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Special Issue Reprint

Featured Articles on Nutrition and Obesity Management (2nd Edition)

Edited by
Javier Gómez-Ambrosi

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Guest Editor

Javier Gómez-Ambrosi

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About the Editor

Javier Gómez-Ambrosi

Javier Gómez-Ambrosi is a Researcher at the Metabolic Research Laboratory, Clínica Universidad de Navarra, and an Associate Professor at the School of Medicine, University of Navarra, Pamplona, Spain. His main area of research is obesity and its related morbidities, examined from both the clinical and molecular perspectives. He combines basic research in experimental animals and cells with clinical studies to elucidate the pathophysiological mechanisms responsible for the impact of adiposity on the comorbidity development. He has published more than 220 articles (h-index 61) and has been a PI for more than 20 research projects.

Preface

Obesity remains a global metabolic disease of significant and increasing prevalence, posing a critical public health challenge. Its impact on reducing life expectancy through heightened risks of comorbidities, such as type 2 diabetes, cardiovascular disease, and certain cancers, underscores the continued importance of understanding the underlying pathophysiology of excess adiposity for effective management.

This expanded second edition builds upon this foundation by incorporating new insights gleaned from recent research. It delves into the intricate interplay of genetic, environmental, and behavioral factors contributing to the development of obesity. Furthermore, the volume provides updated reviews of the latest advancements in lifestyle interventions, pharmacological treatments, and the evolving landscape of bariatric surgery.

Reflecting the dynamic nature of the field, this edition also explores several emerging and critical areas. These include the potential of precision medicine approaches to tailor interventions and the growing influence of digital health tools on obesity treatment outcomes. Moreover, this edition features timely research on specific populations, such as adolescents with obesity and type 2 diabetes, and examines disparities in the cardiometabolic impact of adiposity among different ethnic groups. It also covers investigations into novel therapeutic targets and the predictive roles of physiological markers in obesity-related conditions. The inclusion of focused studies on interventions like chewing gum for satiety, smartphone-delivered programs for adolescents, and the outcomes of bariatric surgery in specific patient groups further enriches the scope of this edition.

With contributions from leading international experts, this book aims to serve as an essential resource for specialists in Endocrinology and Nutrition, as well as for a broader range of healthcare professionals and individuals seeking a comprehensive understanding of the latest advancements and diverse perspectives in obesity research and management.

Javier Gómez-Ambrosi
Guest Editor

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Editorial

Recent Progress in the Management of Obesity: Advances and Insights from the Latest Research

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1. Introduction

The field of obesity research continues to evolve rapidly, driven by new insights into metabolic regulation, behavioral interventions, and disparities in treatment outcomes [1–3]. This Special Issue, "Featured Articles on Nutrition and Obesity Management II" follows up on the previous edition by presenting cutting-edge research on obesity and its related comorbidities [4]. The ten articles featured in this Special Issue explore diverse aspects of obesity management, including pharmacological approaches, metabolic and physiological predictors, disparities in health outcomes, surgical interventions, and behavioral modifications. Together, they highlight the complexity of obesity and the necessity of a multifaceted approach to its treatment [5,6].

2. The Growing Burden of Obesity and the Need for Innovative Approaches

Obesity remains one of the most pressing public health challenges worldwide, contributing to an increased risk of metabolic disorders, cardiovascular diseases, and certain types of cancer [7]. Despite significant advancements in our understanding of the pathophysiology of obesity, effective long-term management remains elusive for many individuals [8,9]. The articles in this Special Issue underscore the need for personalized and multidisciplinary strategies, addressing pharmacological, behavioral, and surgical interventions while also considering socio-demographic disparities.

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3. Advances in Pharmacological and Metabolic Interventions

One promising avenue in obesity treatment is pharmacological intervention [10,11]. Cicuéndez et al. (Contribution 1) explore a novel combination therapy using a dopamine receptor 2 agonist and a kappa opioid receptor antagonist, demonstrating synergistic effects in reducing weight by increasing thermogenic activity in rodents with diet-induced obesity. This study highlights the potential of targeting central nervous system pathways to enhance weight loss outcomes, opening doors for future clinical applications. Zhang et al. (Contribution 2) investigate the predictive roles of the basal metabolic rate (BMR) and skeletal muscle mass in lung function among patients with obesity-exacerbated asthma. Their study suggests that increased BMR and skeletal muscle mass may mediate the detrimental effects obesity has on spirometry in patients with asthma. Their findings underscore the importance of metabolic health for respiratory function, suggesting that personalized metabolic assessments could improve the management of obesity-related comorbidities.

4. Early-Life Influences and Long-Term Consequences

The impact of maternal and early-life nutrition on long-term obesity risk is another critical area of research [12,13]. Holt et al. (Contribution 3) examine how maternal high-fat diets and post-weaning high-carbohydrate diets contribute to increased rates of spontaneous hepatocellular carcinoma in aged mouse offspring. Their findings emphasize the need for targeted nutritional interventions during pregnancy and early childhood to mitigate future metabolic disorders. Similarly, Hernandez et al. (Contribution 6) provide valuable insights into how continuous glucose monitoring metrics in early and late pregnancy predict neonatal adiposity, particularly in mothers with obesity (as compared to normal weight counterparts) and despite following macronutrient-controlled eucaloric diets. This study underscores the complex interplay between maternal glucose regulation and fetal development, reinforcing the importance of metabolic monitoring during pregnancy.

5. Disparities in Cardiometabolic Impact and Potential Treatments in Adolescents with Obesity

The marked global increase in the prevalence of obesity in children and adolescents has intensified the research into potential treatments at early ages [14,15]. Understanding the disparities in obesity-related health outcomes is crucial for developing equitable treatment strategies [16,17]. Velasquez-Mieyer et al. (Contribution 4) analyze the cardiometabolic impact of adiposity among African American and Hispanic adolescents, revealing significant differences in metabolic risk factors. Their work highlights the need for culturally tailored interventions to address these ethnoracial disparities and improve health outcomes in different populations. Niechcial et al. (Contribution 9) provide a comprehensive review of current treatment perspectives for adolescents with obesity and type 2 diabetes. Their work synthesizes the latest evidence on pharmacological and behavioral interventions, advocating for an integrative approach to managing obesity in younger populations.

6. Surgical Interventions and Long-Term Outcomes

Bariatric surgery remains a highly effective intervention for severe obesity, with growing evidence on its metabolic benefits beyond weight loss [9,18]. Martínez-Montoro et al. (Contribution 7) investigate weight loss outcomes after sleeve gastrectomy in patients with metabolic dysfunction-associated steatotic liver disease. The authors find that the presence of steatohepatitis may be independently associated with lower weight loss after the surgery. Their study, based on liver biopsy data, provides crucial insights into how preoperative liver health influences post-surgical weight loss trajectories. On the other hand, Ozeki et al. (Contribution 8) further explore the long-term metabolic effects of laparoscopic sleeve gastrectomy in Japanese patients with obesity and diabetes. Their findings demonstrate sustained improvements in body composition and metabolic profiles three years post surgery, reinforcing the procedure's efficacy as a long-term solution for obesity-related metabolic disorders. The reduction in fat mass and the maintenance of skeletal muscle mass are proposed as possible mediators of these long-term effects.

7. Behavioral and Lifestyle Interventions

Behavioral modifications remain a cornerstone of obesity management [19,20]. Parker et al. (Contribution 5) present a pilot randomized controlled trial testing a smartphone-delivered food attention retraining program in adolescent girls with overweight or obesity. Their findings suggest that digital interventions could play a crucial role in reshaping eating behaviors and reducing unhealthy food cravings in young populations. Finally, Jiménez-Ten Hoevel et al. (Contribution 10) explore the effects of chewing gum on satiety, appetite regulation, energy intake, and weight loss in a systematic review. Their analysis

suggests that this simple behavioral strategy may contribute to reduced energy intake and improved weight management, though further research is needed to establish its long-term efficacy.

8. Conclusions

This second edition of "Featured Articles on Nutrition and Obesity Management" provides a comprehensive overview of recent advancements in obesity research. From novel pharmacological approaches and metabolic predictors to disparities in treatment outcomes and surgical interventions, the studies featured in this Special Issue reflect the multifaceted nature of obesity management [1]. As the field continues to evolve, integrating these diverse strategies will be essential in developing effective, personalized treatments that address both the biological and socio-environmental determinants of obesity. Future research should continue to refine these approaches, ensuring that obesity management strategies are inclusive, innovative, and effective for diverse populations [2].

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Systematic Review

Effects of Chewing Gum on Satiety, Appetite Regulation, Energy Intake, and Weight Loss: A Systematic Review

Claudia Jiménez-ten Hoevel [1,2], Elisabet Llauradó [1,2,*], Rosa M. Valls [1,2], Maria Besora-Moreno [1,2], Judit Queral [1,2], Rosa Solà [1,2,3,*] and Anna Pedret [1,2]

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Abstract: Background: New approaches for the management of obesity, a worldwide problem and a major determinant of disability and mortality, are needed. Mastication influences appetite and satiety mechanisms via actual food or sham feeding. However, the effect of mastication of chewing gum, a type of sham feeding, on appetite regulation has not yet been elucidated. Objectives: Our aim was to evaluate the influence of chewing gum on appetite regulation, satiety, energy intake, and weight loss via randomized controlled Trials. Methods: This study was conducted in accordance with the 2020 PRISMA guidelines, and the protocol was registered in PROSPERO (CRD42023432699). Electronic databases MEDLINE®/PubMed, Scopus, and Cochrane Central Register of Controlled Trials were searched from July 2023 to September 2024. The quality of each included study was assessed using the Cochrane risk of bias tool, RoB 2. Results: A total of eight articles with nine RCTs were included in this systematic review. Seven out of nine RCTs evaluated appetite regulation. Five out of seven RCTs reported a significant suppressing effect of hunger, three out of five RCTs reported a significant reduction in desire to eat, and three out of four reported a significant reduction in the desire to eat a sweet snack, all of them compared to the control group. However, the effects on satiety, energy intake, and weight loss are not conclusive. Conclusions: Chewing gum could be a promising non-pharmacological tool for obesity management through appetite regulation; however, further research, with sustained RCTs evaluating the sustained effects of gum chewing on appetite and weight management, is needed.

Keywords: chewing gum; appetite regulation; satiety; energy intake; weight loss; obesity

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1. Introduction

Obesity is a worldwide problem and a leading cause of cardiovascular diseases and diabetes [1]. Since 1990, worldwide adult obesity has doubled. In 2022, 43% of adults aged 18 years and over lived with overweight and 16% lived with obesity [2].

Overweight and obesity are defined as abnormal or excessive fat accumulation that may impair health [2]. The characteristics of our current society facilitate obesity by overconsumption, such as the increase in ultraprocessed food consumption, the limited availability of healthy sustainable food at affordable prices, and a lack of safe and easy mobility into daily life [2].

Although people with obesity and without obesity do not differ in the frequency of eating, people with obesity consume a greater number of calories than people without obesity [3,4]. This suggests that satiety and appetite regulation may influence meal size, being an important contributor to energy overconsumption and obesity. Moreover, the orosensory stimulation provided by food contributes to the appetite and compensatory dietary responses. The hardness of food, mastication, and time of chewing contribute to satiation and satiety signals [5]. Thus, a greater bite size and increased speed of eating decrease satiation and are considered risk factors for obesity [6,7]. Satiation is described as the process leading to meal termination; it controls meal size and is influenced by several feedback mechanisms such as declining food preference and gastric fullness [8]. However, satiety is defined as the inhibition of further eating as well as the suppression of the feeling of hunger and occurs as a consequence of having eaten [8]. Satiety is influenced by a number of pre- and post-absorptive feedback mechanisms such as macronutrient composition, energy density, physical structure and the sensory qualities of food, and the gastrointestinal peptides that are released following food consumption (CCK, GLP-1, and ghrelin) [4,8].

Additionally, appetite is considered as the sensation that motivates intake and can be present even in the absence of a physiological need [4]. Hunger and the desire to eat represent approach behaviors indicative of appetite or readiness to eat [9].

In recent years, the importance of chewing has been implicated as a strategy for preventing overeating, since reduced masticatory function is associated with obesity [10,11], and a positive correlation has been established between increased speed of eating, higher body mass index (BMI), and higher energy intake [12,13]. Chewing stimulation could reduce subjective appetite and influence the metabolic appetite regulation system [14,15] and, consequently, prevent weight gain. Correspondingly, previous systematic reviews and meta-analyses concluded that increasing masticatory activity reduces food intake and subjective appetite and increases satiety [16–18].

Chewing could be stimulated by actual feeding or by sham feeding. Sham feeding is described as to view, smell, taste, and chew food without ingesting it, promoting gastrointestinal peristalsis and an increase in the salivatory rate [19]. Chewing gum is considered as a type of sham feeding. Actual feeding was compared to sham feeding, and similar results were obtained showing that hunger and preoccupation with food were significantly reduced and fullness significantly increased after feeding or sham feeding [20]. This suggests that chewing stimulation itself, with or without food ingestion, might reduce appetite. Mattes et al. concluded that gum mastication increased resting energy expenditure when compared to no gum chewing, suggesting that chewing without ingestion of food would have an impact on total energy intake and energy expenditure [5].

Chewing gum is very widespread and accepted amongst all populations [21]. In a survey conducted in 2021 in a Spanish population, 14.2% consumed sugars and sweets daily, including chewing gum [22]. Chewing sugar-free gum would increase satiety and reduce subjective appetite without adding extra energy. Furthermore, it could help suppress cravings for high-energy snacks or meals and be considered a promising tool for managing obesity.

Previous systematic reviews evaluated the effects of actual food and sham feeding on satiety, appetite regulation, and energy intake, but not exclusively with chewing gum [16–18]. To the best of our current knowledge, no systematic review has explicitly assessed the influence of chewing gum on satiety, appetite regulation, and energy intake. Furthermore, there has not been an update on the subject, and the existing systematic reviews date back more than five years.

Thus, it was hypothesized that chewing gum affects appetite regulation. Accordingly, the present study aimed to evaluate the effectiveness of chewing gum on satiety, appetite

regulation, energy intake, and, consequently, weight loss determined via randomized control trials (RCTs) through a systematic review.

2. Materials and Methods

This systematic review included RCTs that evaluated the effect of chewing gum on satiety, appetite regulation, energy intake, and/or weight loss in adults. This study is reported in accordance with the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [23], and the protocol was registered in PROSPERO International Prospective Register of Systematic Reviews (CRD42023432699).

2.1. Literature Research

The following electronic databases were searched for eligible studies: MEDLINE®/PubMed, Scopus, and Cochrane Central Register of Controlled Trials. The keywords used were chewing gum, mastication, satiety, satiety regulation, appetite, appetite regulation, hunger, body weight, BMI, weight loss, waist circumference (WC), obesity, energy intake, and caloric intake. The search strategy is described in Supplementary Table S1.

2.2. Eligibility Criteria and Study Selection

The inclusion criteria were as follows: (a) RCTs; (b) limited to the English language and human studies; (c) published from 2000 to 2024; (d) adults aged $\geq$ 18 years from the worldwide general population, healthy or with some comorbidities; (e) assessed only one, two, or all of the outcomes; and (f) mastication of a low-calorie chewing gum, for all timings, frequencies, and doses.

In contrast, the exclusion criteria were as follows: (a) children and adolescents (<18 years); (b) older adults (>65 years); (c) interventions that did not meet one or more of our inclusion criteria; (d) exposure to chewing but not chewing gum; (e) mastication of other low-calorie food; (f) studies that only included individuals with a specific health condition; (g) studies that reported no relevant data on the mentioned outcomes; (h) systematic reviews and/or meta-analyses; (i) case–control studies; (j) cohort studies; (k) protocols; (l) grey literature; (m) correspondence letters; (n) government statistics summaries; (o) book chapters; (p) dissertations; and (q) conference summaries.

The Population, Intervention, Comparison, Outcomes and Study (PICOS) criteria (Table 1) were used to define the inclusion and exclusion criteria of the RCT studies, and the search was conducted from July 2023 to September 2024.

Table 1. PICOS criteria for eligibility of RCTs.

Criteria	Inclusion	Exclusion
Population	- Adults over 18 years old - All sexes and races - Healthy adults or with some comorbidities	- Children and adolescents (<18 years old) - Old adults (>65 years old) - Individuals with a specific health condition
Intervention	- Interventions that analyzed the effect of chewing gum on satiety, appetite regulation, energy intake, and/or weight loss - Any low-calorie chewing gum type - Sustained, postprandial, or short-term interventions	- Studies not involving chewing gum interventions - Studies assessing the effect of chewing gum on other outcomes such as concentration and alertness - Studies involving medicated chewing gum interventions

Table 1. *Cont.*

Criteria	Inclusion	Exclusion
Comparison	- No use of chewing gum or avoiding consumption	- Consumption of low-calorie food - Sham feeding
Outcomes	- Changes in satiety and/or appetite regulation - Changes in energy intake or caloric intake - Weight loss or changes in body weight	- Articles that report no relevant data of the mentioned outcomes
Study type	- Randomized clinical trials (RCTs) involving controlled interventions - Parallel or crossover design	- Systematic reviews and/or meta-analyses - Case–control studies - Cohort studies - Protocols

RCTs: Randomized controlled trials.

2.3. Data Extraction

We selected the included studies using Covidence systematic review software, Veritas Health Innovation, Melbourne, Australia (available at www.covidence.org, accessed on 14 July 2023).

Published studies were selected in the first stage based on a title and abstract screening according to the inclusion and exclusion criteria. In the second stage, full-text articles that had passed the first stage of screening were assessed. Finally, in the third stage, only RCTs that met all the criteria described previously were included for data extraction and quality assessment.

All titles and abstracts were screened for inclusion by two independent researchers (C.J.-t.H. and A.P.). After this first screening, the full text of the studies was read and assessed according to the predefined inclusion criteria by one independent researchers (C.J.-t.H.). In case of discrepancy, a second author helped to extract the article's data (A.P.). Discrepancies were resolved after discussion with a third researcher (E.L.). When any necessary information for inclusion was missing from any study, we contacted the authors to request it.

The variables collected from the included studies were (a) authors; (b) year of publication; (c) country; (d) study design; (e) sample size; (f) age of participants; (g) sex of participants; (h) health state of participants (with or without obesity); (i) aim of the intervention; (j) type of intervention and setting; (k) duration of the intervention and characteristics (time of mastication, frequency a day, etc.); (l) tools used for assessing main and additional outcomes; (m) changes in outcomes.

2.4. Outcomes

The main outcomes included were (1) subjective ratings of hunger and desire to eat as markers of appetite regulation, (2) subjective ratings of fullness as markers of satiety, (3) objective measures of energy intake following food intake, and (4) objective measures of body weight as a marker of weight loss.

The secondary outcomes included were (1) subjective ratings of preoccupation with food as a marker of appetite regulation, (2) objective measures of BMI, and (3) objective measures of WC as secondary markers of weight loss.

2.5. Quality Assessment

The quality of each included study was assessed using the Cochrane risk of bias tool, RoB 2 [24]. This quality tool assesses the risk of bias in 5 domains; the risk of bias classification was (a) low risk of bias; (b) some concerns; (c) high risk of bias. Two authors evaluated the risk of bias of each RCT (C.J.-t.H. and A.P.), and any disagreement

between these authors regarding the risk of bias was resolved through discussion with the other authors.

3. Results

A total of 2829 articles were identified through the databases, 2330 were excluded due to duplication, 499 articles were screened, and 482 were excluded for not meeting the inclusion criteria. The remaining 17 articles were full-text-assessed for eligibility, and 10 were excluded for the following reasons: different study outcomes (n = 5), no results posted (n = 2), study design not accepted according to the eligibility criteria (n = 1), and not available online (n = 1). Finally, eight articles, describing nine RCTs (as one article reported two RCTs), were included in this systematic review [25–32], as shown in Figure 1. In Supplementary Table S2 information regarding the excluded studies is shown.

