actually be too narrow, as within-participant variability is removed by averaging measurements across sessions [20], we calculated adjusted limits of agreement from the variance of the between-subject differences, $\sigma_{\bar{d}}^2$, which in turn can be calculated as $\sigma_{\bar{d}}^2 = s_{\bar{d}}^2 + 0.5\, s_{xw}^2 + 0.5\, s_{yw}^2$. Here, $s_{\bar{d}}^2$ is the variance of the differences between the session means; and s_{xw}^2 and s_{yw}^2 are the within-participant variances of methods x and

y, respectively (staircase and QUEST in our case). The limits of agreement can then be calculated as $\bar{d} \pm 1.96 \times \sigma_d$, with $\bar{d}$ being the mean difference between the session means of both procedures. Again, the interpretation of these limits is straightforward: 95% of the differences between staircase and QUEST measurements can be expected to fall into this interval, and narrower limits indicate a better agreement across the measurement results produced by both procedures. Finally, we derived 95% confidence intervals for these limits ([20], Section 5.1, Equation (5.10)).

Software

The experiments were run via PsychoPy 1.85.4 [22,23] running on Python 2.7.14 (https://www.python.org) installed via the Miniconda distribution (https://conda.io/miniconda.html) on Windows 7 (Microsoft Corp., Redmond, WA, USA). All analyses were carried out with Python 3.7.1, running on macOS 10.14.2 (Apple Inc., Cupertino, CA, USA). We used the following Python packages: correlation coefficients, Bland-Altman and Q-Q plots were derived via pingouin 0.2.2 [24]; confidence intervals for the Bland–Altman plots were calculated with pyCompare 1.2.3 (https://github.com/jaketmp/pyCompare); Shapiro–Wilk statistics were calculated with SciPy 1.2.1 [25,26]; linear regression models were estimated using statsmodels 0.9.0 [27]; and box plots and correlation plots were created with seaborn 0.9.0 (https://seaborn.pydata.org) and matplotlib 3.0.2 [28].

3. Results

3.1. Odor Discrimination and Identification

The average test score was 13.3 (SD = 1.5, range: 11–16; $N = 35$) for odor discrimination, and 13.0 (SD = 1.6, range: 11–16; $N = 36$) for odor identification. When summed with the staircase threshold estimates from the Test and Retest sessions, we observed TDI scores of 33.34 (SD = 3.8; range: 26.5–43) and 33.64 (SD = 3.8; range: 26.75–41.75), respectively. Individual as well as cumulative scores indicate a below-average ability to smell (roughly around the 25th percentile) in our sample compared to recent normative data from over 9000 subjects [8].

3.2. Starting Concentrations

The average starting concentration was pen no. 9.9 (SD = 4.2, range: 1–16) for the Test and 9.6 (SD = 4.1, range: 1–16) for the Retest session of the staircase. The average difference in starting concentrations between sessions was 4.9 (SD = 4.0, range: 0–15). In comparison, we used a slightly higher, fixed starting concentration of pen no. 7 for QUEST.

3.3. Test Duration

The average number of trials needed to complete the staircase measurements was 23.6 (SD = 4.8, range: 13–41), which translates to approx. 11.5 min and is 2 minutes longer than for QUEST, which per our parameters always lasted 9.5 minutes (20 trials). Test duration varied slightly between staircase sessions and was 24.4 trials (SD = 4.2, range: 16–34) for the test and 22.9 trials (SD = 5.4, range: 13–41) for the retest session. Please note that the number of trials and the testing duration for the staircase are based on the time required to reach seven reversal points after the starting concentration had been determined, thereby deviating from the "standard" procedure, which treats the starting concentration as the first reversal.

3.4. Test-Retest Reliability

The mean Test thresholds did not differ from the mean Retest thresholds for the staircase ($M_{\text{Test}} = 6.9$, $\text{SD}_{\text{Test}} = 3.1$; $M_{\text{Retest}} = 7.2$, $\text{SD}_{\text{Retest}} = 3.2$; $W = 268.0$, $p = 0.19$). For QUEST, on the other hand, mean test and retest thresholds differed significantly, with slightly higher sensitivity (higher T unit) in the Retest ($M_{\text{Test}} = 5.2$, $\text{SD}_{\text{Test}} = 3.8$; $M_{\text{Retest}} = 6.2$, $\text{SD}_{\text{Retest}} = 3.4$; $W = 201.5$, $p < 0.01$; see Figure 1).