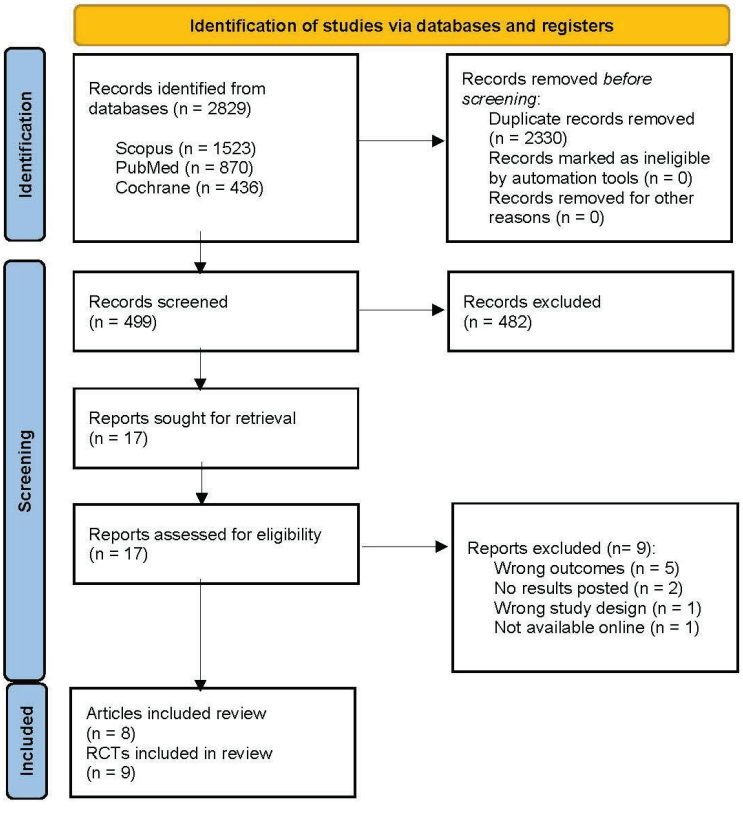

Figure 1. PRISMA flow diagram of the study selection procedure.

3.1. The Characteristics of the Included Studies

Out of the nine RCTs, eight were RCT crossovers with no double blinding [25–29,31,32], and one study was a parallel, double-blind, controlled study [30].

In all the included studies, the study population included both sexes, and the subjects' age was 18–50 years old. The sample size of the studies ranged from 33 to 201, and the duration of the intervention ranged from three days to eight weeks.

The studies were carried out in Europe [26,32] and America [25,27–31]. Out of the nine RCTs, seven evaluated the effect of chewing gum on appetite regulation [25–29,31,32], six RCTs evaluated the effect of chewing gum on satiety [25–29,32], seven RCTs evaluated

the effect of chewing gum on energy intake [25–29,31], and one RCT evaluated the effect of chewing gum on weight loss [30], as shown in Table 2.

Table 2. Characteristics of the RCTs included in this systematic review addressing the effect of chewing gum on appetite regulation, satiety, energy intake, and weight loss in adults.

Author	Participants		Intervention	Results			
	N	BMI	Design	Effect on Appetite Regulation	Effect on Satiety	Effect on Energy Intake	Effect on Weight Loss
Hetherington and Boyland (2007) [26]	60	NOB or OW	Fixed lunch on arrival. Gum chewed 1 h, 2 h, and 3 h after lunch. Access to ad libitum snacks 3 h after lunch. Intake recorded at home by diet records; within subjects.	Hunger * and desire to eat sweet snacks * ↓ in the chewing gum condition. No effect on desire to eat salty snacks.	Fullness * ↑ in the chewing gum condition.	Energy intake * ↓ in the chewing gum condition.	NA
Julis and Mattes (2007) [27]	47	OW	Standard breakfast and lunch. Gum chewed at a fixed time or before meal for 20 min before food consumption. Intake recorded at home by diet records; within subjects.	Hunger $^{\alpha}$ ↑ after gum chewing, but desire to eat $^{\alpha}$ and desire to eat sweet snacks ↓ in the chewing gum condition. No effect on salty snacks, fatty snacks, or preoccupation with food.	Fullness $^{\alpha}$ ↓ after gum chewing.	Energy intake ↓ in the chewing gum condition.	NA
Hetherington and Regan (2011) [32]	60	NOB or OB	Fixed lunch on arrival. Gum chewed 1 h, 2 h, and 3 h after lunch. Access to ad libitum snacks 3 h after lunch. Intake recorded at home by diet records; within subjects.	Hunger **, desire to eat **, desire to eat sweet snacks **, desire to eat salty snacks ** and desire to eat a snack ** ↓ in the chewing gum condition.	Fullness * ↑ in the chewing gum condition.	NA	NA
Shikany J et al. (2012) [30]	201	OB	Eight-week intervention with chewing gum and printed nutrition information. Participants chewed gum six times a day for a total of 90 min/day (20 min after breakfast, lunch, and dinner and chewed an additional 10 min mid-morning, mid-afternoon, and 1–2 h after dinner).	NA	NA	NA	No effect on weight loss.
Swoboda and Temple (study 1) (2013) [31]	44	OW	Gum chewed for 10 min upon arrival at the laboratory. Reinforcement game to earn points in order to earn food (lower or high energy density). Intake recorded at home with diet records; within subjects.	Hunger $^{\beta}$ was ↓ in the chewing gum condition.	NA	Energy intake of healthy food * ↓ in the chewing gum condition but no effect on total daily energy intake.	NA
Swoboda and Temple(study 2) (2013) [31]	54	OW	Gum chewed every single occasion before food for a week. Intake recorded at home using diet records; within subjects.	NA	NA	Energy intake per meal * ↓ in the chewing gum condition but no effect on total daily energy intake.	NA
Park et al.(2016) [29]	50	NOB and OB	Fixed lunch on arrival. Gum chewed 1 h, 2 h, and 3 h after lunch. Access to ad libitum snacks 3 h after lunch. Intake recorded at home using diet records; within subjects.	Hunger * and desire to eat * ↓ in the chewing gum condition.	Fullness ↑ in the chewing gum condition.	Energy intake from snacks ↓ in the chewing gum condition. Carbohydrate intake * was ↓ in the chewing gum condition.	NA
Melanson and Kresge (2017) [28]	33	NOB or OW	Fixed breakfast on arrival. Gum chewed for 20 min, 10 min into ventilated hood indirect calorimetry, and 3 h after breakfast. Access to ad libitum snacks 3 h after breakfast. Intake recorded at home using diet records; within subjects.	Hunger * ↓ in the chewing gum condition only after first chewing period. No effect on desire to eat, desire to eat something sweet, or desire to eat something salty.	Fullness ↑ in the chewing gum condition.	Energy intake * ↓ in the chewing gum condition.	NA
Bobillo et al. (2018) [25]	57	NOB or OW	Fixed lunch on arrival. Gum chewed 1 h, 2 h, 3 h, and 4 h after lunch. Access to ad libitum snacks 4 h after lunch. Intake recorded at home in diet records; within subjects.	Hunger * ↓ in the chewing gum condition.	Fullness * ↑ in the chewing gum condition.	Total energy intake from snacks * ↓ for active gum when compared to no gum. No effect of placebo gum.	NA

NOB = participants without obesity; OW = participants with overweight; OB = participants with obesity; NA = not addressed; ↑ = increase; ↓ = decrease; * $p < 0.05$ comparing gum to no gum chewing; $^{\alpha}$ $p < 0.05$ comparing before and after gum chewing; ** $p < 0.001$ comparing gum to no gum chewing; $^{\beta}$ $p < 0.001$ comparing before and after gum chewing.

The characteristics and duration of the RCTs are very heterogeneous. The common traits among studies are that almost all, except one that was a parallel-controlled trial [30], were crossover studies, most described specific test days when volunteers attended the laboratory or medical center to complete the intervention, and participants received either a fixed meal before chewing gum or a snack 3 h after lunch.

The mastication of chewing gum oscillated between 10 and 20 min, from one to eight times a day, depending on the study protocol, and, in the no gum condition (control group), participants rested or stayed seated for the same amount of time. One RCT did not specify duration of chewing gum mastication [31].

In Supplementary Table S3, detailed information regarding the RCTs is shown.

The results of the RCTs are described as follows: Section 3.2. outlines chewing gum and appetite regulation (Section 3.2.1. relates to hunger; Section 3.2.2. describes desire to eat; Section 3.2.3. describes preoccupation with food); Section 3.3. relates to chewing gum and satiety; Section 3.4. relates to chewing gum and energy intake; Section 3.5. outlines chewing gum and weight loss.

3.2. Chewing Gum and Appetite Regulation

Appetite regulation was measured using hunger, desire to eat, desire to eat a sweet snack, desire to eat a salty snack, desire to eat a fatty snack, and preoccupation with food.

Out of the nine RCTs, seven studies evaluated the effect of chewing gum on hunger [25–29,31,32], five on the desire to eat [26–29,32], four on the desire to eat a sweet snack [26–28,32], four on the desire to eat a salty snack [26–28,32], and one on the desire to eat a fatty snack and preoccupation with food [27].

3.2.1. Hunger

Of the seven RCTs, five RCTs reported that chewing gum had a significant suppressing effect on hunger compared to the control group [25,26,29,31,32], one RCT showed no statistical significance reduction [28], and one RCT described a stronger hunger sensation after gum chewing compared to the no gum chewing group [27].

In the study of Hetherington et al., participants ate a fixed lunch on arrival at the laboratory and chewed gum for 15 min at 1 h, 2 h, and 3 h after lunch, whereas under the no-gum conditions, participants rested for 15 min. After they chewed their last chewing gum, participants rated their hunger and had access to ad libitum snacks. The overall mean hunger ratings were significantly lower after gum chewing compared to after no gum chewing (hunger comparing gum condition to no gum: $F(1,59) = 5.313$, $p = 0.025$). The significant interaction between condition and time indicated that hunger ratings increased to a lesser extent after gum chewing compared to after not chewing gum ($F(3,177) = 2.872$, $p = <0.005$) [26].

In another study conducted by Hetherington et al., participants ate a fixed lunch on arrival at the laboratory and chewed gum for 15 min at 1 h, 2 h, 3 h after lunch and before snack intake; in the no gum condition, participants rested for the same time; hunger was rated after every gum chewing. Overall, hunger was observed to be lower in the gum condition compared to the no gum condition (hunger comparing gum condition to no gum: $F(1,59) = 28.8$; $p < 0.001$). The significant interaction between condition and time indicated that hunger ratings increased to a lesser extent after lunch to before the snack after gum chewing compared to after not chewing gum ($F(3,177) = 10.09$, $p = < 0.001$) [32].

In Swoboda et al., participants chewed gum for 10 min upon arrival to the laboratory and afterward played a reinforcement game to earn points to be exchanged for lower- or high-density snacks. Before eating the earned food, participants rated their hunger, and then they could eat as much or as less of the food that they earned. Hunger was significantly

lower in the gum conditions, with no difference between fruit or mint gum, compared to the no gum conditions (hunger after chewing mint gum: F (3,129) = 5.9; $p = 0.001$; hunger after chewing fruit gum: F (3,129) = 5.5; $p = 0.001$; comparison of hunger between mint and fruit gum: F (3,129) = 0.49; $p = 0.68$) [31].

Additionally, in Park et al., participants ate a fixed lunch upon arrival at the laboratory and chewed gum for 15 min at 1 h, 2 h and 3 h after lunch. After they chewed their last chewing gum, participants rated their hunger and had access to ad libitum snacks. Hunger was significantly lower in the gum conditions compared to the no gum conditions (hunger comparison between groups: $p = 0.006$) [29].

Furthermore, in Bobillo et al., participants ate a fixed lunch upon arrival at the laboratory and chewed gum (either active gum or placebo gum) for at least 15 min every hour starting 45 min after breakfast and 1 h, 2 h, 3 h, and 4 h after lunch, for a total of eight pieces of gum. In the no gum condition, participants rested for at least 15 min every hour, instead of chewing gum. Hunger was rated before and after lunch every 30 min up to 4 h after lunch. A significant decrease in hunger was observed in the active and placebo gum conditions compared to the no gum condition, without differences between active and placebo gum, pre- and post-lunch (pre-meal ± SE mean difference between active gum versus no gum: -7.89 ± 2.96; $p = 0.01$; pre-meal mean ± SE difference between placebo gum versus no gum: -10.55 ± 2.96; $p = 0.004$; pre-meal mean ± SE difference between active gum and placebo gum: $p = 0.41$; post-lunch mean ± SE difference active gum versus no gum: -5.32 ± 2.25; $p = 0.02$; post-lunch mean ± SE difference placebo gum versus no gum: -5.83 ± 1.95; $p = 0.004$; post-lunch mean ± SE difference between active gum and placebo gum: $p > 0.05$) [25].

In Melanson et al., participants underwent a 45 min measurement of resting metabolic rate (RMR) using ventilated hood indirect calorimetry. Ten minutes into the RMR measurement, in the gum condition, participants chewed gum for 20 min; in the no gum condition, the passing and collection of gum were simulated. After the 45 min of RMR, participants received a standardized breakfast. The hood of the indirect calorimeter remained in place throughout the 3 h postprandial measurement, except for brief time periods when subjects rated their appetite as well as delivered and collected gum, or during which these processes were simulated in the no gum condition. In the gum condition, volunteers chewed gum two additional times for 20 min each time (60–80 min and 150–170 min after eating), for a total 60 min of chewing time during the morning of testing. At the end of the 3 h postprandial measurement period, subjects completed a final rating of appetite and were then offered an ad libitum pasta lunch with water. A significant reduction in hunger was observed after the first chewing period (hunger before first chewing period comparison between groups: t = 1.37; $p = 0.18$; hunger after first chewing period comparison between groups: t = 2.66; $p = 0.01$), but no significant changes were observed at 90 min and 180 min after eating when comparing the gum condition to the no gum condition (hunger 90 min postprandial comparison between groups: t = 0.05; $p = 0.96$; hunger 180 min postprandial comparison between groups: t = 0.78; $p = 0.44$) [28].

In Julis et al. on arrival at the laboratory, participants either chewed gum for 20 min after a 2 h fixed-time meal or after consuming a calorie-containing food or drink after lunch. Ratings of hunger were completed on arrival at the laboratory, after lunch, every 30 min after leaving the laboratory until the next eating occasion, after gum chewing, and after the intake of food and caloric beverages. The mean post-lunch hunger was higher in the gum condition compared to the no gum condition (mean ± SE post-lunch hunger for fixed-time gum treatment: 27 ± 2, $p > 0.05$; mean ± SE post-lunch hunger with pre-meal gum treatment: 29 ± 1, $p > 0.05$; mean ± SE post-lunch hunger, no gum 25 ± 2, $p > 0.05$), and hunger significantly increased after chewing gum (mean ± SE hunger fixed-time treatment

before gum: 31 ± 2, $p < 0.05$; after gum: 32 ± 3, $p < 0.05$; mean ± SE hunger pre-meal before gum: 45 ± 3, $p < 0.05$; after gum: 48 ± 3, $p < 0.05$) [27].

3.2.2. Desire to Eat

The desire to eat information included the desire to eat a sweet snack or a salty snack or a fatty snack.

Three out of the five RCTs that evaluated the desire to eat and chewing gum described a significant reduction in the desire to eat after chewing gum compared to not chewing gum [27,29,32]. One RCT described a positive correlation between the desire to eat, energy intake, and the gum condition [26], and one RCT described no effect on the desire to eat [28].

Hetherington et al. observed a significant decrease in the desire to eat after gum chewing compared to not chewing gum (desire to eat comparing gum condition to no gum condition ($F(1,59) = 21.3$, $p < 0.001$). A significant interaction between the condition and time indicated that the desire-to-eat ratings increased to a lesser extent after gum chewing compared to after not chewing gum ($F(3,177) = 7.259$, $p < 0.001$) [32].

Furthermore, Julis et al. described a reduction of desire to eat was seen in the fixed time gum treatment after the gum, however in the pre-meal treatment a significant increase in desire to eat after chewing gum was observed (mean ± SE desire to eat fixed time gum treatment before gum chewing: 31 ± 3 mm, $p < 0.05$; after gum chewing: 27 ± 3 mm, $p < 0.05$; mean ± SE desire to eat pre-meal gum treatment before gum chewing: 43 ± 3 mm, $p < 0.05$; after gum chewing: 46 ± 3 mm, $p < 0.05$) [27].

Additionally, Park et al. described a significant decrease in desire to eat was observed after chewing gum compared to no gum (desire to eat comparison in between groups: $p = 0.002$) [29].

Three out of the four RCTs that evaluated the desire to eat a sweet snack described a significant reduction after gum chewing compared to no gum chewing [26,27,32] and one described no effect on the desire to eat a sweet snack [28]. Hetherington et al. described that there was a significant reduction in desire to eat a sweet snack after gum chewing compared to no gum chewing (desire to eat a sweet snack comparing gum condition to no gum condition: $F(3,177) = 4.530$; $p = 0.004$) [26].

In another study conducted by Hetherington et al., a reduction of the desire to eat something sweet was observed after gum chewing compared to no gum (desire to eat something sweet in gum condition compared to no gum condition: $F(1,59) = 22.5$; $p < 0.001$) [32]. A significant interaction between condition and time indicated that ratings on the desire to eat a sweet snack increased to a lesser extent after gum chewing compared to no gum chewing: ($F(3,177) = 2.7$, $p = 0.048$).

Julis et al. observed a non-significant reduction in the desire to eat something sweet in the fixed-time gum treatment and pre-meal gum treatment comparing before and after gum chewing (mean ± SE desire to eat something sweet, fixed-time gum treatment before gum: 22 ± 3, $p < 0.05$; after gum: 21 ± 3, $p > 0.05$; mean ± SE desire to eat something sweet, pre-meal gum treatment before gum: 31 ± 3, $p < 0.05$; after gum: 29 ± 3, $p > 0.05$) [27].

Four RCTs evaluated the effect of chewing gum on the desire to eat a salty snack [26–28,32]; only one showed a significant reduction in the desire to eat a salty snack after chewing gum chewing compared to no gum chewing (desire to eat something salty in gum condition compared to no gum condition: $F(1,59) = 20.6$; $p < 0.001$) [32]. Additionally, a significant interaction between condition and time indicated that the ratings of the desire to eat a salty snack increased to a lesser extent after gum chewing compared to no gum chewing ($F(3,177) = 2.9$, $p = 0.036$) [32].

Only one RCT evaluated the effect of chewing gum on the desire to eat a fatty snack, and no effect was found [27].

3.2.3. Preoccupation with Food

Only one RCT evaluated the effect of chewing gum on the preoccupation with food, and the results showed a significantly lower preoccupation with food after gum chewing in the fixed-time gum treatment and pre-meal gum treatment (mean ± SE preoccupation with food, fixed-time gum treatment, before gum: 25 ± 3, $p > 0.05$; after gum: 23 ± 3, $p < 0.05$; mean ± SE preoccupation with food, pre-meal gum treatment, before gum: 32 ± 3, $p > 0.05$; after gum: 36 ± 4, $p < 0.05$) [27].

3.3. Chewing Gum and Satiety

Six RCTs evaluated the effect of chewing gum on satiety measured as fullness [25–29,32]. Three studies found a significant increase in fullness in the chewing gum condition compared to the control group [25,26,32], two RCTs found a non-significant increase in fullness compared to no gum [28,29], and one RCT found a significant decrease in fullness after gum chewing [27].

Hetherington et al. reported a significant increase in fullness in the gum condition compared to the no gum condition (fullness comparing gum to no gum condition: $F(1,59) = 4.545$, $p = 0.04$) [26].

In another study conducted by Hetherington et al., fullness was significantly higher after gum chewing compared to no gum chewing (fullness in gum condition compared to no gum condition: $F(1,59) = 5.18$; $p = 0.026$). Furthermore, a significant interaction between condition and time indicated that the ratings of fullness decreased to a lesser extent after gum chewing compared to no gum chewing: $F(3,177) = 3.96$, $p = 0.009$ [32].

Lastly, Bobillo et al. described a significant increase in fullness after chewing either active and placebo gum when compared to no gum chewing before a meal (mean ± SE difference between active gum versus no gum: 7.89 ± 3.22 mm; $p = 0.018$; mean ± SE difference between placebo gum versus no gum: 5.80 ± 2.98 mm; $p = 0.057$) and after lunch (mean ± SE difference active gum versus no gum: 5.54 ± 2.50 mm; $p = 0.03$; mean ± SE difference placebo gum versus no gum: 6.12 ± 2.23 mm; $p = 0.008$; active gum versus placebo gum: $p > 0.05$) [25].

However, Julis et al. found a significantly lower sensation of fullness in the gum condition between before and after gum chewing (mean ± SE fullness fixed-time gum treatment before gum: 51 ± 3 mm, $p < 0.05$; after gum 47 ± 4 mm, $p < 0.05$; mean ± SE fullness pre-meal gum treatment before gum: 37 ± 3 mm, $p < 0.05$; after gum: 34 ± 2 mm, $p < 0.05$), but no information on the control group was given [27].

3.4. Chewing Gum and Energy Intake

Seven out of the nine included RCTs evaluated the effect of chewing gum on energy intake [25–29,31]. Only two RCTs reached significance in the reduction in energy intake in the gum condition compared to the control group [26,32].

Hetherington et al. described a reduction in energy intake in the gum condition compared to the no gum condition (energy intake comparing gum to no gum condition: $F(1,58) = 4.344$, $p = 0.04$) [26].

Similar findings were described by Melanson et al., where energy intake was lower in the gum condition compared to the no gum condition (energy intake comparison between groups: $t = 3.130$; $p = 0.004$) [28].

Four studies did not find any significant effect on energy intake. Swoboda et al. described a significant reduction in the energy intake of healthy food but not for total daily energy intake in the gum condition when compared to the no gum condition (interaction between gum chewing and intake of healthy food: $F(2,86) = 5.8$; $p = 0.004$) [31].

Furthermore, in a second RCT conducted by Swoboda et al., a significant reduction in energy intake per meal was observed (chewing gum and energy intake per meal:

F (2,106) = 10.3; $p < 0.0001$), but no significance changes in the total daily energy intake were observed in the gum condition compared to the no gum condition [31].

Additionally, Park et al. described a significant reduction in carbohydrate intake in the gum condition compared to no gum but a non-significant reduction in energy intake from snacks (carbohydrate intake; total snack intake comparison between groups: $p = 0.08$) [29].

Lastly, Bobillo et al. described a significant reduction in the total energy intake from snacks for the active gum when compared to no gum, but no effects were found for the placebo gum when compared to no gum (total energy intake from snacks comparing active gum and no gum: $p < 0.001$; total energy intake from snacks comparing placebo gum and no gum: $p > 0.05$) [25].

3.5. Chewing Gum and Weight Loss

Only Shikany et al. assessed the effect of chewing gum on weight loss and BMI, finding no effects of the chewing of gum on weight loss or BMI compared to no gum chewing. A significant intertreatment reduction in WC was observed in the gum condition, although no significant reduction was observed when compared to the no gum condition (mean ± SD change in WC in the gum condition: −1.7 ± 5.7 cm; $p < 0.05$; mean ± SD change in WC in the no gum condition: −0.7 ± 5.5 cm; $p > 0.05$; comparison between groups: $p = 0.27$) [30].

3.6. Quality of the RCTs Included in This Systematic Review

According to the Cochrane risk of bias tool RoB2 [24], of the nine RCTs included, one was classified as having a low risk of bias [30] and eight as having some concerns in domain 1 (randomization process) [25–29,31,32] (Figure 2).

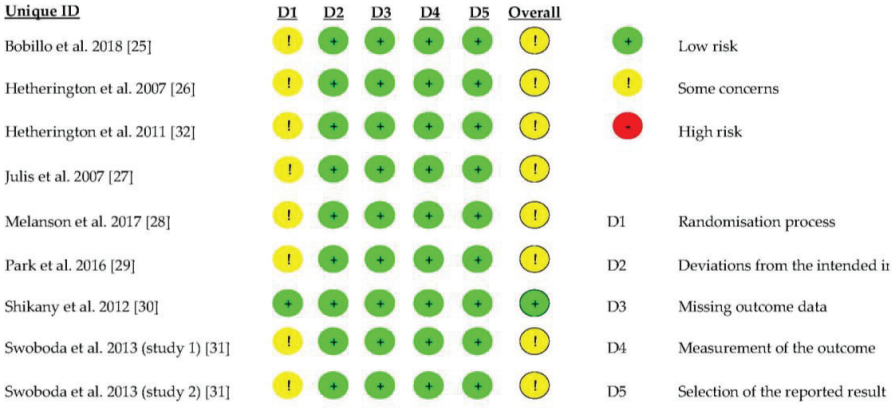

Figure 2. Quality of the RCTs included in this systematic review according to the Cochrane risk of bias tool RoB2.

4. Discussion

The present systematic review showed that chewing gum has an influence on appetite regulation by reducing hunger, the desire to eat, and the desire to eat a sweet snack, but the effect on the desire to eat a salty and a fatty snack, preoccupation with food, satiety measured as fullness, energy intake, and weight loss is not conclusive.

Our results suggest that the mastication effect of chewing gum has an impact on appetite regulation and that there might be a suppressor effect on appetite sensations, specifically on hunger. Similar findings were observed in a systematic review and meta-analysis [16], stating that prolonged chewing, regardless of whether the mastication of food or sham feeding with chewing gum, reduces self-reported hunger. Additionally, in a

meta-analysis, Krop et al. concluded that prolonged orosensory exposure to food reduced subjects' appetite and increased chewing reduced food intake [17]. Furthermore, in a study conducted by Ikeda et al., chewing stimulation reduced subjective appetite, suggesting that chewing, even without ingestion, may affect reward circuits and reduce subjective appetite ratings that reflect cravings [20]. The consistency across these trials highlights the potential utility of gum chewing as a non-pharmacological intervention to control hunger.

Reducing the consumption of sweet or salty snacks is a key strategy for managing obesity. These foods are often high in energy, low in nutrients, and easily accessible, which contribute to excessive energy consumption and, consequently, weight gain [33,34]. These findings suggest that chewing gum could serve as an effective measure to curb general cravings and manage overall food intake. In a systematic review conducted by Cooke et al., discretionary snack consumption was associated with energy intake, suggesting that the increased consumption of snacks might contribute to increased energy intake; however, there was a lack of consistent associations with increased weight and BMI [35].

Concerning satiety, measured as fullness, three out of six RCTs showed a significant increase in fullness after chewing gum when compared to no gum chewing [25,26,32]. However, one RCT found a significant decrease in fullness between before and after chewing gum chewing [27]. These mixed results suggest that chewing gum might influence satiety signals; however, the non-significant results in these studies might be due to the variations in study design, participant characteristics, or sensitivity of the measures used to assess fullness. A study conducted by Komai et al. described tendencies toward increases in satiety and fullness after 20 min of gum mastication [36]. Similar results were observed in another study where chewing stimulation, via chewing gum, was compared to actual feeding to reduce attentional bias toward food and concluded that actual feeding significantly increased fullness. Chewing gum showed the same results but to a lesser extent [20]. However, a systematic review and meta-analysis by Miquel-Kergoat et al. showed that only a minority of studies demonstrated significant increases in fullness and satiety after chewing without considering if it was food or sham feeding [16].

Increasing satiety is a crucial factor in obesity management, as it can help in controlling food intake and contribute to weight loss and the maintenance of a healthy weight [37]. Therefore, more high-quality interventions are needed to elucidate the effectiveness of chewing gum on increasing satiety.

Regarding appetite, increased masticatory cycles, either by thorough the mastication of food or sham feeding with chewing gum, have been described to reduce postprandial appetite and influence appetite hormones such as GLP-1, CCK, and ghrelin [14,38,39]. In a study conducted with 12 healthy male volunteers, a decrease in GLP-1 concentration was associated with an increase in satiety after 30 min of chewing gum [38]. Various studies associated an increase in CCK and a reduction of ghrelin after an increased number of chewing cycles before swallowing food [14,39]. These results show that thorough mastication may increase satiety.

These results and the results obtained in this systematic review show that chewing gum might influence appetite regulation and, consequently, may increase satiety and therefore reduce energy intake. These findings suggest that chewing gum could enhance feelings of fullness in various experimental setups, particularly when consumed in regular intervals after meals. Taking into account that current antiobesity drugs target satiety signaling in the brain, often do not produce the expected effect, can cause chronic disorders and side effects, and do not have a sufficient level of safety [40], the use of chewing gum to increase satiety signals would be a novel tool.

Concerning energy intake, the mixed findings across these studies suggest that while chewing gum may help reduce energy intake under certain conditions, its overall impact

on daily energy consumption is inconsistent [26,28,31]. Factors such as the timing of gum chewing, types of meals, and individual differences likely play critical roles in these outcomes [41]. Furthermore, only one RCT was a long-term study; all other RCTs were acute studies. This suggests that the results might have been influenced, and it would be interesting to carry out more long-term studies that evaluate energy intake in order to see changes over time.

The evidence collected and the current bibliography suggest that chewing gum could be a novel tool to reduce hunger and desire to eat, whereas its effect on body weight has not been proven. These findings support our hypothesis stating that chewing gum affects appetite regulation, specifically hunger and desire to eat, but the effects on satiety and energy intake are inconclusive. Taking into account that there are already drugs on the market that are used for weight loss, our results suggest that chewing would have the same influence as these drugs, with lesser side effects. However, more studies with a longer duration are needed to evaluate the chronic effect of chewing gum to establish a frequency of chewing gum chewing that does not cause side effects such as abdominal distention and to analyze whether the decreases in hunger and desire to eat translate into a decrease in energy intake and ultimately decreases in weight, BMI, and WC.

To the best of our current knowledge, this is the first systematic review that has explicitly assessed the influence of chewing gum on satiety, appetite regulation, and energy intake in RCTs.

One of the key limitations of this systematic review is the heterogenicity of the studies. The differences in the intervention design, the evaluation of the outcomes, and the duration of the intervention make it very difficult to compare these studies and establish conclusions. There is a need to carry out intervention studies with the same type of blinding, duration of intervention, and outcomes to be studied to be able to draw conclusions and define the role of chewing gum on appetite regulation and satiety. The purpose of these studies should be to conduct randomized controlled studies that evaluate the effect of chewing gum on satiety and appetite, as well as chewing it for a certain time and a certain period of time, compared to not chewing gum. Comparisons between chewing gum and actual food should be avoided, since the hormonal responses differ [6]. These studies are essential to determine whether chewing gum is an effective tool for obesity and to be able to use it in clinical practice. Lastly, most of the articles included in this review lacked statistical data, such as mean differences, standard deviation, and standard error or confidence intervals for each intervention, as well as their *p*-values. Consequently, a meta-analysis, which would have provided more conclusive results, as well as a forest plot, which would have provided a clearer presentation of the results, could not be performed. Finally, we also point out that the data were extracted from the articles by only one researcher, and although a second researcher was consulted in case of doubt, the fact of not performing the entire extraction independently by at least two researchers could have led to biases.

Further research on the effect of chewing gum on populations with overweight, obesity, or abdominal obesity is needed to establish if it is a good co-adjuvant tool for weight loss and, consequently, for obesity management.

Future research should aim to elucidate the mechanisms, optimal conditions, and long-term effects of gum chewing on appetite and weight management.

5. Conclusions

The results of this systematic review confirm that chewing gum influences appetite regulation parameters, particularly leading to a clear decrease in the feeling of hunger, desire to eat, and desire to eat a sweet snack. While the findings regarding chewing gum are promising, further research to reduce the heterogenicity between RCTs is needed to

elucidate the mechanisms, optimal conditions, and long-term effects of gum chewing on appetite and weight management.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu17030435/s1, Table S1: Search strategy; Table S2: Summary of characteristics of excluded studies; Table S3: Summary of characteristics of included RCTs.

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Review

Current Perspectives for Treating Adolescents with Obesity and Type 2 Diabetes: A Review

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Abstract: Background: Childhood obesity is an epidemic and a significant health concern all over the world. Several factors can influence excess weight gain, including eating behaviors, physical inactivity, and genetics. Children and adolescents with obesity have a four-times greater risk of developing type 2 diabetes (T2D) compared with their normal-weight peers. The management of obesity before the development of its comorbidities may prevent its escalation into significant medical and psychosocial problems. However, treatment options for obesity and T2D in youth remained limited for many years, and moreover, available drugs were characterized by low efficacy. The Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study showed that metformin in monotherapy failed in almost 52% of children with T2D, while adjuncts to rosiglitazone and lifestyle intervention failed in 38.6% and 46.6%, respectively. Recently approved antiobesity medications and/or bariatric surgery are revolutionizing the management of adolescents with obesity and T2D. This work aims to provide a comprehensive overview of the current treatment possibilities for childhood obesity and T2D. **Methods**: An in-depth review of articles with evidence-based research from different countries discussing novel management options for adolescents with obesity and/or T2D was conducted in this review paper. **Results**: The new medications, such as SGLT2 receptor agonists and GLP-1 agonists, are highly effective in treating T2D in adolescents with obesity. **Conclusions**: Based on the performed literature review, the recent approval of a novel generation of drugs seems to be the dawn of a new era in childhood obesity and T2D treatment.

Keywords: obesity; weight gain; type 2 diabetes; adolescents; weight management; pharmacotherapy; pharmacological treatment; metabolic surgery

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1. Introduction

Obesity is the most prevalent nutritional disorder among children and adolescents. In recent decades, the prevalence of childhood obesity increased dramatically, reaching epidemic dimensions. According to the World Health Organization (WHO), over 340 million youth aged 5–19 years and almost 40 million children under the age of 5 years suffered from overweight or obesity in 2016 and 2020, respectively [1,2]. The occurrence of obesity in children and adolescents is increasing in many low-income and middle-income countries, while in high-income countries, it has plateaued, usually at high levels [3]. Nowadays, the WHO and national and international scientific societies recognize obesity as a chronic progressive disease [2,4–7]. Several factors can lead to excess weight gain, such as poor eating habits, physical inactivity, and unhealthy sleeping manners. Health-related risk behaviors, genetic background, and obesogenic environment also are vital factors. As the prevalence of obesity grows, so does the prevalence of associated comorbidities, including prediabetes and type 2 diabetes (T2D) [8]. Adolescents with obesity face four times the risk of developing T2D than those with a body weight within the normal range [9]. However, trends in T2D occurrence in youth with obesity vary considerably due to different degrees of obesity, age range of the sampled population, or racial/ethnic variation [10].

Abnormal glucose metabolism in individuals with obesity results from peripheral and hepatic insulin resistance followed by a progressive deterioration in beta-cell function. The relationship between obesity and T2D is even stronger in adolescents than in adults. Data from the SEARCH for Diabetes in Youth (SEARCH) study demonstrated a high prevalence of T2D in adolescents with excess weight. In accordance, T2D developed in 79.4% and 10.4% of children with obesity and overweight, respectively [11]. Moreover, higher BMI during adolescence is linked to a significant risk of T2D development in adulthood. Interestingly, the pathogenesis of T2D in youth is similar to T2D in adults; however, youth-onset T2D is associated with lower insulin sensitivity, insulin hypersecretion, and more rapid loss of beta-cell function [12–14]. The Treatment Options for Type 2 Diabetes in Adolescents and Youth (TODAY) study demonstrated through studies with hyperglycemic clamp methodology and oral glucose tolerance tests (OGTTs) that in adolescents with T2D beta-cell function declined relatively quickly, about 20–35% per year [14]. This is in contrast to adults with T2D, in which loss of beta-cell function was reported at 7–11% per year [13]. In addition, early-onset T2D is linked to significantly higher mortality, more T2D complications, and risk of adverse cardiovascular events compared to type 1 diabetes [15]. In the TODAY study, microalbuminuria was found in 6.3% of participants at the beginning of the study, while at the end of follow-up, it was already observed in 16.6% of adolescents. The cumulative incidence of T2D-related comorbidities such as nephropathy was 54.8%, and neuropathy and retinopathy were 32.4% and 51%, respectively, at the end of the study, with an average duration of T2D being 13.3 ± 1.8 years [12].

Finally, recent evidence has shown that the stigmatization of adolescents with obesity is a relatively common problem. Young people very often experience victimization and bullying, which altogether may lead to binge-eating disorders, decreased physical activity, and even isolation from society and loneliness [4,5,16,17]. Then, obesity and T2D are conditions that can significantly reduce life expectancy, negatively affect quality of life, and raise healthcare expenditure. This highlights the significance of early intervention in attaining better weight control and preventing the frequency of obesity and T2D complications in youth [4,17]. This review provides a comprehensive overview of recently published evidence-based studies on novel agents and surgical options for childhood obesity and T2D management.

2. Materials and Methods

This review is narrative, and no systematic literature search was performed; each author identified and critically reviewed the most relevant papers. The work presents several evidence-based studies on recently approved drugs by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for treating adolescents with obesity and T2D and bariatric surgeries performed in pediatric populations. The following electronic databases were searched for relevant full-text articles in the English language: PubMed, Google Scholar, Scopus, EMBASE, and Web of Science. The search period was from May 2023 to July 2024 using the following keywords: obesity; type 2 diabetes; obesity-induced type 2 diabetes; childhood obesity; children; adolescents; youth; complications; comorbidities; nephropathy; neuropathy; retinopathy; weight management; treatment; management; regimen; guidelines; nonpharmacological therapy; lifestyle interventions; nutritional modification; behavioral intervention; physical activity; pharmacotherapy; pharmacological agents; antidiabetic medications; anti-obesity medications drugs; metformin; 1,1-dimethylbiguanide; insulin; glucagon-like peptide-1 receptor agonists; liraglutide; exenatide; dulaglutide; semaglutide; orlistat; phentermine; topiramate; sodium-glucose co-transporter-2 inhibitor; empagliflozin; dapagliflozin; approval; the U.S. Food and Drug Administration; the European Medicines Agency; effectiveness; sides effects; bariatric surgery; weight-loss surgery. All articles published between January 2001 and July 2024 were checked by title, abstract, and full text. Reviews, guidelines, and experimental studies (in vitro/ex vivo or animal studies) were excluded. As a result, 11 studies were selected, including 1 large study on bariatric surgery and 6 and 4 studies

regarding new-generation drugs currently approved for use in youth with T2D and obesity, respectively. It aimed to detect the most clinically significant papers related to the topic and provide a theoretical point of view, considered a valuable educational tool in continuing medical education.

3. Treatment Options for T2D and Obesity in Children and Adolescents

3.1. Nonpharmacologic Therapy

Lifestyle interventions, including nutritional and behavioral modifications and enhancing physical activities, are a cornerstone of managing childhood obesity and T2D [4,5,17]. The main goal in changing lifestyle behaviors is to promote body mass reduction and, consequently, reduce insulin resistance. It has been reported that decreases in BMI of 0.5 kg/m^2 or more lead to improvements in insulin sensitivity [18]. International societies such as the American Diabetes Association (ADA) and International Society for Pediatric and Adolescent Diabetes (ISPAD) advise that young individuals with obesity and/or T2D should at least have 60 min of moderate to vigorous physical activity daily [6,7]. The nutrition of youth with obesity should be focused on personalized nutrition approaches that ensure adequate nutritional content tailored to physiological conditions, ages, and sex-specific needs. Dietary components such as ultraprocessed foods and sugar-sweetened beverages should be eliminated from the diet at the baseline in individuals with obesity [6]. However, lifestyle intervention studies in youth with obesity provide conflicting data. Some studies, including behavioral components, showed substantial weight reduction compared to typical management or passive control, with long-term interventions having the greatest benefit [19–21]. Conversely, the TODAY study demonstrated no difference in weight loss between the group receiving intensive lifestyle interventions in conjunction with metformin and the group treated with metformin only [12,14]. Moreover, the benefits observed in randomized clinical trials might be difficult to translate to real-world settings.

3.2. Metformin

Metformin (1,1-dimethylbiguanide) is one of the oldest antidiabetic medications, which is used as the first-line therapy for the treatment of T2D in metabolically stable children aged 10 years and older, as adjuncts to diet and exercise and sometimes in combination with other glucose-lowering agents [6,7]. For many years, metformin, along with insulin, were the only drugs approved for treating T2D in youth. Metformin is a complex medication that acts through several molecular mechanisms. It differs from other antihyperglycemic agents because of its unique mechanisms of action. Primarily, it reduces hepatic glucose production and intestinal absorption of glucose and decreases insulin resistance by increasing peripheral glucose uptake and utilization [22].

It is predominantly used as a medication for managing T2D, although it has also been studied for its effects on lipid profiles in various populations, including youth. Research has indicated that metformin can have a positive effect on lipid profiles, such as reducing total cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides while potentially increasing high-density lipoprotein (HDL) cholesterol [23–25]. Moreover, it has been described that metformin may have a beneficial effect on the normalization of ovulatory abnormalities in girls with polycystic ovary syndrome (PCOS), which affects around 20% of girls with T2D [24,25]. While data are limited for children, studies conducted in adult populations have shown that metformin also protects against cardiovascular disease [26]. In adult trials, metformin decreased the risk of myocardial infarction (39%), coronary deaths (50%), and all-cause mortality (36%) compared with standard therapy [27]. Moreover, in adults, metformin is also assumed to have positive effects in reducing the risk of developing diabetes mellitus among individuals at risk for the disease. The findings suggest that metformin might potentially be a valuable intervention for diabetes prevention, particularly in high-risk populations. However, this indication is considered as off-label use [28,29]. Finally, it is worth mentioning that metformin does not increase the risk of hypoglycemia during therapy [23,30].