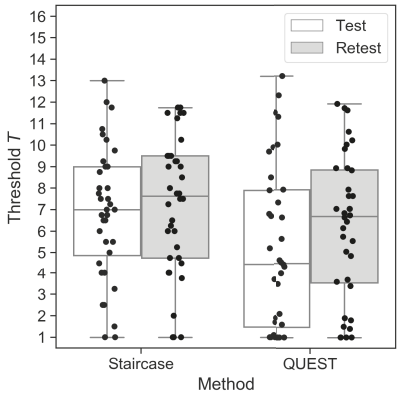

Figure 1. Threshold estimates for the staircase and QUEST procedures during Test and Retest sessions. Each dot represents one participant. Horizontal lines show the median values, and whisker lengths represent 1.5 × inter-quartile range.

The test and retest thresholds correlated significantly for both procedures, with QUEST demonstrating a stronger relationship between measurements than the staircase (staircase: $\rho_{34} = 0.49$, $p < 0.01$; QUEST: $\rho_{34} = 0.66$, $p < 0.001$; Figure 2A).

As already pointed out, correlation gives an indication of the strength of the monotonic relationship between values, but only provides limited information on their agreement. We therefore calculated the repeatability coefficient RC and created Bland–Altman plots to generate a better understanding of the measurement differences. The prediction of the RC is that two measurements (test and retest) will differ by the value of RC or less for 95% of participants. We found that RC was about 16% smaller for QUEST than for the staircase ($RC_{Staircase} = 6.44$, $RC_{QUEST} = 5.43$), suggesting a slightly better agreement between Test and Retest measurements for the QUEST procedure. Accordingly, the Bland–Altman plot (Figure 2B) showed narrower limits of agreement for QUEST (staircase: -6.79 $[-8.89, -5.63]$ and 6.09 $[4.93, 8.18]$; QUEST: -6.42 $[-8.18, -5.44]$ and 4.44 $[3.46, 6.29]$; 95% CIs in brackets). The mean of the differences between measurements was relatively small and deviated less than 1 T unit from zero—the "ideal" difference—for both methods ($M_{\Delta T,Staircase} = -0.35$ $[-1.43, 0.72]$; $M_{\Delta T,QUEST} = -0.99$ $[-1.89, -0.08]$). This systematic negative shift indicates that participants, on average, reached higher T units in the second session than in the first. The differences between Test and Retest measurements for three (staircase) and two participants (QUEST), respectively, fell outside their respective limits of agreement, which corresponds to the expected proportion of 5% of outliers ($3/36 = 8.3\%$; $2/36 = 5.6\%$), demonstrating the appropriateness of the estimated limits. Considering the confidence intervals of the limits of agreement, an equal number of measurement differences (four) fell outside the predicted range for both procedures.

To test whether the time between Test and Retest sessions might be linked to the observed differences between Test and Retest threshold estimates, we computed correlations between those measures. We found no relationship for either method (staircase: $\rho_{34} = -0.12$, $p = 0.50$; QUEST: $\rho_{34} = 0.03$, $p = 0.85$).

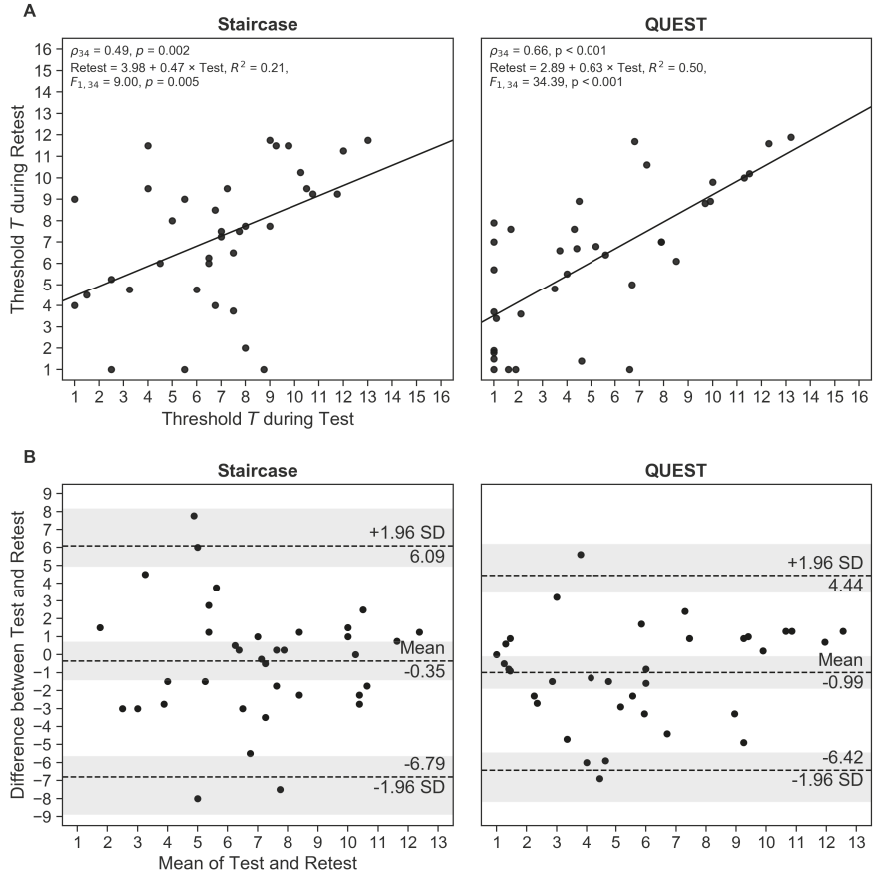

Figure 2. (**A**) Correlation between Test and Retest threshold estimates for the staircase and QUEST procedures. (**B**) Bland–Altman plots showing mean differences between Test and Retest, and limits of agreement corresponding to 95% confidence intervals (CIs) as mean ± 1.96 × SD. The shaded areas represent the 95% CIs of the mean and the limits of agreement. Each dot represents one participant.

3.5. Comparison between Procedures

Although the threshold estimates, averaged across sessions, for the staircase were significantly higher than those for QUEST ($M_{\text{staircase}} = 7.0$, $\text{SD}_{\text{staircase}} = 2.7$; $M_{\text{QUEST}} = 5.7$, $\text{SD}_{\text{QUEST}} = 3.3$; $W = 101.0$, $p < 0.001$; Figure 3A), we found a strong correlation between the procedures ($\rho_{34} = 0.80$, $p < 0.001$; Figure 3B). The regression slope was close to 1, providing an indication of agreement across procedures. The Bland-Altman plot based on the session means (Figure 3C) shows a systematic difference between both procedures; specifically, QUEST thresholds were, on average, 1.38 [0.78, 1.97] T units smaller than the staircase estimates (95% CIs in brackets). The limits of agreement reached from −2.20 [−3.37, −1.56] to 4.95 [4.31, 6.12], meaning the difference between the two procedures will fall into this range for 95% of measurements. Only for 1 participant the observed differences between staircase and QUEST fell outside the limits of agreement ($1/36 = 2.8\%$; when considering the CIs of the limits, 3 participants fell outside the expected range ($3/36 = 8.3\%$)

The corrected limits of agreement, taking into account individual measurements (as opposed to session means only), were −4.20 [−23.6, 15.3] and 6.96 [−12.5, 26.4], which is substantially larger than

the uncorrected limits. The large confidence intervals that expand even beyond the concentration range reflect the relatively large within-participant variability across sessions in both threshold procedures.