Metformin is typically prescribed at daily doses ranging from 500 to 2000 mg divided twice daily. Generally, this oral medication is safe and, in most cases, well tolerated. The main adverse effects include nausea, vomiting, abdominal bloating, diarrhea/constipation, weakness, or a metallic taste in the mouth [6,22].

Metformin is not approved for weight loss in the pediatric population. However, it has been extensively used as an off-label for weight management in young individuals with excessive weight [31]. In some controlled trials among children with obesity, metformin demonstrated a favorable short-term effect on weight reduction and improvement of insulin sensitivity compared with a placebo and lifestyle modification [32]. However, there are limited studies on the treatment of T2D in children and adolescents. The largest clinical trial, the TODAY study, showed that metformin in monotherapy failed in almost 52% of children with T2D, while adjuncts to rosiglitazone (the thiazolidinedione class) and lifestyle intervention failed in 38.6% and 46.6%, respectively [12,14,33,34]. It suggests that metformin is insufficient to produce durable glycemic control and, therefore, is not an optimal treatment option in youth with T2D. The management of childhood-onset T2D must be optimized and should consider not only pharmacologic regimens but also lifestyle modification, along with close monitoring and follow-up. Notably, metformin was reported to have a much higher failure rate in adolescents compared to adults treated with metformin alone [6,7,12–14].

3.3. Insulin

In children and adolescents with diabetic ketoacidosis or HbA1c $\geq$ 8.5% (69 mmol/mol), insulin treatment is an alternative for initial management. There are several different insulin regimens. However, an initial dosing of 0.25–0.5 units/kg of basal insulin is usually effective in achieving good T2D control [6,7]. The main drawback of insulin treatment is weight gain, which results mainly from an increased appetite, insulin's anabolic effect, and lowering glucose levels under the renal threshold [35]. Insulin-induced weight gain may negatively impact recommended management. Therefore, patients may experience dissatisfaction, which can cause treatment discontinuation or decreased adherence [36]. Another issue that might occur during insulin therapy is hypoglycemia, which is not very common in youth with T2D because of observed insulin resistance [6]. After treating acidosis, metformin should be started and slowly titrated. Transition to metformin takes approximately 2 to 6 weeks by reducing the total daily dose of insulin by around 30–50% with a parallel increase in metformin dosing [6,7]. Data from the TODAY study showed that, in most cases, insulin can be eliminated from the regimen without losing glycemic control [37,38].

3.4. Sodium-Glucose Transporter 2 (SGLT2) Inhibitors

Yet, in the pediatric population, there are limited therapeutic options for treating T2D compared to adults. Therefore, subsequent approvals of newer agents used in childhood-onset T2D treatment are promising and extend potential health benefits to patients facing difficulty controlling the disease. Recently, the FDA expanded the indication of two SGLT2 inhibitors (empagliflozin and dapagliflozin) to include children aged $\geq$10 years with T2D combined with diet and exercise to attain better metabolic control. These antidiabetic drugs act by inhibiting renal SGLT2, located in the proximal collecting tubule of the nephron. Then, SGLT2 inhibitors increase glucosuria, reducing blood glucose concentration and improving HbA1c. To date, this pharmacological class is assumed to significantly improve cardiovascular outcomes among high-risk patients and slow the progress of kidney disease in individuals with T2D. Beyond benefits, SGLT2 inhibitors also are related to some side effects. The most common adverse events are female genital mycotic infections, urinary tract infections, increased urination, nausea, and constipation. However, it might also be associated with some severe effects, such as volume depletion, acute kidney injury, hypoglycemia, or diabetic ketoacidosis (DKA) [39,40].

Previously, empagliflozin was tested in one phase trial assessing the pharmacokinetic and pharmacodynamic characteristics in adolescents with T2D to identify the appropriate doses for further pediatric use. The main outcome of this study confirmed that the pharmacokinetic profile of the 10 and 25 mg doses of empagliflozin already used in adult-onset T2D is similar in youth with T2D [41]. However, empagliflozin received a positive opinion (June 2023) based on the phase 3 DINAMO trial. This double-blind, placebo-controlled trial included 157 patients between 12 and 17 years of age with uncontrolled T2D (HbA1c ranging between 6.5 and 10.5% (mmol/mol) [42–84] initially taking metformin and/or insulin. Participants were randomly assigned to three groups, receiving empagliflozin 10 mg, linagliptin 5 mg (dipeptidyl peptidase-4 [DPP-4)] inhibitor), or placebo orally once a day for 26 weeks. At week 12, individuals in the empagliflozin group who did not experience a lowering of HbA1c to less than 7.0% (<53 mmol/mol) were again randomized at week 14 to remain on 10 mg dose or increased to 25 mg. The primary endpoint was a significant reduction in HbA1c level from the beginning of the on-treatment period to the end (26 weeks) compared with the placebo. This study demonstrated that empagliflozin as an adjunct to other treatment methods (diet, exercise, metformin, and/or insulin) significantly reduced HbA1c by 0.8% at week 26 compared with the placebo. Meanwhile, those on linagliptin did not experience statistical significance in HbA1c decrease compared to the placebo. When it comes to the overall safety profile of empagliflozin, it is comparable to the profile observed in adults. The most-reported side effect was hypoglycemia, followed by urinary tract infections. No cases of DKA or necrotizing fasciitis were reported [85].

The second SGLT2 inhibitor, dapagliflozin, was approved by the FDA in June 2024. The approval was supported by the results from the T2NOW phase 3 trial. In the trial, children between 10 and 17 years of age with insufficient control of T2D (HbA1c ranging between 6.5 and 10.5% (mmol/mol) [42–84] already on metformin, insulin, or both were randomized to receive 5 mg dapagliflozin, 2.5 mg saxagliptin (dipeptidyl peptidase-4 [DPP-4)] inhibitor), or placebo as an add-on treatment for 26 weeks. Participants in active treatment groups who did not attain HbA1c less than 7% (<53 mmol/mol) at week 12 were again randomly assigned to continue the dose or to receive an increased dose (10 mg of dapagliflozin or 5 mg of saxagliptin). Data from the T2NOW phase 3 trial confirmed that dapagliflozin is related to significant improvement in metabolic control in children and adolescents with T2D. At 26 weeks, participants assigned to dapagliflozin had a 0.62% point reduction in HbA1c compared with a 0.41% point increase for the placebo group. Moreover, subanalysis has presented a 1.1% HbA1c drop in adolescents who reported consistent use of dapagliflozin [86]. Safety outcomes in the T2NOW phase 3 trial study aligned with those reported in adults with T2D. The most common adverse effect was headache, followed by hypoglycemia [87].

Apart from the beneficial effect on glycemia control in individuals with T2D, as it has been demonstrated in adult studies receiving SGLT2 inhibitors, those drugs are related to a reduction in cardiovascular disease risk by decreasing blood pressure and increasing HDL cholesterol [47]. Then, in adults, SGLT2 inhibitors have a wider indication profile compared to youth. These agents may be indicated for adults with TD2 and established cardiovascular disease to reduce the risk of major adverse cardiovascular events. Moreover, SGLT2 inhibitors are also indicated for adults with heart failure, particularly those with reduced ejection fraction (HFrEF), regardless of the presence of diabetes, to reduce the risk of hospitalization and cardiovascular death or for those with chronic kidney (CKD) disease at risk of progression [42,88].

3.5. Glucagon-like Peptide-1 (GLP-1) Receptor Agonists

Glucagon-like peptide-1 (GLP-1) agonists represent a group of medications primarily aimed at treating T2D; however, they are now more widely used in children and adolescents with obesity. GLP-1, an incretin hormone, is produced mainly by enteroendocrine L cells in the distal ileum and colon, alpha cells of the pancreatic islets, and hindbrain neurons in the central nervous system. GLP-1 agonists stimulate insulin secretion from pancreatic

beta-cells in response to carbohydrates absorbed from the gut. Then, the main function of GLP-1 is thought to be enhancing postprandial insulin release. Moreover, GLP-1 agonists slow gastric emptying, protect beta-cells of the pancreatic islets against the inflammation and apoptosis caused by cytokines, and inhibit hepatic gluconeogenesis. These drugs also improve insulin sensitivity through two potential mechanisms: directly augmenting glucose uptake by peripheral tissues such as skeletal muscles and indirectly promoting weight reduction [43]. A beneficial effect of these agents on weight loss is mainly related to suppressed gastric emptying, which stimulates satiety and appetite reduction at the level of the hypothalamus. Symptoms of delayed gastric emptying, including nausea and vomiting, are the main side effects caused by GLP-1 agonists.

Moreover, GLP-1 agonists in adult population studies are well documented to have a similar magnitude of effect on major adverse cardiovascular events, all-cause mortality, and cardiovascular-related death in overweight/obese individuals with and without T2D [44,45]. Yet, while GLP-1 agonists have been approved for use in children with T2D aged 10 and older for the treatment of obesity, their cardiovascular benefits have not been established in pediatric populations to the same extent as in adults [46].

Numerous studies also suggested that GLP-1 agonists generally have a positive effect on blood lipid profiles [47]. For instance, liraglutide combined with metformin decreased levels of cholesterol and LDL cholesterol in adult patients with T2D and cardiovascular disease already taking statins [48]. Furthermore, the specified analysis revealed that liraglutide significantly lowers blood concentrations of multiple lipid species, including ceramides, phosphatidylcholines, phosphatidylethanolamines, and triglycerides [49]. Some studies also suggested that GLP-1 agonists may improve the HDL cholesterol profile, although the results can vary depending on the specific agent and individual patient factors [50,51]. The exact mechanisms by which GLP-1 agonists affect lipid levels are still being studied, but they may involve effects on hepatic fat metabolism, appetite regulation, and overall weight reduction.

Finally, it must be highlighted, besides several beneficial effects, that these medications might increase the risk of thyroid cancer. The potential link between the new onset of thyroid cancer and GLP-1 agonist use is not yet fully explored. Firstly, this issue was highlighted in the premarketing phase after animal studies demonstrated raised rates of thyroid C-cell tumors in rodents [52]. GLP-1 receptor agonists receptors are expressed in different organs, including the lung, kidney, stomach, heart, and intestine; α, β, and d cells of the pancreatic islets; and multiple regions of the CNS. Also, this receptor might be widely expressed on the parafollicular C-cell membrane. After binding to the receptor, native GLP-1 stimulates cell proliferation, creating a favorable environment for tumor development [53]. Chronic stimulation of C-cells may lead to overproduction of calcitonin in a cAMP-dependent manner [54]. Therefore, this is a possible mechanism in which GLP-1 may promote rodent thyroid carcinogenesis, but there is insufficient evidence indicating the potential cancerogenic effect in humans [52]. Nevertheless, *GLP-1 cannot be used in individuals with their own or family medical history of medullary thyroid carcinoma or patients with multiple endocrine neoplasia syndrome type 2* [5,17,55].

Liraglutide in a dose of 0.6–1.8 mg/daily (Victoza) is indicated in pediatric patients $\geq$ 10 years with uncontrolled T2D as additions to lifestyle modification and oral metformin to attain sufficient metabolic control of the disease. The efficacy of liraglutide in youth-onset T2D was widely studied in the Evaluation of Liraglutide in Pediatrics with Diabetes (Ellipse) trial. The Ellipse study was a double-blinded trial in which 135 participants between 10 and 17 years with T2D were enrolled and randomly assigned to receive liraglutide or placebo for a 26-week period, followed by a 26-week open-label extension. Compared to the placebo, this study demonstrated superiority in improving metabolic control in the liraglutide group. In detail, at week 26, the mean HbA1c level decreased to 0.64% from the beginning of the study among those on liraglutide and only 0.42% in the placebo group. Furthermore, at week 52, the drop in HbA1c concentration was even more significant in the liraglutide patients than in placebo. Regarding the beneficial effect of GLP-1 agonist on weight loss,

in the first part of the trial, there was no difference in BMI between the two groups of participants; however, by week 52, individuals on liraglutide experienced a reduction in BMI [56]. Then, adding liraglutide to therapy seems to be a relevant and promising treatment option for youth with obesity-induced T2D. Nevertheless, when choosing a second-line drug, one should consider the glycemic target, dosage schedule, route of administration, impact on weight, adverse effects, and possible comorbidities.

A higher dose of liraglutide (3 mg a day, Saxenda) is recommended in children $\geq$12 years of age with obesity defined as a BMI $\geq$ 30 kg/m^2 or if $\geq$27 kg/m^2 in the presence of one or more comorbidities for weight management. Recent clinical trials showed that liraglutide 3.0 mg yielded significant weight loss when combined with lifestyle intervention. In a double-blind study, a total of 251 youth between 12 and 17 years of age with obesity and insufficient weight management were randomized to receive 3.0 mg of subcutaneous liraglutide once a day (125 adolescents) for 26 weeks, followed by a 26-week observational period or a placebo in addition to lifestyle intervention for a 56-week treatment period. In this study, participants in the liraglutide group had a greater decrease in BMI compared to individuals assigned to the placebo group (43.3% vs. 18.7%, respectively). Nonetheless, after treatment withdrawal in youth on liraglutide, a greater rise in BMI was noticed compared to placebo. It suggests that young patients with obesity should receive a solid education on lifestyle modifications, which must be repeated to avoid the child's demotivation regarding eating and physical activity habits. Also, it highlights the importance of introducing new pharmacological agents to weight management because, in most cases, lifestyle intervention is not enough to achieve set goals. Available data suggest that liraglutide 3.0 mg significantly affects weight reduction. However, individuals receiving liraglutide experienced more frequent gastrointestinal side effects than those in the placebo group. Then, adverse effects of GLP-1 were associated with treatment discontinuation in 10.4% of participants in this study [57]. Nevertheless, liraglutide 3.0 mg seems to be a reasonable option for the treatment of youth with obesity.

In 2021, medicine agencies for the drug administration (FDA and EMA) gave a positive opinion on an extended-release version of the GLP-1 drug, exenatide (BYDUREON BCise); since then, this agent has also been recommended in children and adolescents aged 10 to 17 years old with T2D in conjunction with nutritional management and exercise to achieve and maintain good metabolic control. The new approval for exenatide follows results from a clinical trial called BCB114, in which the safety of BYDUREON BCise was investigated among young people aged 10–17 years old. This study demonstrated that exenatide has a greater effect on HbA1c level reduction than the placebo [58].

In some countries, dulaglutide (Trulicity) is also available, which is approved for youth aged 10 years or older who suffer from T2D, in addition to lifestyle modification. In the AWARD-PEDS trial, treatment with dulaglutide agonist was associated with significant decreases in HbA1c levels compared to placebo among youth aged 10–17 years. This study included 154 individuals aged 10–17 with T2D taking metformin alone or without insulin or lifestyle modifications. Individuals were randomly assigned to three groups receiving a placebo, a 0.75 mg dose of dulaglutide, or a 1.5 mg dose of dulaglutide. Findings from this study showed that dulaglutide injected once a week is significantly more effective in achieving good metabolic control in adolescents with T2D than placebo. Over 26 weeks of treatment, HbA1c and fasting glucose levels were greatly reduced in the dulaglutide groups. However, there was no beneficial effect on BMI reduction [59].

Finally, semaglutide (Wegovy) was approved to manage childhood obesity in individuals aged 12 years and older [17,60]. The new indication for semaglutide follows results from the STEP TEENS clinical trial. This phase 3 study included 201 teens aged between 12 and 17 years, with obesity or overweight and at least one obesity-associated comorbidity. Participants were randomly selected to receive once-weekly semaglutide 2.4 mg injections or placebo in conjunction with diet and physical activity modifications for a 68-week period. This on-treatment phase was followed by a 7-week follow-up period during which the participants from both groups continued only lifestyle intervention. Efficacy endpoints

were evaluated from the time of randomization to the end of the treatment period. The primary endpoint was the percentage change in BMI, and the secondary endpoint was a decrease in body weight of at least 5%. In the STEP TEENS clinical trial, semaglutide achieved superior mean percentage change in BMI in adolescents with obesity compared to the placebo group. The mean percentage change in BMI from the beginning to the end of the on-treatment phase was estimated at 16.1% with semaglutide and only 0.6% with placebo. Moreover, when participants were still on lifestyle interventions, the BMI stayed below the baseline level in individuals receiving semaglutide and above the baseline value in those assigned to a placebo. Similarly, body weight decrease from the study's beginning to week 68 was significantly higher in the semaglutide group than in the placebo group. At the end of the treatment period, 73% of adolescents on semaglutide experienced at least a 5% reduction in their initial body weight. In contrast, for those who received a placebo, such reduction was only observed in 18% of the participants. The improvement in body weight resulted in a reduction in HbA1c, lipid profiles, and waist circumference. Therefore, semaglutide seems to decrease the risk of cardiovascular disease. Again, this study confirmed that GLP-1 agonists are an optimal treatment option for youth with obesity having a poor response to standard management [60].

GLP-1 receptor agonists undoubtedly benefit youth with morbid obesity and T2D. However, some concerns regarding the unintended side effects of these drugs need to be highlighted. Cooper et al. pointed out that inappropriate reductions in energy intake associated with GLP-1 during a child's critical stage of growth and development might negatively impact their health later in life. Also, the authors suggested the possibility of abuse in individuals suffering from eating disorders, as well as youth involved in competitive sports such as gymnastics or ballet [61]. Finally, some postmarketing reports suggested the association between GLP-1 agonist use and suicidal thoughts, prompting the FDA to evaluate a causal relationship. However, after an investigation, the FDA did not find evidence that the use of these medicines causes suicidal thoughts or actions. So, implementing GLP-1 treatment should be carried out by an experienced clinical professional with further monitoring of adverse consequences for children's health. Table 1 gives an overview of the effects and mechanism of action of antidiabetes drugs used recently in pediatric populations.

Table 1. Overview of effects and mechanisms of action of approved antidiabetic agents in youth [22–29,37,38,40–47,52,85–88].

	Biguanides (Metformin)	**SGLT2 Receptor Agonists (Empagliflozin/Dapagliflozin)**	**GLP-1 Agonists (Liraglutide/Exenatide)**
Effects/Mechanism of actions	Acts via AMP kinase in liver, muscle, and fat. Inhibit hepatic gluconeogenesis and increase peripheral glucose uptake and insulin sensitivity.	Inhibit renal tubular reabsorption of glucose and lower the renal threshold for glucose, thereby increasing urinary glucose excretion.	Increase insulin secretion proportionate to blood glucose concentrations, suppressing glucagon, prolonging gastric emptying, and promoting satiety.
Percent HbA1c lowering	1–2%	1–2% Dapagliflozin use in youth with T2D did not show benefit relative to metformin ± insulin, although subanalysis presented a 1.1% HbA1c drop in adolescents that reported consistent use.	Ellipse trial showed the liraglutide group had 1% and 1.5% HbA1c reduction at 26 and 52 weeks, respectively. Exenetide (Bydureon) 2 mg once a week lowered HbA1c by 0.85% compared to a placebo.
Cardiovascular benefits and risk	Reduce MI by 39% and coronary deaths by 50%.	Positive CV effect due to reduction in Na and UA absorption and reduction in BP.	Reduce CV risk.
Effect on lipid profile			
HDL cholesterol level	↑	↑	↑

Table 1. *Cont.*

	Biguanides (Metformin)	SGLT2 Receptor Agonists (Empagliflozin/Dapagliflozin)	GLP-1 Agonists (Liraglutide/Exenatide)
LDL cholesterol level	↓	↔ or ↑	↓
Triglycerides level	↓	↔	↓
Weight loss	↔ or ↓	↓	↓↓
Risk of hypoglycemia	↔	↔	↔

Abbreviations: SGLT2, sodium-glucose transporter 2 inhibitors; GLP-1, glucagon-like peptide-1; AMP kinase, Adenosine monophosphate-activated protein kinase; HbA1c, glycated hemoglobin; T2D, type 2 diabetes; MI, myocardial infarction; CV, cardiovascular; Na, sodium; UA, uric acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BP, blood pressure; ↑, increasing trend; ↓, decreasing trend; ↓↓, strong decreasing trend; ↔, stable trend.

3.6. Challenges and Special Considerations of Youth-Onset T2D Management

Youth-onset T2D presents unique challenges and considerations both in research and clinical practice due to its distinct characteristics compared to adult onset. Scientific research on T2D is limited in the childhood population, and it is mostly related to difficulties in including appropriate participant numbers, stringent eligibility criteria, limited sites worldwide suitable for such studies, and the growing number of trials vying for the few eligible patients. In addition, research involving children and adolescents requires strict ethical oversight. Obtaining consent or assent from minors and managing potential risks are more complex than in adult populations [62–64]. These important concerns were highlighted by the SEARCH for Diabetes in Youth study, which pointed out that the time between diagnosis and enrollment in clinical trials for youth T2D was more than double that for children with T1D [65]. Therefore, medical knowledge on the mechanism of action, use indications, and beneficial or adverse effects are generally based on results obtained in studies on adults with T2D. In consequence, in adults, there is a wider range of medications that might be included in T2D management.

Generally, the mechanisms of action of antidiabetic agents are generally comparable across different age groups, including children, adolescents, and adults, as these drugs target specific biological pathways involved in glucose metabolism and insulin regulation. However, there are potential metabolic differences between adults and children, which can affect pharmacokinetics and pharmacodynamics. It might result from differences in body composition, organ function, and enzyme activity; therefore, it theoretically impacts the dosing, frequency, and efficacy of medications [66].

Another issue is the developmental consideration, which makes T2D treatment more complicated in children than adults. Puberty is a dynamic period of development marked by rapid changes in body size, shape, and composition, all of which might influence the treatment of T2D in children and adolescents. Moreover, youth have special nutritional requirements, making it fundamental to find a balance that avoids deficiencies and potential consequences like stunted growth while maintaining glycemic control [67,68].

Finally, adherence to T2D in youth might be lower compared to adults. Some studies have shown that adults with T2D do not have satisfactory levels of adherence to lifestyle modifications and/or medication regimens due to the complexity of treatment. Adolescents are undergoing significant physical and emotional changes, which can impact their ability to manage a chronic condition. Additionally, motivation to follow the medical recommendations might be lower in children; thus, youth need more support for motivation from parents or healthcare providers [69,70]. Still, data on treatment adherence in youth with T2D are scarce; however, some studies conducted in children with T1D showed that adherence to therapy declines over time [71]. Then, it might be assumed that youth with T2D can also be characterized by similar patterns of adherence.

3.7. Other Antiobesity Medications

3.7.1. Orlistat

Orlistat (Xenical) is another agent that is used in some countries together with a healthy diet and exercises in youth older than 12 years with a BMI greater than 27 kg/m^2 who have already developed hypertension, T2D, or hyperlipidemia [17]. This drug prevents the absorption of dietary fat by reversibly inactivating gastrointestinal and pancreatic lipases. The inhibition of lipases reduces the hydrolysis of triglycerides. Therefore, free fatty acids are not absorbed [72]. The effectiveness of orlistat was investigated in a double-blind study enrolling 539 individuals with excessive weight who were randomly assigned to the orlistat group or the placebo. After 54 weeks of treatment, a modest BMI decrease was observed in patients on orlistat compared to the control group. Interestingly, there were no significant changes in glucose concentration between the studied groups. Gastrointestinal side effects in those receiving orlistat were high at 50%, and most individuals had very low tolerability of the drug [73]. By inhibiting fat absorption, orlistat could be one of the promising drugs used for weight management, but the rate of adverse effects is relatively high, then it might be discouraging for the patients.

The adverse events of orlistat are closely related to its mechanism of action. Across the studies, the most frequently observed side effects include abdominal pain, fecal spotting, flatus with discharge, fecal urgency, and fatty/oily stool. However, individuals who start following a low-fat diet experienced less severe side effects. Therefore, orlistat might indirectly positively impact eating patterns. It should also be mentioned that patients on this treatment have a greater risk in developing vitamin deficiencies because orlistat affects the absorption of fat-soluble vitamins (A, D, E, and K) [74]. Multivitamin supplements, mainly vitamins A, D, E, and K, are recommended for patients taking orlistat. Finally, postmarketing reports of severe liver injury were reported. After evaluating the observed cases, the FDA did not find a causal relationship between orlistat and acute liver disease; however, relabeling the product and outlining the risks of potential liver injury with its use was recommended.

3.7.2. Phentermine and Topiramate

Phentermine is one of the most recommended antiobesity drugs in adults, yet data on its effectiveness in youth are limited. This drug is an amphetamine derivative that inhibits simulation in the central nervous system. The main effect of phentermine is appetite reduction. Thereby, this medication contributes to weight loss [17,75,76]. In a retrospective medical chart review of children on a weight treatment program in an outpatient clinic, phentermine with lifestyle intervention was superior in weight reduction at one month, three months, and six months among youth with obesity relative to the control group. At three and six months, most participants receiving phentermine experienced a significant BMI reduction [76].

While topiramate is an antiepileptic typically used in adolescents with epilepsy and migraine, this drug also shows a profound effect on weight reduction. Some animal studies indicated that topiramate increases energy expenditure and reduces food intake, decreasing caloric intake. Still, the proper mechanism of topiramate on weight loss has yet to be fully understood [76,77]. In a double-blind trial among adults with obesity complicated by T2D, topiramate was associated with a great decrease in body weight and improvement in glucose profile (fasting and postprandial) and free fatty acid [78]. Recently, the combination of phentermine and extended-release topiramate (Qsymia) was included in weight treatment by the FDA in conjunction with diet and exercise in children $\geq$ 12 years of age with obesity. The pediatric indication was based on data from a 56-week, double-blind, placebo-controlled study conducted in the United States. In this study, 223 children with obesity between 12 and 16 years of age were selected to take mid-dose phentermine/topiramate 7.5 mg/46 mg or high-dose phentermine/topiramate 15 mg/92 mg or placebo as adjunct dietary restriction and exercise. After 56 weeks of treatment, the drop in BMI in individuals receiving high-dose phentermine/topiramate was more significant than in those taking

placebo and mid-dose phentermine/topiramate [79]. Therefore, combining phentermine and extended-release topiramate could be a good option for supporting weight reduction in young individuals with obesity.

The side effects observed in the pediatric population while taking phentermine/topiramate are generally in line with those reported in adult trials testing this agent. The occurrence of adverse drug reactions of phentermine/topiramate depends on its dose. In accordance, treatment side effects were reported in 37% and 52.2% of patients in phentermine/topiramate 7.5 mg/46 mg and 15 mg/92 mg, respectively. The most common adverse events related to phentermine/topiramate are nausea, pyrexia, dizziness, arthralgia, influenza, ligament sprain, and depression [79]. The results of the included evidence-based studies regarding new-generation drugs used in childhood obesity and T2D management are demonstrated in Tables 2 and 3.

Table 2. Summary of included studies on T2D management in youth.

Study ID	Drug	Study Design	Endpoints	Outcome
Zeitler et al. [34]	Metformin (biguanides)	N = 699 youth with overweight and T2D receiving metformin (1000 mg b.i.d.) with HbA1c up to 8% aged 10–17 years Group 1: Metformin (1000 mg × 2/24 h) for 6 months Group 2: Metformin plus RZ (4 mg × 2/24 h) Group 3: Metformin plus lifestyle program	The primary endpoint was a loss of glycemic control, defined as HbA1c > 8% for 6 months or sustained metabolic decompensation requiring insulin	Metformin failed to control T2D in 51.7% of participants. Metformin plus RZ was more effective than metformin alone (51.7% vs. 38.6%). Metformin plus lifestyle intervention significantly differed from metformin alone or metformin plus RZ (46.6%).
Laffel et al. [37]	Empagliflozin (SGLT2 receptor agonists)	N = 157 adolescents with uncontrolled T2D (HbA1c $\geq$ 6.5 to $\leq$10.5%) despite metformin and/or insulin aged 10–17 years Group 1: Empagliflozin 10 mg (o.d.) for 26 weeks. At week 12, when HbA1c $\leq$ 7.0%, individuals were again randomized to remain on 10 mg or increase to 25 mg Group 2: Linagliptin 5 mg (o.d.) Group 3: placebo (o.d.)	The primary outcome was a change from baseline in HbA1c at 26 weeks	Empagliflozin as an adjunct to other treatment methods (diet, exercise, metformin, and/or insulin) significantly reduced HbA1c by 0.8% at week 26 compared with the placebo.
Shehadeh et al. [87]	Dapagliflozin (SGLT2 receptor agonists)	N = 256 adolescents with uncontrolled T2D (HbA1c $\leq$ 10.5%) despite diet and exercise and/or metformin and/or insulin aged 10–17 years Group 1: Dapagliflozin 5 mg (o.d.) for 26 weeks. At week 12, when HbA1c $\leq$ 7.0%, individuals were again randomized to remain on 5 mg or increase to 10 mg Group 2: Saxagliptin 2.5 mg (o.d.) for 26 weeks. At week 12, when HbA1c $\leq$ 7.0%, individuals were again randomized to remain on 2.5 mg or increase to 5 mg. Group 3: placebo for 26 weeks	The primary endpoint was a change in HbA1c at week 26	At 26 weeks, participants assigned to dapagliflozin had a 0.62% point reduction in HbA1c compared with a 0.41% point increase for the placebo group.
Tamborlane et al. [56]	Liraglutide (GLP-1 agonists)	N = 134 adolescents with BMI $\geq$ 85th percentile and uncontrolled T2D [HbA1c < 7.0 to $\leq$11.0% if participants treated with diet and exercise or HbA1c < 6.5 to $\leq$11.0% if they were on metformin (with or without insulin)] aged 10–17 years Group 1: S.C. liraglutide (dose increased 0.6, 0.9, 1.2, and 1.8 mg/24 h) for 26 weeks Group 2: placebo (26 weeks blinded and 26 weeks unblinded)	The primary outcome was a change from baseline in HbA1c at 26 weeks. Secondary endpoints included the change in FPG	At week 26, the mean HbA1c level decreased to 0.64% from a baseline in those on liraglutide and only 0.42% in the placebo group. FPG had reduced at both time points in the liraglutide group but had increased in the placebo group.
Tamborlane et al. [58]	Exenatide (an extended -release version of GLP-1 agonist)	N = 83 adolescents with uncontrolled T2D (HbA1c < 6.5 $\leq$ 11.0% if participants were not taking insulin, or HbA1c% < 6.5 $\leq$ 12.0% if they were insulin, or treated with diet and exercise or in combination with a stable dose of an oral antidiabetic drug and/or insulin for at least 2 months. Group 1: S.C. exenatide (2 mg q.w.) for 24 weeks Group 2: placebo for 24 weeks	The primary endpoint was a change in HbA1c at week 24	At 24 weeks, the least squares mean change in HbA1c was −0.36% for the exenatide and +0.49% for the placebo.

Table 2. *Cont.*

Study ID	Drug	Study Design	Endpoints	Outcome
Arslanian et al. [59]	Dulaglutide (GLP-1 agonists)	N = 154 adolescents with uncontrolled T2D, HbA1c > 6.5% to <11%, treated with diet and exercise, with or without metformin and/or basal insulin, aged 10–17 years Group 1: S.C. dulaglutide (0.75 mg q.w.) for 26 weeks Group 2: S.C. dulaglutide (1.5 mg q.w) Group 3: placebo	The primary endpoint was the change from baseline in HbA1c level at 26 weeks; secondary endpoints included HbA1c < 7.0% and changes from baseline in the FPG and BMI	At 26 weeks, more participants on dulaglutide achieved HbA1c < 7% than in the placebo group (51% vs. 14%). FPG increased in the placebo group and decreased in the pooled dulaglutide groups, and there were no between-group differences in the change in BMI.

Abbreviations: T2D, type 2 diabetes; b.i.d., twice a day; HbA1c, glycated hemoglobin; RZ, rosiglitazone; SGLT2, sodium-glucose transporter 2 inhibitors; o.d., once a day; BMI, body mass index; GLP-1, glucagon-like peptide-1; S.C., subcutaneous; FPG, fasting plasma glucose; q.w., once a week.

Table 3. Summary of included studies of new agents for treating adolescents with obesity.

Study ID	Drug	Study Design	Endpoints	Outcome
Kelly et al. [57]	Liraglutide (GLP-1 agonists)	N = 251 adolescents with BMI ≥ 95th percentile and a poor response to lifestyle therapy aged 12–17 years Group 1: S.C. liraglutide (3 mg/24 h) for 26 weeks, followed by a 26-week observational period Group 2: placebo in addition to lifestyle intervention for a 56-week treatment period	The primary endpoint was the change from baseline in the BMI-SD at week 56	Participants on liraglutide had a greater decrease in BMI-SD than those from the placebo group (43.3% vs. 18.7%).
Weghuber et al. [60]	Semaglutide (GLP-1 agonists)	N = 201 adolescents with BMI ≥95th percentile or >85th percentile with 1 or more weight-related comorbidities: HA, dyslipidemia, obstructive sleep apnea, or T2D, and a poor response to lifestyle, aged 12–17 years Group 1: S.C. semaglutide (2.4 mg q.w.) for 68 weeks Group 2: placebo in conjunction with diet and physical activity modifications for a 68-week period	The primary endpoint was the percentage change in BMI from baseline to week 68; the secondary endpoint was a decrease in body weight of at least 5%	The mean change in BMI was −16.1% with semaglutide and 0.6% with placebo. At the end of the study, 73% of participants on semaglutide had a weight loss of 5% or more, as compared with 18% in the placebo group.
Chanoine et al. [73]	Orlistat (lipase inhibitor)	N = 593 adolescents with BMI ≥ 2 units above the 95th percentile, aged 12–16 years Group 1: a 120 mg dose of orlistat 3 times daily for 1 year, as an adjunct to a hypocaloric diet (30% fat calories) and lifestyle modifications Group 2: placebo for 1 year, as an adjunct to hypocaloric diet (30% fat calories) and lifestyle modifications	The primary endpoint was the percentage change in BMI from baseline to the end of the study; secondary measures included changes in WHR, weight loss, lipid measurements, and glucose and insulin responses to the OGTT	At the end of the study, BMI had decreased by 0.55 with orlistat but increased by 0.31 with placebo. WHR decreased in the orlistat group but increased in the placebo group.
Kelly et al. [79]	PHEN/TPM (anorectics/ anticonvulsants)	N = 223 adolescents with obesity, BMI ≥ 95th percentile, and a poor response to lifestyle therapy alone aged 12–16 Group 1: mid-dose PHEN/TPM (7.5 mg/46 mg, o.d.) plus lifestyle therapy for 56 weeks Group 2: top-dose PHEN/TPM (15 mg/92 mg, o.d.) plus lifestyle therapy for 56 weeks Group 3: placebo plus lifestyle therapy for 56 weeks	The primary endpoint was the mean percent change in BMI from baseline to week 56	The primary outcome of percent change in BMI at week 56 showed differences from placebo of −10.44 percentage points and −8.11 percentage points for the top and mid doses of PHEN/TPM, respectively.

Abbreviations: GLP-1, glucagon-like peptide-1; BMI, body mass index; BMI-SD, body mass index- standard deviation; HA, hypertension; T2D, type 2 diabetes; q.w., once a week; S.C., subcutaneous; WHR, waist-to-hip ratio; OGTT, oral glucose tolerance test; o.d., once a day; PHEN/TPM, phentermine/topiramate.

The characteristics of the most recent pharmacologic agents approved for treating obesity and T2D in youth are summarized in Table 4.

Table 4. Most recent pharmacologic agents approved for treating obesity and T2D in youth [34,35,38–41,47,85].

Medication	Indication	Benefits	Potential Side Effects	Approval FDA/EMA
SGLT2 inhibitors (empagliflozin/dapagliflozin)	As an adjunct to diet and exercise to improve glycemic control in children aged 10 years and older with T2D	Beneficial effect on HbA1c	UTI, female/male genital mycotic infections, URTIs, polyuria, back pain, nausea, dyslipidemia, increases serum creatinine and decreases eGFR, renal impairment, necrotizing fasciitis of the perineum, and DKA.	FDA/EMA
	Liraglutide (Victoza, 0.6–1.8 mg daily): T2D in children $\geq$ 10 years	Beneficial effect on HbA1c and weight loss, reduction in the risk of T2D complications	Gastrointestinal nausea, vomiting, and diarrhea. Hypoglycemia (S). Warnings: personal or family history of medullary thyroid carcinoma or in patients with MEN2, pregnancy.	FDA/EMA
GLP-1 receptor agonist	Liraglutide (Saxenda, 3 mg a day): weight management in adolescents $\geq$ 12 years	Beneficial effect on weight loss	Cautions: acute pancreatitis, acute events of gallbladder disease, and renal impairment related to dehydration.	
	Exenatide (BYDUREON BCise): T2D in children $\geq$ 10 years	Beneficial effect on HbA1c	The same as liraglutide plus injection site nodule.	
	Dulaglutide (Trulicity): T2D in children $\geq$ 10 years	Beneficial effect on glycemic control	The same as liraglutide plus DR progression among patients with a history of DR.	
	Semaglutide (Wegovy): weight management in adolescents $\geq$ 12 yrs	Beneficial effect on weight loss	The same as liraglutide plus hypoglycemia.	
Orlistat (Xenical)	Weight management for adolescents 12 years and older	Beneficial effect on weight loss	Oily spotting, abdominal pain, nausea, fatty/oil stool, reduced absorption of fat-soluble vitamins, and liver failure. Contraindications: chronic malabsorption syndrome, cholestasis. Cautions: If a meal is missed or contains no fat, the dose should be omitted, and a multivitamin supplement is recommended.	FDA
Phentermine/ topiramate (Qsymia)	Weight management for adolescents 12 years and older	Beneficial effect on weight loss	Insomnia, dry mouth, increased heart rate, anxiety, increased blood pressure, cognitive dysfunction, metabolic acidosis, teratogenicity, and kidney stones. Contraindications: history of CVD, glaucoma, agitated states, hyperthyroidism, history of DA, use of MAOIs within the preceding 14 days.	FDA

Abbreviations: SGLT2, sodium-glucose transporter 2 inhibitors; T2D, type 2 diabetes; HbA1c, glycated hemoglobin; UTI, urinary tract infection; URTIs, upper respiratory tract infections; eGFR, estimated glomerular filtration rate; DKA, diabetic ketoacidosis; FDA, U.S. Food and Drug Administration; EMA, European Medicines Agency; GLP-1, glucagon-like peptide-1; MEN2, multiple endocrine neoplasia syndrome type 2; DR, diabetes retinopathy; CVD, cardiovascular disease; MAOIs, monoamine oxidase inhibitors; DA, drug abuse.

3.8. Bariatric Surgery

In children experiencing obesity and its health consequences, weight-loss surgery was proven to be a safe and efficient option to manage weight. Nowadays, bariatric surgery is one of the most effective approaches for substantial and persistent weight reduction and improving coexisting complications. However, the few existing guidelines on bariatric surgery for children and adolescents give somewhat different recommendations. In 2015, the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) advised bariatric surgery as an option for adolescents who had achieved 95% of the expected height and a BMI $\geq$ 40 kg/m^2 with severe comorbidities and BMI $\geq$ 50 kg/m^2 with mild comorbidities [80]. According to the latest evidence on weight management and prevention of cardiovascular risk factors, the American Society for Metabolic and Bariatric Surgery (ASMBS) and the American Academy of Pediatrics (AAP) produced a consensus that recommends antiobesity surgery in children and adolescents who suffer from moderate obesity (BMI of 35 kg/m^2 or higher) with significant health complications related to obesity or severe obesity (40 kg/m^2) [4,81]. However, the decision regarding bariatric surgery is typically a multidisciplinary process and involves various healthcare professionals to ensure comprehensive evaluation and care for the patient. Usually, this multidisciplinary team includes pediatricians, endocrinologists, pediatric surgeons, dietitians, psychologists,

and psychiatrists. The team ensures that the child meets specific criteria, including age, weight, health status, and psychosocial factors. Parental consent and involvement in the decision-making process are also crucial. The ultimate goal is to ensure that the benefits of the surgery outweigh the risks and that it is in the best interest of the child's long-term health [5,80,81].