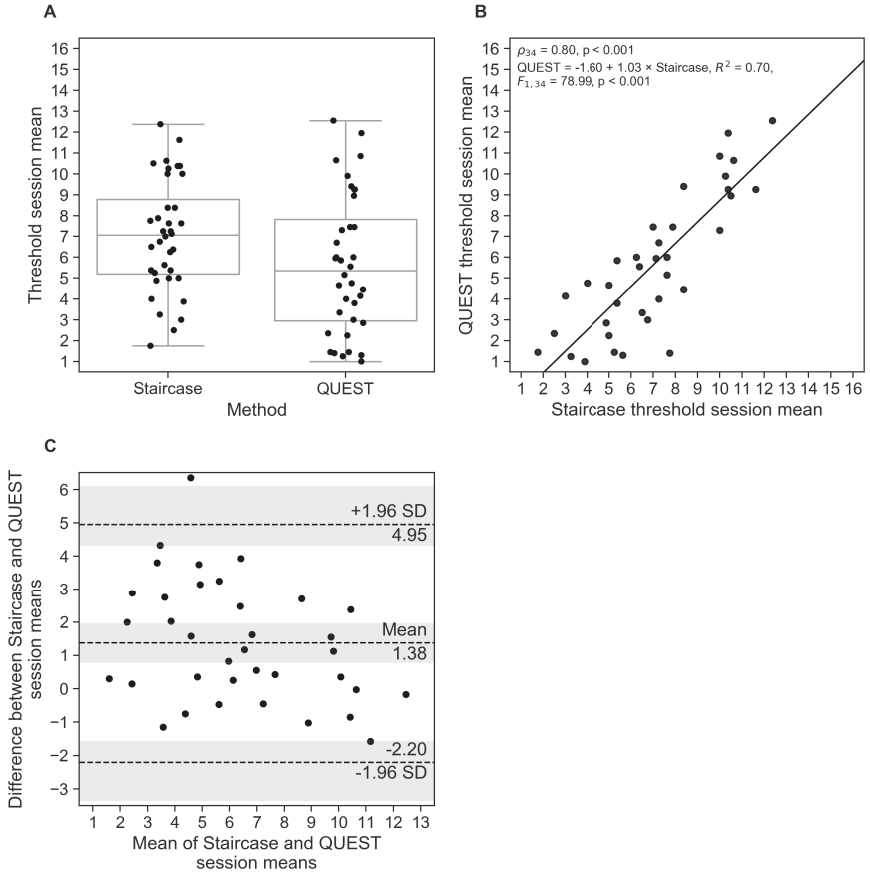

Figure 3. (**A**) Mean threshold estimates, averaged across Test and Retest sessions for the staircase and QUEST procedures. Horizontal lines show the median values, and whisker lengths represent 1.5 × inter-quartile range. (**B**) Correlation between mean staircase and QUEST threshold estimates. (**C**) Bland–Altman plot showing mean differences between session means in both procedures, and limits of agreement corresponding to 95% confidence intervals (CIs) as mean ± 1.96 × SD. The shaded areas represent the 95% CIs of the mean and the limits of agreement. Each dot represents one participant.

4. Discussion

In the presented study we used a QUEST-based algorithm to estimate olfactory detection thresholds for 2-phenylethanol with the aim to provide a reliable test result as it had recently been demonstrated for taste thresholds [13] with reduced testing time. The results were compared to a slightly modified version of the widely-used testing protocol based on a one-up/two-down staircase procedure [6,7,9,15,16].

Test–retest reliability was assessed using multiple approaches. Comparison of Test and Retest thresholds revealed a small yet significant mean difference for QUEST: threshold estimates during retest were higher than in the test, indicating an increase in participants' sensitivity. A similar effect

was reported in a previous study [6]. However, with a mean difference of approx. 1 *T* unit or pen number, the practical relevance of this effect is debatable, even more so when considering the large variability of measurement results within individual participants.

Following common practice of establishing test-retest reliability of olfactory thresholds (see e.g., [6,9,29]), we calculated correlations between Test and retest sessions. The correlation coefficient for QUEST ($\rho = 0.66$) indicated solid, but not exceptionally great test–retest reliability. Reliability of the staircase procedure was only moderate ($\rho = 0.49$) and lower than reported in previous studies for *n*-butanol ($r = 0.61$; [6]) and 2-phenylethanol ($r = 0.92$; [9]) thresholds.