Currently, antiobesity surgery is related to substantial body mass reduction and remission of T2D in youth. In pediatric populations, two main types of weight-loss surgery are commonly recommended: the laparoscopic sleeve gastrectomy and the Roux-en-Y gastric bypass. The largest study testing bariatric surgery's effectiveness in youth, the Teen Longitudinal Assessment of Bariatric Surgery (Teen-LABS), showed sustainable effects on weight reduction, improved cardiometabolic health, and slowed disease progress. The Teen-LABS study is the first trial, which included 242 youth with obesity who underwent Roux-en-Y gastric bypass or sleeve gastrectomy between 2007 and 2012. At the beginning of the study, the mean age of participants was 17 years, and the initial BMI was estimated at around 53 kg/m^2. Three years after the bariatric procedure, the body mass, the appearance of obesity-related conditions, and other risk factors of cardiometabolic diseases were evaluated. At the end of the follow-up period, a significant weight reduction was observed in both groups. However, the two procedures performed had no difference in weight loss. Then, this study showed that Roux-en-Y gastric bypass and sleeve gastrectomy have similar efficacy in treating children and adolescents with obesity. In addition, coexisting conditions such as dyslipidemia, hypertension, and T2D were significantly decreased or even in remission within three years [89]. Finally, compared to the outcomes of two large studies in adolescents with obesity, the TODAY study (intensive nonsurgical treatment) vs. the Teen-LABS study, bariatric surgery was significantly more effective than pharmacological management. Adolescents with T2D who underwent bariatric surgery experienced a greater decrease in BMI than those matched by baseline age (13–18 years), race, sex, ethnicity, and baseline BMI (>35 kg/m^2) medical controls from the TODAY study (29% vs. 3.7%). Also, participants from the Teen-LABS study had a greater reduction in the average HbA1c levels. In detail, HbA1c concentration declined from 6.8 to 5.5% in Teen-LABS participants, while in the TODAY group, HbA1c rose from 6.4 to 7.8% during two years [83]. Finally, it seems that having earlier bariatric surgery might have better results than waiting until adulthood. Five years after surgical intervention, adolescents had greater results in maintaining weight and treating other weight-related conditions than adults receiving the same procedure in the LABS (Longitudinal Assessment of Bariatric Surgery) study. Most adults from the LABS study already suffered from obesity in childhood but did not receive surgery until they were adults [84]. Nutritional deficiencies are the main concern in developing adolescents who receive surgical treatment. Over the period of 2 years after surgery, low ferritin levels were found in 57%, while deficiency of B12 or any other vitamins was reported in 35% and 16% of participants, respectively [85]. Therefore, after bariatric surgery, most adolescents will require vitamin supplementation. Moreover, bariatric surgery in adolescents might develop other long-term postoperative complications. The most observed complications include gastroesophageal reflux disease (GERD), hiatal hernia, and treatment failure requiring operative revision. Less common adverse effects are liver necrosis, gallbladder disorders, pancreatic disorders, acute kidney failure, and neuromuscular or skin complications. Finally, GERD and hiatal hernia may lead to Barrett's esophagus; therefore, youth after bariatric surgery are at risk in developing esophageal adenocarcinoma [89].

4. Strengths and Limitations of the Review

The strength of the present narrative review is that it provides a practical summarization of the available knowledge on the most recent therapeutic options for treating adolescents with obesity and T2D. In detail, this work discusses the most significant trials assessing the efficacy and effectiveness of the lately approved agents. It also provides the indication for each drug and evaluates its potential benefits as well as adverse effects. Thus,

it gives a broader perspective and hints at the possible treatment methods in the pediatric population affected by obesity and T2D. Mainly, it might be valuable for everyday clinical practice. However, concerning the limitations of our study, this work is not a systematic review. It may not cover all relevant issues or include every pertinent study, potentially leaving out some aspects of the complex management of obesity and T2D in youth. The review's findings are based on selected literature and expert opinion, which may not fully represent the breadth of evidence available in comprehensive guidelines. Consequently, while the review provides valuable insights, it should be considered alongside more exhaustive systematic reviews and clinical practice standards for a complete understanding of childhood obesity and T2D management.

5. Conclusions

The incidence of T2D in the pediatric population is rising, driven by increasing levels of excess weight and obesity. Youth-onset T2D is characterized by lower insulin sensitivity, insulin hypersecretion, and rapid deterioration in beta-cell function compared to adult-onset T2D. In consequence, children and adolescents with early-onset T2D have a more rapid progression of microvascular and cardiovascular disease than young individuals with type 1 diabetes and those with adult-onset T2D. Unfortunately, the benefits of lifestyle interventions observed in clinical trials might be challenging to translate to real-world settings, and most young people do not achieve set goals in everyday clinical practice. Early recognition and management of childhood obesity should aim at the prevention of its comorbidities, including T2D. A multifaceted approach to childhood obesity and T2D is essential, and young people should receive intensive treatment as early as possible, including the use of newer pharmacological agents in conjunction with behavior and lifestyle modifications. Nevertheless, when choosing a second-line drug, one should consider the glycemic target, dosage schedule, route of administration, impact on weight, adverse effects, and possible comorbidities. In certain circumstances, treatment might include weight-loss surgery. In particular, antiobesity surgery is related to significant weight reduction, remission of T2D, and lower overall mortality in youth.

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A Combination of a Dopamine Receptor 2 Agonist and a Kappa Opioid Receptor Antagonist Synergistically Reduces Weight in Diet-Induced Obese Rodents

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Abstract: As the global obesity rate increases, so does the urgency to find effective anti-obesity drugs. In the search for therapeutic targets, central nervous system (CNS) mechanisms engaged in the regulation of energy expenditure and food intake, such as the opioid and dopamine systems, are crucial. In this study, we examined the effect on body weight of two drugs: bromocriptine (BC), a D2R receptor agonist, and PF-04455242, a selective κ opioid receptor (KOR) antagonist. Using diet-induced obese (DIO) rats, we aimed to ascertain whether the administration of BC and PF-04455242, independently or in combination, could enhance body weight loss. Furthermore, the present work demonstrates that the peripheral coadministration of BC and PF-04455242 enhances the reduction of weight in DIO rats and leads to a decrease in adiposity in a food-intake-independent manner. These effects were based on heightened energy expenditure, particularly through the activation of brown adipose tissue (BAT) thermogenesis. Overall, our findings indicate that the combination of BC and PF-04455242 effectively induces body weight loss through increased energy expenditure by increasing thermogenic activity and highlight the importance of the combined use of drugs to combat obesity.

Keywords: obesity; pharmacological treatment; weight loss; synergistic treatment

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1. Introduction

Obesity is the most common metabolic disorder worldwide, and its prevalence has reached epidemic levels [1]. Projections indicate that the global population's obesity prevalence will increase and reach approximately 60% by 2030 [2], which highlights the necessity of developing new preventive and therapeutic anti-obesity drugs.

Over the past few years, the search for new treatments for weight loss has led to the development of several drugs. Some of them, including orlistat [3], phentermine–topiramate, naltrexone–bupropion, liraglutide, and semaglutide [4], have received approval from the FDA. In recent years, GLP-1 agonists, such as liraglutide and semaglutide, have evolved, and dual glucose-dependent insulinotropic polypeptides (GIP/GLP-1), dual GLP-1/glucagon receptor agonists, and triple agonists have recently been developed [5]. All these compounds promote weight loss and improve glucose tolerance in patients with diabetes by stimulating insulin production and promoting satiety [6,7]. The strategy employed by two of the other FDA-approved drugs involves the combined administration of substances affecting the central nervous system (CNS). Specifically, phentermine–topiramate combines a sympathomimetic, which suppresses appetite through the elevation of norepinephrine levels, with a gamma-aminobutyric acid (GABA) receptor agonist [6,8]. Another example is naltrexone–bupropion, which combines an opioid receptor antagonist with a dual norepinephrine and dopamine reuptake inhibitor, thereby promoting weight loss [9]. The combination of drugs in the treatment of obesity has prompted extensive research into both homeostatic and hedonic/reward mechanisms controlled by the CNS.

The dopamine system can modulate food intake through both reward (hedonic) and hypothalamic (homeostatic) pathways [10]. Indeed, the availability of the dopamine D2 receptor (D2R) showed a proportional decrease in obese individuals corresponding to their body mass index (BMI) [11]. Specifically, the significance of D2R agonists, namely cabergoline and bromocriptine (BC), to reduce body weight has been demonstrated [10,12], and they have been approved in the United States as an adjunctive treatment for type 2 diabetes [13]. In particular, rodent studies have proven that central administration of BC reduces body weight and fat accumulation, increasing energy expenditure, and promoting thermogenesis in brown adipose tissue (BAT) in obese animals [10]. It also enhances glucose tolerance and reduces levels of fasting and postprandial plasma glucose in individuals with diabetes [12,14].

Another well-known central system involved in hedonic/reward mechanisms is the opioid system, which regulates appetite and energy balance [15]. The μ, δ, and κ opioid receptors (MOR, DOR, and KOR) are a family of G-coupled protein receptors that are extensively spread across the CNS [16,17]. Recent data indicate that opioid receptors could modulate and control energy balance. For example, naltrexone, mentioned earlier, used with bupropion, produces weight loss [18]. This highlights the beneficial effects of using opioid antagonists in conjunction with other treatments for weight management. Specifically, the role of KOR in the control of energy homeostasis has been proven, as it has been observed that dynorphin (an endogenous ligand of KOR) controls food intake by increasing CNS activity [19]. Additionally, KOR controls the metabolic response to a high-energy diet [20], and hypothalamic KOR modulates the orexigenic effects of ghrelin [21] and melanin-concentrating hormone (MCH) [22]. Furthermore, it has been demonstrated that KOR receptors mediate the action of nicotine by inducing thermogenesis and browning [23]. KOR receptors can also ameliorate obesity caused by estrogens by increasing energy expenditure [24], as was demonstrated using a selective pharmacological blocker of the KOR system, PF-04455242 [25].

Exploring combined therapies that leverage synergistic mechanisms to increase energy expenditure is crucial. It is important to consider the existence of cooperative systems, which are essential for the development of innovative drugs to address obesity [26]. For this reason, we decided to explore the synergistic effect of BC, a D2R agonist, and a selective antagonist of KOR named PF-04455242. Both compounds can reduce body weight in animals, but it is currently unknown whether they have combined action on body weight metabolism or energy intake. In this study, we demonstrated that peripheral coadministration of both compounds is able to increase weight loss in diet-induced obese (DIO) rats independently of food intake. Furthermore, we show that the combined action of BC and PF-04455242 on body weight occurs through an increase in energy expenditure, triggering the thermogenic program in the BAT. Due to the synergistic effects of both drugs, we observed a remarkable enhancement in body weight reduction in DIO rats, suggesting a significant advancement in the development of a promising combination therapy for the treatment of obesity.

2. Materials and Methods

2.1. Animal Model and Diets

Adult male Sprague Dawley rats (8–10 weeks old, 250–350 g) were employed in the study. The rats were kept in an environment with a 12 h light/12 h dark cycle, maintaining controlled temperature and humidity conditions. Throughout the experimental period, the rats were provided unrestricted access to water and either a standard laboratory chow diet (Scientific Animal Food & Engineering; comprising 16% protein, 60% carbohydrate, and 3% fat) or a high-fat diet (HFD) (Research Diets 12492; containing 60% calories from fat, 5.24 kcal/g; Research Diets, New Brunswick, NJ, USA) for 12 weeks. All protocols and interventions involving animals were subjected to thorough review and approval by the Ethics Committee of the University of Santiago de Compostela (15010/14/007) adhering to European Union regulations governing the utilization of experimental animals.

2.2. Treatments and Surgeries

2.2.1. Intracerebroventricular Treatment

Rats were anesthetized through an intraperitoneal injection of ketamine (100 mg per kilogram body weight (BW)) + xylazine (15 mg per kilogram BW). A stereotaxic surgery procedure was employed to implant an intracerebrovascular (ICV) cannula in the lateral ventricle of the hypothalamus (coordinates: 1.3 mm posterior to bregma and 1.9 mm lateral to the midsagittal suture at a depth of 3.5 mm), as previously described [27]. After this procedure, the animals were individually housed for a 4-day acclimatation period prior to the experiment, allowing them to recover from surgery. The administration of ICV vehicle (DMSO 100 mM), bromocriptine mesylate (40 µg per rat; Tocris, St. Louis, MO, USA), or PF-04455242 hydrochloride (1.39 µg per rat; Tocris, St. Louis, MO, USA) was performed using a 22-gauge needle (Hamilton; Reno, NV, USA) through the implanted cannulas.

2.2.2. Intraperitoneal Treatment

Rats received an acute (24 h) or chronic daily (10 days) intraperitoneal administration (IP) of vehicle (DMSO 100 mM), bromocriptine mesylate (0.625, 1.25, 2.5, 5, 10, and 20 mg/kg per rat; Tocris, St. Louis, MO, USA), or PF-04455242 hydrochloride (0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/kg per rat; Tocris, St. Louis, MO, USA).

2.2.3. Weight Measurements

In all experiments, daily measurements were taken for food intake and body weight. The animals were euthanatized, and the BAT and liver were weighed rapidly postmortem. Additionally, the 24 h fecal output was measured, and the tissue weights as well as faecal output values were corrected for the body weight of the animal for subsequent analysis.

2.2.4. Nuclear Magnetic Resonance

We recorded body composition, including fat and lean mass, using nuclear magnetic resonance imaging (Whole Body Composition Analyzer; EchoMRI; Houston, TX, USA) as previously described [23,28].

2.2.5. Temperature Measurements and Thermal Imaging

The recording of body temperature was performed using a rectal probe connected to a digital thermometer (BAT-12 Microprobe-Thermometer; Physitemp, Clifton, NJ, USA). The measurement of interscapular temperature was accomplished utilizing a high-resolution infrared camera (E60bx: Compact Infrared Thermal Imaging Camera; FLIR, Wilsonville, OR, USA). The subsequent analysis of the images was conducted using an FLIR Tools-specific software package (version number 5.13) [29].

2.2.6. Indirect Calorimetry

Animals underwent analysis for energy expenditure (EE), respiratory quotient (RQ), and locomotor activity (LA) using a calorimetric system (LabMaster; TSE Systems; Bad Homburg, Germany) [10,27]. The animals were positioned in a temperature-controlled (24 °C) chamber supplied with air circulation. Following the calibration of the system with reference gases (20.9% O_2, 0.05% CO_2, and 79.05% N_2), metabolic rate measurements were recorded at 30 min intervals. Prior to starting the measurements, the animals were acclimated for 48 h, and data collected during the final 48 h were utilized for calculating all metabolic parameters.

2.3. Statistical Analysis

Data are expressed as the mean ± standard error of the mean (SEM). Group differences were assessed for statistical significance using a two-tailed unpaired Student's t-test or one- or two-way analysis of variance (ANOVA) combined with Tukey's post hoc test. Statistical significance was established at a p-value < 0.05 (GraphPad Prism 8.0). Specific statistical information and experimental sample sizes (n) are provided in the figure legends.

3. Results

3.1. Central Administration of BC and PF-04455242 Decreases Body Weight in Combination and Individually

Initially, ICV cannulas were implanted, and the rats were systematically grouped into four categories to ensure uniformity in initial body weight, as depicted in Figure 1A. In keeping with previous studies [10,30], central BC (40 µg/rat) and PF-04455242 (1.39 µg/rat) administration significantly decreased body weight (Figure 1B). To assess whether both compounds could improve body weight in DIO rats when administered together, we ICV coinjected them, and we observed a similar weight loss compared to the single-drug treatments and a concordant increase compared to the control group (Figure 1B). This same pattern is observed when the data are represented as the percentage of body mass loss (Figure 1B). Despite the apparent similarity in the decreasing trend of intake and body weight, the difference in intake is not statistically significant compared to the control group, suggesting that the effects on body weight balance occur in a food-independent manner (Figure 1C). Thus, the effects of the two compounds did not synergize, and we did not find a cumulative reduction in body mass when coadministered centrally in obese animals.

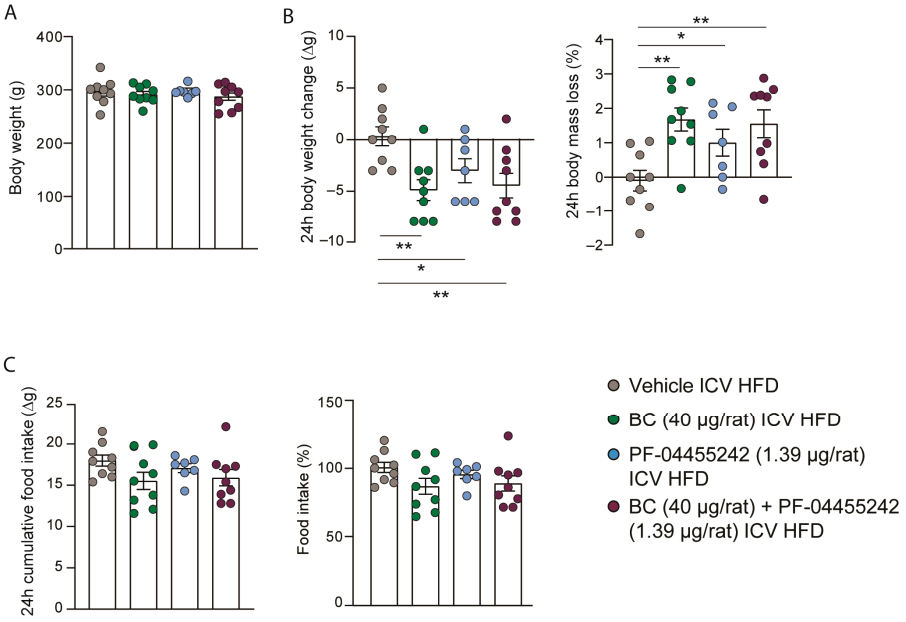

Figure 1. (**A**) Initial weight of the rats evenly distributed among the experimental groups; (**B**) effect at 24 h of intracerebroventricular (ICV) bromocriptine (BC) (40 µg/rat), PF-04455242 (1.39 µg/rat), and BC (40 µg/rat) + PF-04455242 (1.39 µg/rat) injection on body weight change and body mass loss; (**C**) cumulative food intake in 24 h and percentage of food intake compared to control rats in diet-induced obese (DIO) rats. Data are expressed as the mean ± SEM; statistical differences were evaluated using a two-tailed Student's *t*-test; $n = 7–9$ animals per group. * $p < 0.05$, ** $p < 0.01$ vs. vehicle.

3.2. Peripheral Administration of BC and PF-04455242 Increases Body Weight Loss in a Dose-Dependent Manner

Next, we aimed to assess whether these compounds, which were already demonstrated to reduce body weight when administered centrally, were capable of influencing body weight when administered peripherally. Initially, a homogeneous distribution of the animals' weights was carried out to ensure no initial differences in the peripheral

BC dose-response experiment (Figure 2A). A single intraperitoneal (IP) injection of BC (2.5 and 5 mg/kg) significantly reduced body weight and increased body mass loss after 24 h independently of food intake (Figure 2B,C), while higher doses of BC (10 and 20 mg/kg) reduced body weight accompanied by a decrease in food intake (Figure 2B,C). On the other hand, in the dose-response study for PF-04455242, which again was performed with rats with a consistent weight distribution (Figure 2D), we observed that the acute IP administration of PF-04455242 (2.5 and 5 mg/kg) induced a decrease in body weight and, therefore, a higher body mass loss without affecting intake (Figure 2E,F); however, higher doses (10 mg/kg) were incapable of affecting either body weight or intake (Figure 2E,F).