To acknowledge previous criticism of correlation analysis – which focuses on the agreement, but not on the differences between measurements [18–20] – we calculated repeatability coefficients and generated Bland–Altman plots for the analysis of session differences. Repeatability was higher for QUEST than for the staircase; however, measurement results of both procedures varied considerably across sessions for many participants. This inter-session variability is further substantiated by the differences in starting concentrations assessed for the staircase, which varied up 15 pen numbers in the most extreme case. The effect was not universal: some participants performed better in the Test than in the Retest session, whereas for others performance dropped across sessions, and remained almost unchanged in others. Since both sessions had been scheduled within a relatively short time period and all measurements have been performed by the same experimenter, measurement variability can be mostly attributed to variability within participants themselves.

The comparison of the staircase and QUEST procedures via the session means of each participant showed that the staircase yielded slightly higher pen numbers (i.e., lower thresholds) than QUEST. This was expected as the procedures were assumed to converge at approx. 71% and 80% correct responses, respectively. We found a strong correlation between the session means of the procedures ($\rho = 0.80$), and regression analysis showed an almost perfect linear relationship, which some would interpret as a good agreement between QUEST and staircase results. The 95% limits of agreement, taking into account the within-participant variability, showed a large expected deviation between both procedures (range: QUEST thresholds almost 7 *T* units smaller or more than 4 *T* units greater than staircase results), with the corresponding CIs of those boundaries even exceeding the concentration range. This result is indicative of the large variability we found within participants in both procedure. The limits of agreement based on the within-participant session means were much narrower, as variability is greatly reduced through averaging.

A potential source of variability might be guessing. In fact, the probability of responding correctly merely by guessing is ⅓.

In a series of simulations, it could be shown that with an increasing number of trials the frequency of correct guesses might get unacceptably high, potentially leading increased variability in the threshold estimates [30]. The author determined that, for a staircase procedure like the one in our study, the expected proportion of such false-positive responses exceeds 5% with the 23rd trial. For our staircase experiments, the average number of trials was 23.6; and the procedure finished after 23 or more trials for 24 of the 36 participants in the Test, and for 20 participants in the Retest session. Therefore, the large variability between Test and Retest threshold estimates in the staircase could, at least partially, be ascribed to correct guesses "contaminating" the procedure. However, QUEST—which always finished after 20 trials—only had slightly better test-retest reliability according the the repeatability coefficient, suggesting that the largest portion of test-retest variability in our investigations was probably not caused by (too) long trial sequences and related false-positive responses alone.

Surprisingly, a number of participants were unable to correctly identify pen no. 1 at least on one occasion, and this effect was more pronounced during QUEST compared to the staircase. It seems plausible that the variable step size used by QUEST made it possible to approach even the extreme concentration ranges quickly, whereas the staircase requires a longer sequence of incorrect responses to reach pen no. 1.

Despite careful selection of healthy participants who reported no smell impairment, olfactory performance was lower than recently reported in a sample comprising over 9000 participants [8]. This coincidental finding highlights the need for a comprehensive smell screening before enrollment. To what extend olfactory function contributed to the present results and limits their generalizability remains to be explored.

All QUEST runs completed after 20 trials for all participants. The procedure could be further optimized by introducing a dynamic stopping rule. For example, [13] set the algorithm to terminate once the threshold estimate had reached a certain degree of confidence. Such a rule can reduce testing time, as the run may finish in fewer than 20 trials, and should be considered in future studies. Although the reduction or omission of a minimum trial number bears potential to reduce the testing time further, it needs to be shown first that the algorithm performs well under these conditions and, most importantly, large-scale studies need to show whether such a reduced or faster protocol is appropriate to assess odor sensitivity in participants with odor abilities at the extremes (particularly insensitive/sensitive).

Inspection of the data showed that some staircase runs had not fully converged although seven reversal points were reached. In these cases, participants exhibited a somewhat "fluctuating" response behavior (or threshold) that caused the procedure to move in the direction of higher concentrations throughout the experiment (see Figure A1 in the appendix and supplementary data for an example). QUEST proved to behave more consistently, at least in some cases, by either converging to a threshold or by reaching pen no. 1, which would then sometimes not be identified correctly. These interesting differences between procedures require further investigation to fully understand their cause and influence on threshold estimates and, ultimately, diagnostics.