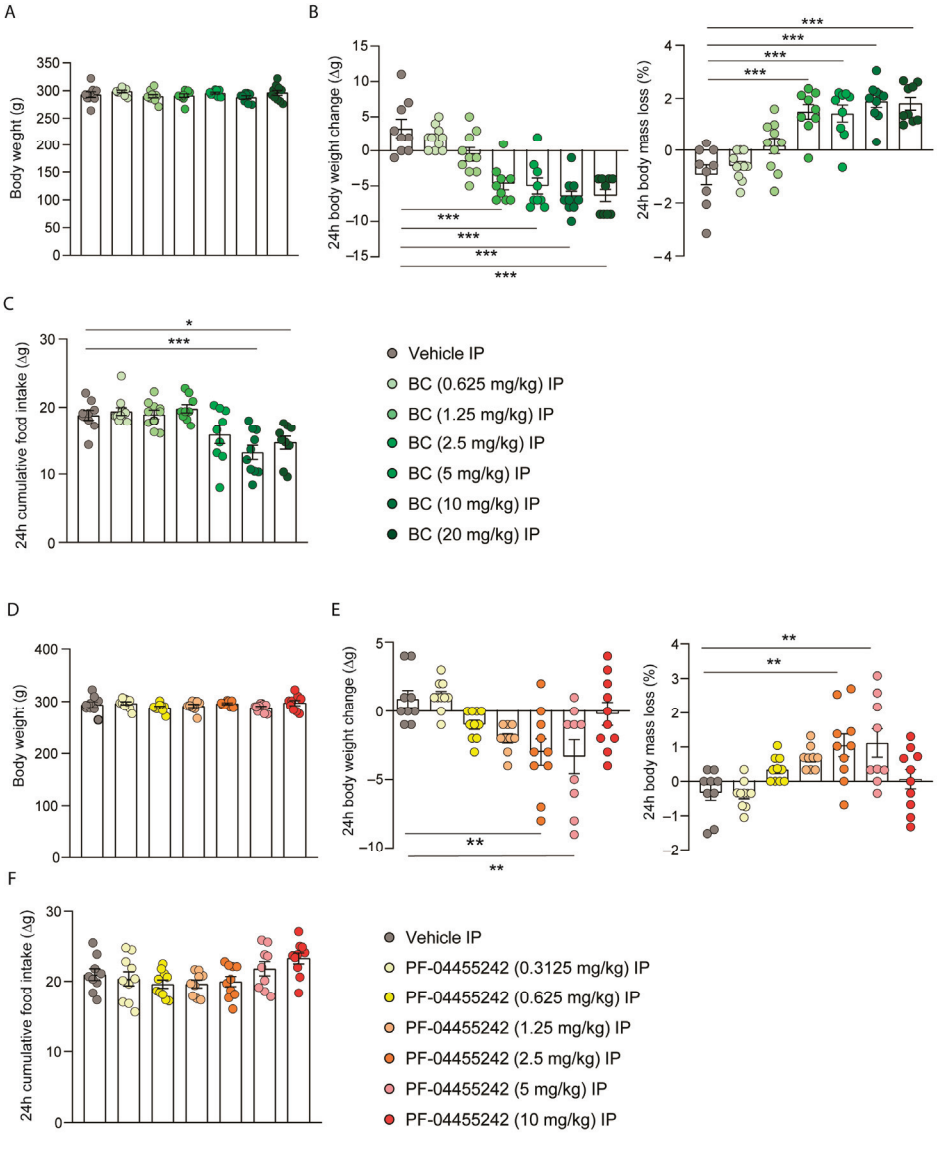

Figure 2. (**A**) Initial weight of the rats distributed among the BC experimental groups. (**B**) Effect at 24 h of a dose-response to intraperitoneal (IP) injection of bromocriptine (BC) (0.625, 1.25, 2.5, 5, 10,

and 20 mg/kg) on body weight change and body mass loss and (**C**) cumulative food intake in male rats fed a chow diet (n = 9–11 each group). (**D**) Initial weight of the rats distributed among the PF-04455242 experimental groups. (**E**) Effect at 24 h of a dose response to intraperitoneal injection of PF-04455242 (0.3125, 0.625, 1.25, 2.5, 5, and 10 mg/kg) on body weight change and body mass loss and (**F**) cumulative food intake in male rats fed a chow diet (n = 9–10 each group). Data are expressed as the mean ± SEM; statistical differences were evaluated using a one-way ANOVA followed by Tukey's multiple comparisons test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle.

3.3. Acute Peripheral Combination of BC and PF-04455242 Causes Synergistic Increased Body Weight Loss in Diet-Induced Obese (DIO) Rats

According to the previously obtained IP dose-response data for both compounds, we selected the dose of BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg). We decided to choose these two doses because they represent the minimum doses of BC and PF-04455242 at which we observed a trend toward a decrease in body weight, although without statistically significant changes. Additionally, we aimed to investigate whether, at these doses, the compounds (independently or when administered together) could impact body weight in obesity. To address this, we administered an HFD to rats for 12 weeks, during which we observed a significant increase in their body weight compared to rats on a normal diet (Figure 3A). Next, we organized the rats into four groups with comparable average body weights (Figure 3B). Following the intraperitoneal administration of the two compounds separately to DIO rats over a 24 h period, we observed no significant effect on body weight, body weight loss, or food intake (Figure 3C,D). However, the acute peripheral combination of both BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg) resulted in a synergistic decrease in body weight accompanied by a consequentially higher body mass loss (Figure 3C) in a food-intake-independent manner (Figure 3D) 24 h after the IP administration of both compounds in DIO rats. These data highlight that the peripheral administration of BC and PF individually at the chosen doses is unable to affect the energy metabolism in both lean and obese rats. By contrast, the combined administration of both drugs at these minimal doses can effectively reduce body weight in obese rats. Therefore, we aimed to investigate whether the cotreatment of rats with BC and PF-04455242 alters body composition. We observed that, in parallel with the increased weight loss, fat mass loss was also significantly higher in IP-cotreated animals for 24 h compared with the control group (Figure 3E), without alterations in lean mass (Figure 3F). Because of the decreased weight gain and adiposity, we next explored the thermogenic profile, and we found a significant increase in the interscapular BAT temperature (Figure 3G), whereas body temperature remained unaltered (Figure 3H). These results suggest that acute BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg) combination treatment controls body weight by inducing thermogenesis in DIO rats.

3.4. Chronic Peripheral Coadministration of BC and PF-04455242 Reduces Body Weight and Adiposity Independently of Food Intake in DIO Rats

We next investigated whether the effects of the peripheral combination of BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg) may be long-lasting. Initially, we organized the groups to ensure no differences in weight before initiating the chronic treatment in DIO rats (Figure 4A). Subsequently, we chronically administered both compounds for 10 days in rats fed an HFD. We found that body weight loss was significantly lower in rats treated with BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg) independently (Figure 4B). By contrast, we observed that the weight loss was much greater when we coadministered both compounds, BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg), together (Figure 4B). Furthermore, the percentage of body mass loss compared to the control was only significant when we cotreated with the two drugs and not with their individual administration to DIO rats (Figure 4B). However, cumulative food intake did not exhibit any statistically significant differences between treatment groups (Figure 4C). Moreover, we did not observe changes in fat mass with the individual treatment of BC (1.25 mg/kg) and PF-04455242

(0.625 mg/kg) independently, but we found a significant reduction in adiposity after 10 days of peripheral cotreatment with both compounds (Figure 4D) in obese rats. We also evaluated the possible role of the vagus nerve in the body weight changes observed. Interestingly, we did not find alterations in the fecal output in DIO rats (Figure 4E) treated with BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg), nor did we find changes in liver weight (Figure 4F). Nevertheless, in keeping with the lower body weight seen with the coadministration of BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg), these animals displayed increased BAT weight in comparison with the control group (Figure 4G). These findings indicate that cotreatment with BC and PF-04455242 increases the activity of BAT in DIO rats, promoting weight loss in a feeding-independent manner.

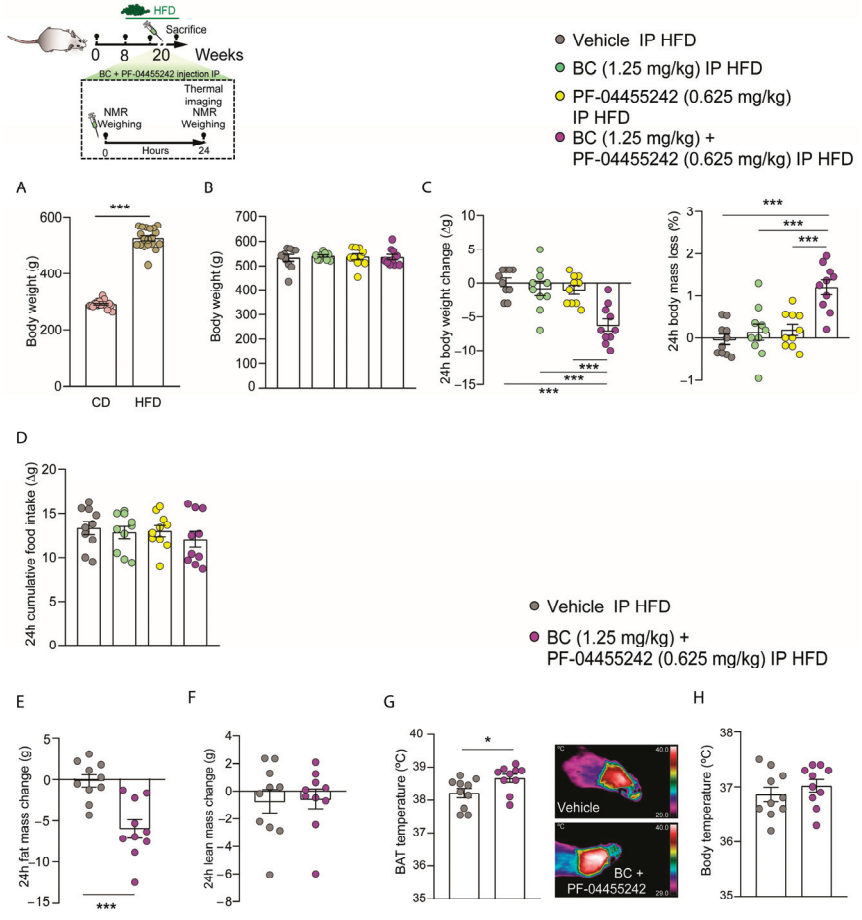

Figure 3. Schematic representation of the in vivo protocol. (**A**) Comparison of total body weight between rats fed a normal diet and those on an HFD for 12 weeks. (**B**) Initial weight of the rats distributed among the experimental groups. (**C**) Effect at 24 h of acute intraperitoneal (IP) bromocriptine (BC) (1.25 mg/kg), PF-04455242 (0.625 mg/kg), and BC (1.25 mg/kg) + PF-04455242 (0.625 mg/kg) injection on body weight change, body mass loss, and (**D**) cumulative food intake in diet-induced obese (DIO) rats. Effect at 24 h of acute intraperitoneal (IP) BC (1.25 mg/kg) + PF-04455242 (0.625 mg/kg) injection on (**E**) fat mass change, (**F**) lean mass change, (**G**) brown adipose tissue (BAT) temperature, and (**H**) body temperature in diet-induced obese (-DIO) rats. Data are expressed as the mean ± SEM; statistical differences were evaluated using a one- or two-tailed Student's *t*-test; n = 10–20 animals per group. * $p < 0.05$, *** $p < 0.001$ vs. vehicle.

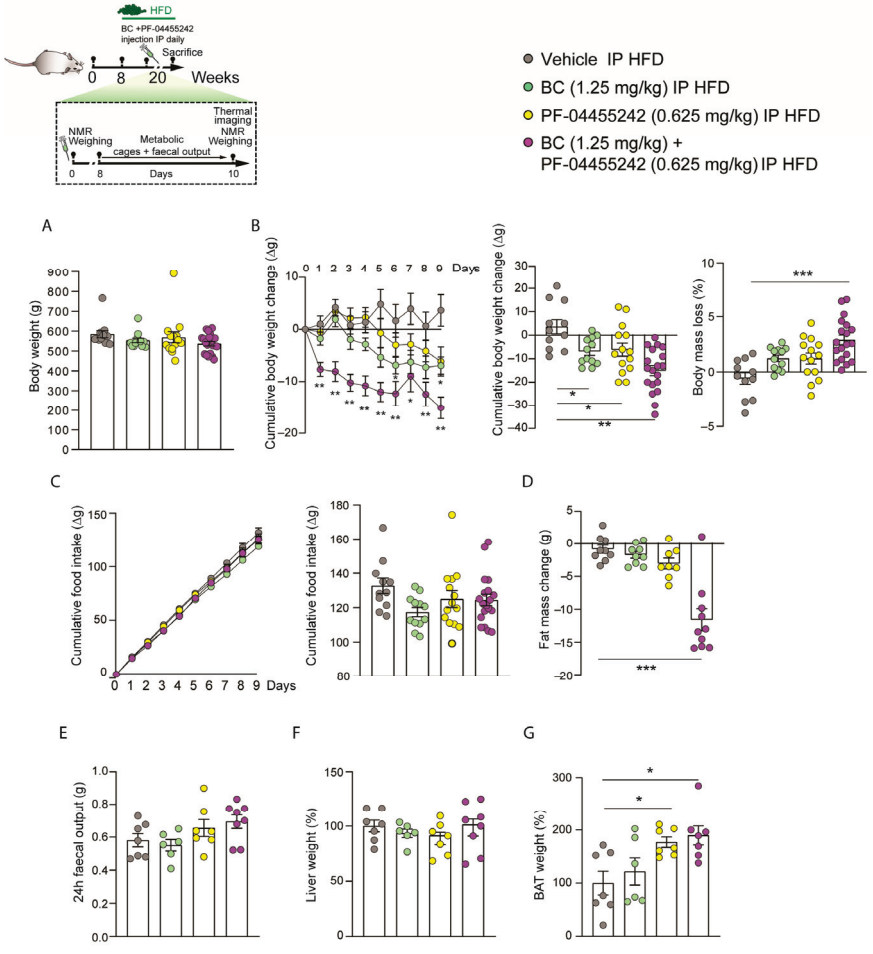

Figure 4. Schematic representation of the in vivo protocol. (**A**) Initial weight of the rats distributed among the experimental groups. (**B**) Effect of a 10-day intraperitoneal (IP) injection of bromocriptine (BC) (1.25 mg/kg), PF-04455242 (0.625 mg/kg), and BC (1.25 mg/kg) + PF-04455242 (0.625 mg/kg) on body weight change and body mass loss (**C**) cumulative food intake, (**D**) fat mass change, (**E**) fecal output, (**F**) liver weight, and (**G**) brown adipose tissue (BAT) weight in diet-induced obese (DIO) rats. Data are expressed as the mean ± SEM; statistical differences were evaluated using a one- or two-way ANOVA followed by Tukey's multiple comparisons test; n = 6–19 animals per group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs. vehicle.

3.5. Chronic Peripheral Cotreatment with BC and PF-04455242 Induces Negative Energy Balance, Triggering Thermogenesis in DIO Rats

To clarify how the coadministration of BC and PF-04455242 exerted its effects on reducing body weight and adiposity in DIO rats, the animals were monitored through the indirect calorimetry system. No differences were noted in the respiratory quotient (Figure 5A) or locomotor activity (Figure 5B) when compared to animals treated with the vehicle. However, in agreement with the decreased weight, energy expenditure was increased in DIO rats cotreated with BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg) for 10 days (Figure 5C). Although no differences were found in body temperature (Figure 5D), analyzing the interscapular temperature of BAT reveals that the individual treatment with both drugs, BC and PF-04455242, is capable of increasing BAT temperature

(Figure 5E). However, the most remarkable finding is that the increase in BAT temperature after 10 days of treatment was higher in DIO rats treated with BC (1.25 mg/kg) and PF-04455242 (0.625 mg/kg) simultaneously (Figure 5E), resulting in a synergistic achievement of negative energy balance and a heightened activation of thermogenesis in DIO rats.

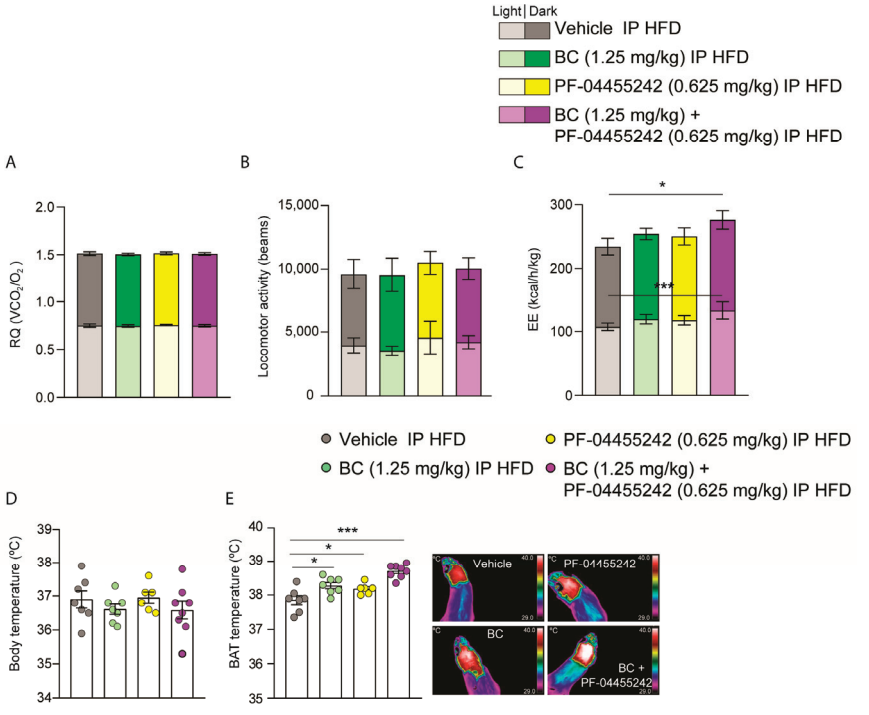

Figure 5. (**A**) Effect of a 10-day intraperitoneal (IP) injection of bromocriptine (BC) (1.25 mg/kg), PF-04455242 (0.625 mg/kg), and BC (1.25 mg/kg) + PF-04455242 (0.625 mg/kg) on respiratory quotient (RQ), (**B**) locomotor activity (LA), (**C**) energy expenditure (EE), (**D**) body temperature, and (**E**) brown adipose tissue (BAT) temperature in diet-induced obese (DIO) rats. Data are expressed as the mean ± SEM; statistical differences were evaluated using one- or two-way ANOVA followed by Tukey's multiple comparisons test; n = 6–8 animals per group. * $p < 0.05$, *** $p < 0.001$ vs. vehicle.

4. Discussion

The present study provides findings that evidence the efficacy of a novel combination therapy involving BC, a D2R agonist, and PF-04455242, a selective KOR antagonist. This synergistic approach was observed to activate BAT thermogenesis, leading to increased energy expenditure. As a result, there was a notable decrease in body weight and adiposity in DIO rats.

While the existing literature lacks direct evidence concerning the effects of these two drugs together, recent studies have indicated that the central activation of their respective receptors independently is involved in the control of energy balance and body weight [10,22,29]. However, when both substances were coadministered centrally in DIO rats, we did not observe a significant decrease in body weight compared to that in animals treated with BC or PF-04455242 independently. Nonetheless, these observations were made only 24 h after central coadministration; continuous administration over a longer period may indeed result in a reduction in body weight.

On the other hand, when we coadministered both compounds peripherally, which is a much less invasive approach, we observed a reduction in body weight both in the short and long term. This change in body weight occurred even though we administered both

drugs at a dosage at which no significant changes in body weight were observed when given separately. However, using these compounds together, we found that the DIO rats lost weight, leading to an increased loss of body mass and a reduction in adiposity. These findings suggest a synergistic effect of the combination treatment. It is noteworthy that when administered individually, neither BC nor PF-04455242 induced changes in body weight or adiposity in the rats. Instead, it is crucial for them to be administered together to unlock this synergy, triggering a remarkable reduction in body weight and enhancing weight loss exclusively in the rats treated with the combination. In addition, the observed data on body weight with the chosen doses, which did not significantly decrease body weight in lean rats and did not affect the body weight in DIO rats after 24 h of separate administration, should be highlighted. While it is true that we did not administer both compounds together peripherally to normal-weight rats, given that the focus of this study was to explore new tools and therapeutic targets for weight reduction, once the doses were optimized, we directly administered both compounds, BC and PF-04455242, peripherally together, demonstrating the synergistic effect of both drugs. Interestingly, although some previous studies have suggested that alterations in KOR may modify food intake [15,31], in our research, we did not observe changes in the food consumption with the doses of PF-04455242 used, which were significantly lower than those needed in previous experiments to observe a decrease in food intake.

Kappa opioid receptors exhibit a widespread distribution within the CNS, encompassing regions such as the ventromedial nucleus of the hypothalamus (VMH), the arcuate nucleus (ARC), and other brain regions, including the ventral tegmental area (VTA) [32]. In addition, several studies have identified significant repercussions on energy balance resulting from the central blockade of opioid receptors [20,33]. This includes effects on NPY neurons [34,35], AgRP neurons [36], or MCH neurons [22,30] as well as their interactions with hormones like ghrelin [21]. In most of these brain regions, the dopaminergic system is crucial, suggesting a potential neurochemical overlap between the dopaminergic and opioid systems. Although most studies linking KOR with the dopaminergic system focus on reward signaling or behavior [37–39], it has recently been demonstrated that brain regions where D2R is highly expressed play a pivotal role in the central regulation of thermogenesis and body weight control [10,29]. In this study, we observed that the coadministration of a D2R agonist and a KOR antagonist can lead to a decrease in body weight, enhancing thermogenesis in the BAT. However, the neural connections occurring when both compounds are coadministered peripherally remain unknown. Further studies are required to elucidate the specific brain areas and neuronal populations implicated in these effects.