5. Conclusions

The present study compared the reliability of olfactory threshold estimates using two different algorithms: a one-up/two-down staircase and a QUEST-based procedure. The measurement results of both procedures showed considerable overlap. QUEST thresholds were more stable across sessions than the staircase, as indicated by a smaller variability of test-retest differences and a higher correlation between session estimates. QUEST offered a slightly reduced testing time, which may be further minimized through a variable stopping criterion. Yet, QUEST also tended to present the highest concentration, pen no. 1, more quickly than the staircase, which may induce more rapid adaptation and habituation during the procedure and, eventually, produce biased results. Further research is needed to better understand possible advantages and drawbacks of the QUEST procedure compared to the staircase testing protocol.

6. Data and Software Availability

The data analyzed in this paper along with graphical representations of each individual threshold run are available from https://doi.org/10.5281/zenodo.2548620. The authors provide a hosted service for running the presented experiments online at https://sensory-testing.org; the sources of this online implementation can be retrieved from https://github.com/hoechenberger/webtaste.

Author Contributions: conceptualization, R.H. and K.O.; programming, analysis, and visualization, R.H.; interpretation and writing, R.H. and K.O.; supervision and project administration, K.O.

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Appendix A

Example threshold runs of the same participant: while the QUEST runs did converge, the staircase runs obviously did not fully converge although seven reversal points were reached. Intriguingly, the staircase provided more consistent results (more similar thresholds across runs) than QUEST. We speculate that this participant exhibited a fluctuating response behavior during the staircase procedure.

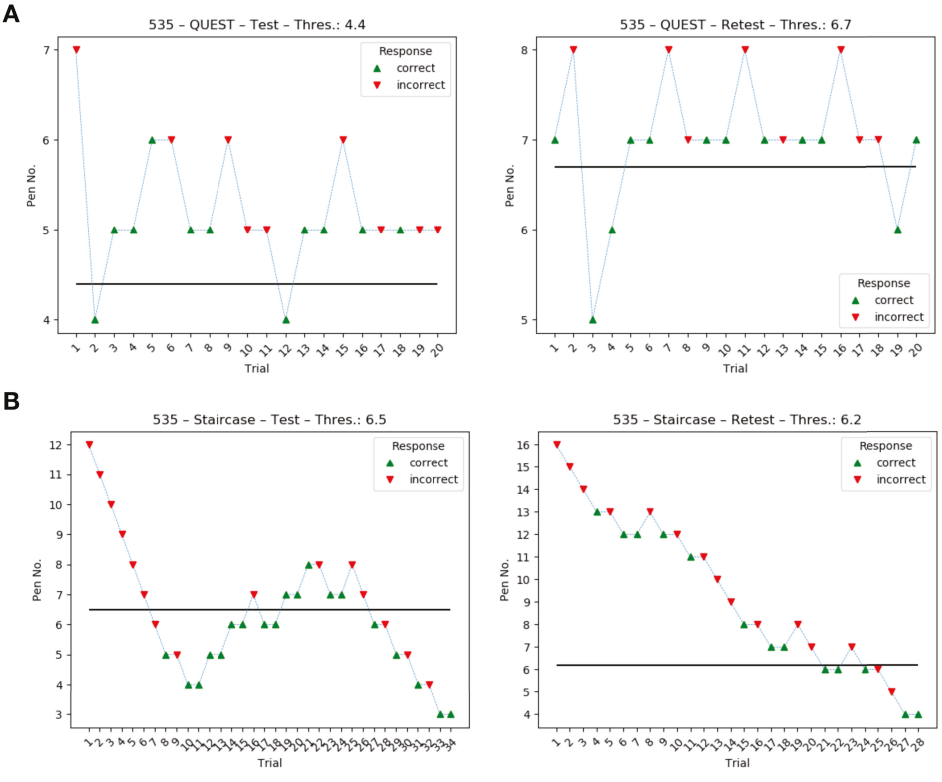

Figure A1. Comparison of threshold estimation runs of the same participant during test and retest sessions for QUEST (**A**) and the staircase (**B**).

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