In recent years, there has been a growing concern regarding the discovery of an effective drug for fighting obesity. While several have been proposed, only five are currently approved for use by the FDA: orlistat, phentermine/topiramate, naltrexone/bupropion, liraglutide, and semaglutide. A sixth drug, lorcaserin, obtained approval for weight loss but was subsequently withdrawn from clinical use due to concerns about an elevated risk of cancer [4].

In this search, the importance of coadministering two drugs (as in the case of phentermine/topiramate and naltrexone/bupropion) has become evident, as it seems to be a promising approach to combat this disease. In our study, we observed that the coadministration of BC (approved in the United States since 2009 as an adjunctive treatment for type 2 diabetes [40]) and the KOR antagonist PF-04455242 (which was tested in a double-blind study for the treatment of bipolar disorder in 2010 [25,41]) triggered weight loss in our animals. This could potentially represent a new effective combination therapy against obesity. However, our study was limited to a 10-day treatment period, and further assessment is needed to evaluate the potential side effects of BC and PF-04455242 combination therapy on the rest of the organism.

5. Conclusions

This work demonstrates for the first time that coadministration of BC and PF-04455242 exerts beneficial effects in DIO rats, leading to a reduction in body weight and adiposity. These effects stimulate the BAT thermogenic program and increase energy expenditure, indicating that this combination therapy holds promise as a novel strategy for obesity treatment.

Author Contributions: B.C., J.P.-G. and C.F. contributed to the experiments and data analysis. B.C., J.P.-G. and C.F. contributed to the development of the analytical tools and discussion. C.F. contributed to the experimental design and writing of the manuscript. C.F. served as the guarantor of this work. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of the University of Santiago de Compostela (15010/14/007) in accordance with the European Union normative for the use of experimental animals (15012/2023/014) approval date 31 July 2023.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy reasons.

Conflicts of Interest: The authors declare no conflicts of interest.

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Predictive Roles of Basal Metabolic Rate and Muscle Mass in Lung Function among Patients with Obese Asthma: A Prospective Cohort Study

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Abstract: Background: The metabolic-status-related mechanisms underlying the deterioration of the lung function in obese asthma have not been completely elucidated. Objective: This study aimed to investigate the basal metabolic rate (BMR) in patients with obese asthma, its association with the lung function, and its mediating role in the impact of obesity on the lung function. Methods: A 12-month prospective cohort study (n = 598) was conducted in a real-world setting, comparing clinical, body composition, BMR, and lung function data between patients with obese (n = 282) and non-obese (n = 316) asthma. Path model mediation analyses for the BMR and skeletal muscle mass (SMM) were conducted. We also explored the effects of the BMR on the long-term lung function in patients with asthma. Results: Patients with obese asthma exhibited greater airway obstruction, with lower FEV_1 (1.99 vs. 2.29 L), FVC (3.02 vs. 3.33 L), and FEV_1/FVC (65.5 vs. 68.2%) values compared to patients with non-obese asthma. The patients with obese asthma also had higher BMRs (1284.27 vs. 1210.08 kcal/d) and SMM (23.53 vs. 22.10 kg). Both the BMR and SMM mediated the relationship between obesity and the lung function spirometers (FEV_1, %FEV_1, FVC, %FVC, and FEV_1/FVC). A higher BMR or SMM was associated with better long-term lung function. Conclusions: Our study highlights the significance of the BMR and SMM in mediating the relationship between obesity and spirometry in patients with asthma, and in determining the long-term lung function. Interventions for obese asthma should focus not only on reducing adiposity but also on maintaining a high BMR.

Keywords: obese asthma; basal metabolic rate; muscle mass; lung function

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1. Introduction

The complex interplay between obesity and asthma poses a significant challenge in understanding the pathophysiological mechanisms underlying this comorbidity [1]. Obesity, as a primary risk factor, exacerbates asthma symptoms and the disease severity, giving rise to the phenotype known as "obese asthma." This particular asthma subtype is often unresponsive to standard therapeutic approaches [2], emphasizing the need for more tailored treatment strategies. Abnormal lung function, particularly in patients with obese asthma, serves as a strong predictor for uncontrolled asthma and asthma exacerbations [2]. Decades of research have focused on elucidating the physiological effects of obesity on the lung function [3], yet the association between asthma and obesity has sparked renewed interest in exploring the mechanical impacts of obesity on the lung function. These mechanisms remain controversial and not fully understood [4]. One such mechanism involves the impact of the adipose tissue mass around the rib cage, abdomen, and visceral cavity. This additional mass alters the balance of the inflationary and deflationary pressures on

the lungs, ultimately leading to a reduction in the functional residual capacity (FRC) [5–7]. Furthermore, as the body mass index (BMI) increases in patients with asthma, a worsening of the airway obstruction is observed, as measured by spirometric parameters such as the forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) [8–11].

Our previous research identified a critical limitation in exploring the role of obesity in asthma: the singular reliance on the BMI as the sole measure of obesity. The BMI, a commonly used metric calculated by dividing weight by height squared (kg/m^2), serves as a general indicator of nutritional status [12]. However, despite its association with the fat mass (FM) and percentage of body fat (PBF), the BMI falls short as a comprehensive proxy for fat distribution and composition [12]. The sensitivity and specificity of the BMI in detecting individuals with excessive PBFs are limited [12–14]. Moreover, the BMI fails to distinguish between muscle and fat tissue [12,15], offering an incomplete picture of a person's body composition. Obesity results from an imbalance between energy intake and expenditure, and a given BMI may represent significantly different metabolic and energetic profiles [16]. Therefore, relying solely on the BMI to assess obesity and its metabolic impact in patients with asthma is insufficient. A more comprehensive approach, incorporating additional metrics, such as body composition analysis, is necessary to accurately assess the role of obesity in asthma and develop targeted therapeutic strategies.

The basal metabolic rate (BMR)—the energy expended by the body at rest to maintain vital functions—has traditionally been a key metric in assessing the metabolic rate and energy expenditure in obesity [17]. Understanding the contribution of the individual BMR to daily energy expenditure is crucial for developing and implementing weight management interventions in patients with obese asthma [17]. Typically, the BMR is estimated using prediction equations that consider various factors, such as age [18–25], gender, race [26], and body composition (including fat mass (FM), muscle mass, and fat-free mass (FFM)) [19]. Among these variables, FFM and muscle mass are the primary determinants of the BMR. Previous research has shown a positive correlation between the BMR and lung function in healthy individuals [27]. Moreover, individuals with asthma tend to have higher BMRs compared to healthy people [28]. However, despite this knowledge, it remains unclear whether the BMR and its associated body composition factors, particularly muscle mass, play a significant role in the airway obstruction in patients with obese asthma.

In this prospective cohort study, we aimed to address the limitations of relying solely on the BMI to define obesity in individuals with asthma. To achieve this, we employed a multifaceted approach that incorporated the BMI, PBF, and waist circumference (WC) as the metrics to define obese asthma. Crucially, we performed body composition analysis (BCA) on the patients, which allowed us to assess their BMRs, muscle mass, adiposity, and fat-free mass (FFM). This comprehensive analysis enabled us to explore the intricate relationships between the BMR and muscle mass with obese asthma. Furthermore, we delved into the potential mediating roles of the BMR and muscle mass in the association between obesity and the lung function. By doing so, we hoped to gain a deeper understanding of the mechanisms underlying the relationship between obesity and asthma. Lastly, we investigated the longitudinal relationship between the BMR and muscle mass with the lung function over the following year. This allowed us to assess the prognostic values of these metrics in patients with obese asthma. By incorporating multiple metrics and exploring their interactions, we hope to provide valuable insights that can inform the development of nutrition-related multidimensional assessments and interventions for patients with obese asthma.

2. Methods

2.1. Study Design and Participants

This prospective real-world cohort study ran from March 2016 to January 2022, with a 12-month follow-up period. Eligible participants were adults (aged 18 years and above) who had been diagnosed with stable asthma and were receiving optimal treatment according to the Global Initiative for Asthma (GINA) criteria at West China Hospital in China [2].

Stable asthma was defined as the absence of respiratory tract infection, exacerbation, or the use of systemic corticosteroids in the preceding four weeks. Patients with other conditions known to affect the BMR, such as hypoadrenocorticism, nephrotic syndrome, pathological starvation, diabetes, erythrocytosis, leukemia, cardiac disease with respiratory distress, and thyroid pathology (e.g., hypothyroidism, hyperthyroidism) indicated by thyroid function tests were excluded from the study. Additional exclusion criteria included an inability to understand the questionnaires and perform spirometry or sputum induction, as well as pregnancy and breastfeeding. Consistent with a real-world study design, patient treatment decisions were guided by the GINA recommendations [2]. This involved a continuous cycle of assessment, treatment, and review, with adjustments made to step up or step down treatments as necessary. The study protocol was approved by the Institutional Review Board (IRB) at West China Hospital, Sichuan University (Chengdu, China) (No. 2014-30). Prior to participation, all individuals provided written informed consent. The study was also registered with the China Clinical Trial Registry (ChiCTR-OOC-16009529; http://www.chictr.org.cn (accessed on 7 April 2024)).

By examining the associations between the BMR, body composition, and lung function in patients with asthma, this study aims to contribute to a more comprehensive understanding of the pathophysiology of asthma in patients with obesity.

2.2. Multidimensional Clinical Assessment and Data Collection

We conducted multidimensional data collection, utilizing a standardized case report form to gather information on demographics and clinical characteristics. This encompassed detailed assessments that encompassed anthropometric measurements and body composition analysis, BMRs, muscle mass, medication histories, asthma control assessments, quality-of-life surveys, comorbidity screenings, spirometry tests, fractional exhaled nitric oxide (F_ENO) measurements, atopy and skin-prick tests, sputum induction procedures, peripheral blood collections, and asthma exacerbation detections.

2.3. Definition of Obesity

The BMI, widely regarded as the standard metric for defining general obesity, has been the focal point of many obesity studies. However, exploring alternative adiposity indicators like WC, a measure of abdominal obesity, or PBF (PBF-defined obesity) could hold significant clinical value and enhance our comprehension of the intricate relationship between obesity and asthma [29]. Therefore, in this study, we employed three adiposity measures—BMI, WC, and PBF—to define obesity based on WHO recommendations. The BMI was calculated by dividing an individual's body weight (kg) by the square of their height (m^2). A BMI exceeding 25 kg/m^2 was considered obese for both Asian men and women [30]. WC (cm) was measured at the level of the umbilicus using an inelastic tape measure. A WC greater than 90 cm for men or 80 cm for women was classified as abdominal obesity [31]. Furthermore, drawing from previous studies and the WHO Technical Report, we adopted a PBF threshold of 25% for men and 30% for women as the cutoff for defining PBF-defined obesity [29,32–34]. A diagnosis of obesity in our study was made if any one of these three criteria was met. Based on this assessment, patients were categorized into either the obese asthma group or the non-obese asthma group at the start of the study. This comprehensive approach allowed us to capture a broader spectrum of obesity phenotypes and their potential impact on asthma outcomes.

2.4. Body Composition and BMR Measurements

The body composition and BMR were measured using indirect calorimetry under strictly standardized conditions. These measurements were conducted early in the morning (08:00 a.m.), following a minimum of 10–12 h of fasting and at least 30 min of rest. The environment was maintained in absolute silence and at a thermoneutral temperature, with the room temperature set at 25 °C to ensure optimal conditions for accurate measurements [35]. To assess the body composition, including the FM (kg), PBF (%), visceral fat area (VFA)

(cm^2), skeletal muscle mass (SMM) (kg), and appendicular lean mass (ALM) (kg), we utilized a multifrequency bioimpedance analysis (BIA) with the InBody S10 analyzer (Body Composition Analyzer; Biospace Co., Ltd., Seoul, Republic of Korea). This device employs six different frequency impedance measurements (1, 5, 50, 250, 500, and 1000 kHz) and three frequencies of phase angle measurement (5, 50, and 250 kHz) across five segments of the body (right arm, left arm, trunk, right leg, and left leg), providing a comprehensive and accurate assessment of the body composition. The BIA measurements were performed by a trained nutritionist from our research group, who followed the InBody S10 user's manual and adhered to the recommended guidelines for the clinical application of bioelectrical impedance analysis [36]. Prior to the measurements, patients were instructed to fast overnight, empty their bladders, and wear light indoor clothing. They were then positioned in a standing posture.

The ALM was calculated as the sum of the muscle mass in the arms and legs [37]. Additionally, the skeletal muscle mass index (SMI) (kg/m^2) was derived by dividing the ALM by the square of the patient's height [38]. The BMR was determined using a validated formula derived from BIA, specifically the Cunningham formula, which has been confirmed through comparisons with indirect calorimetry measurements. To further normalize the BMR, we divided it by both height squared (cm^2) and the BMI, eliminating the confounding effects of height and weight. While dual-energy X-ray absorptiometry (DXA) is recognized as the gold standard for body composition measurement, BIA has been shown to exhibit a strong correlation with DXA, making it a reliable and practical alternative for assessing body compositions in research settings [39–41].

2.5. Definition of Low Muscle Mass

Currently, there is no consensus definition on low muscle mass, leading to a diversity of proposed criteria and definitions within the field. In our study, we employed several such definitions to assess low muscle mass, including the European Working Group on Sarcopenia in Older People (EWGSOP1,2) [42,43], the Asian Working Group for Sarcopenia (AWGS) [44], the International Working Group on Sarcopenia (IWGS) [45], and the Foundation for the National Institutes of Health Biomarkers Consortium Sarcopenia Project (FNIH) [46].

2.6. Lung Function and F_ENO

All subjects participating in the study were instructed to refrain from using long-acting β_2-agonists or anticholinergics for at least 24 h prior to their attendance, and to avoid short-acting β_2-agonists for 12 h or more [47]. In accordance with the standards set by the American Thoracic Society and the European Respiratory Society (ATS/ERS), we utilized a standardized spirometer (CPES/D USB, MedGraphics, Saint Paul, MN, USA) to measure the FEV_1 and FVC before and 15 min after administering 400 μg of salbutamol (GSK, Burgos, Spain) through a metered-dose inhaler and spacer (150 mL, Vanbo Technology Corp., Shanghai, China) [47]. To ensure accuracy, all subjects were required to perform at least three acceptable and reproducible maneuvers, and the largest FEV_1 and FVC values were used for analysis. The predicted FEV_1 and FVC were calculated based on data from the Chinese population [48]. Additionally, the F_ENO levels were measured using a NIOX analyzer (Aerocrine, Solna, Sweden), adhering to the ATS/ERS recommendations [49,50].

2.7. Asthma Control, Quality of Life, and Exacerbation

Asthma control was evaluated using the Asthma Control Questionnaire (ACQ) [51,52], a six-item survey that delves into asthma symptoms, activity limitations, and the frequency of rescue medication usage. The Asthma Quality-of-Life Questionnaire (AQLQ), encompassing 32 questions, provides a thorough assessment of the individual's quality of life, encompassing activity limitations, asthma symptoms, emotional distress, and environmental stimuli [52,53]. Both questionnaires have established minimal clinically important

differences (MCIDs) of 0.5. Notably, these questionnaires have been validated for use in the Chinese population [54,55].

Asthma exacerbation (AE) was defined in accordance with the guidelines by the American Thoracic Society and the European Respiratory Society (ATS/ERS). Specifically, severe AE (SAE) was characterized by the need for systemic corticosteroid treatment lasting at least three days, or an asthma-related hospitalization or emergency department visit resulting in the administration of systemic corticosteroids for a minimum of three days. To maintain consistency in the assessments, any courses of corticosteroids separated by one week or more were deemed separate SAE events [50,56].

2.8. Peripheral Blood and Sputum Induction

Peripheral venous blood samples were collected from fasting individuals into vacuum tubes or tubes containing ethylenediaminetetraacetic acid (EDTA). These samples were then processed (the Sysmex XN-9000 hematology analyzer, Sysmex Corporation, Kobe, Japan) to obtain differential white blood cell counts. Serum total IgE levels were measured (Beckman Image 800 immunoassay analyzer, Beckman Coulter Inc., Brea, CA, USA), with a minimum detectable level of 5.0 IU/mL [52].

Sputum samples were also collected and processed using a standardized method described in our previous studies [57–59]. This involved inducing sputum production using 0.9% saline, selecting mucus plugs from the saliva, and dispersing them with dithiothreitol. The supernatant of the processed sputum was aspirated and stored at −80 °C for subsequent analysis. Total and differential cell counts were determined using CytoPro 7620 centrifugation-smear (Wescor, Inc., Logan, UT, USA) [52].

2.9. Statistical Analysis

Categorical variables were summarized as frequencies and proportions, while continuous variables were tested for Gaussian distribution and expressed as means and standard deviations or medians and interquartile ranges. When possible, all continuous data were transformed into a normal distribution. The difference between cohorts for each variable was evaluated using the Student *t*-test or Mann–Whitney U test for the continuous variables and the chi-square test for the categorical variables, as appropriate. The area under the receiver operating characteristic (ROC) curve was used to determine the appropriate cutoff values of the BMR, ALM, and SMM for detecting obese asthma. Model 1 was an unadjusted model. Model 2 was adjusted for potential confounders (age and sex). Spearman correlation analysis was employed to investigate the association between the BMR and muscle mass in patients with obese and non-obese asthma.

Mediation analysis was performed to gain a deeper understanding of the relationship between obesity and lung function (PROCESS Macro [version 3.3] for SPSS [version 23.0, SPSS, Chicago, IL, USA] [50,60]. Using post hoc parallel multiple-mediation models, the study examined whether the association between obesity and the spirometric measures (FEV_1, FVC, and FEV_1/FVC) was mediated by the BMR or muscle mass. Adjustments were made for factors such as age, sex, ICS dosage, and SAE in the past year [27,61]. Specifically, the unstandardized path coefficients were the beta (β) coefficients of the multivariable regression models and represent the magnitude and direction of the associations between the variables included in the model. The total unstandardized β effect (path c) represents the effect of obesity on measurements of the lung function when no other mediators were included in the model, while the direct unstandardized β effect (path c') represents the effect of obesity on measurements of the lung function when mediators were included. Finally, indirect effects (path a_1b_1) represent the effect of obesity on measurements of the lung function through the BMR or muscle mass, respectively. If the indirect-effect path is statistically significant, it can be concluded that mediation has occurred. The significance of the indirect effects was tested by bootstrapped 95% confidence intervals (CIs). PROCESS Macro produces bootstrap estimates and bias-corrected 95% confidence intervals (CIs) for indirect effects, and a 95% CI that does not cross zero indicates a statistically significant

indirect effect. For the other tests, two-tailed p-values < 0.05 were considered statistically significant. Measurements of the lung function between groups at each visit within the 12-month follow-up were compared using analysis of covariance (ANCOVA) adjusted for age. Statistical analysis was performed using SPSS (Version 23.0; IBM Corp., Armonk, NY, USA).

3. Results

3.1. Subject Characteristics

A total of 598 patients were included in the study (obese asthma, n = 282; non-obese asthma, n = 316) (Figure 1). Table 1 lists the demographic and clinical characteristics of the patients grouped by their obesity status. Obese asthma was more likely in elderly patients (48.0 [41.0, 60.0] vs. 40.0 [31.0, 48.0] years; $p < 0.001$) and constituted a greater proportion of the uncontrolled asthma (25.5 vs. 17.1%; $p = 0.011$). Patients with obesity and asthma had greater airway obstruction, assessed by the FEV_1 (1.99 vs. 2.29 L, $p < 0.001$; 71.1 vs. 75.7%, $p = 0.018$), FVC (3.02 vs. 3.33 L, $p < 0.001$; 89.3 vs. 92.8%, $p = 0.011$), and FEV_1/FVC (65.5 vs. 68.2%; $p = 0.012$). There were no significant differences in the percentages of sputum inflammatory cells. In the peripheral blood, patients with obesity had increased neutrophils (4.60 vs. 3.24 $\times$ 10^9/L; $p = 0.001$) but reduced eosinophils (3.63 vs. 3.76%; $p = 0.016$) compared with patients with non-obesity (Table 2). Lower levels of IgE (108.5 vs. 152.19 IU/mL; $p = 0.051$) and F_ENO (35.5 vs. 42.5 ppb; $p = 0.011$) were observed in patients with obese asthma compared with patients with non-obese asthma (Table 2).

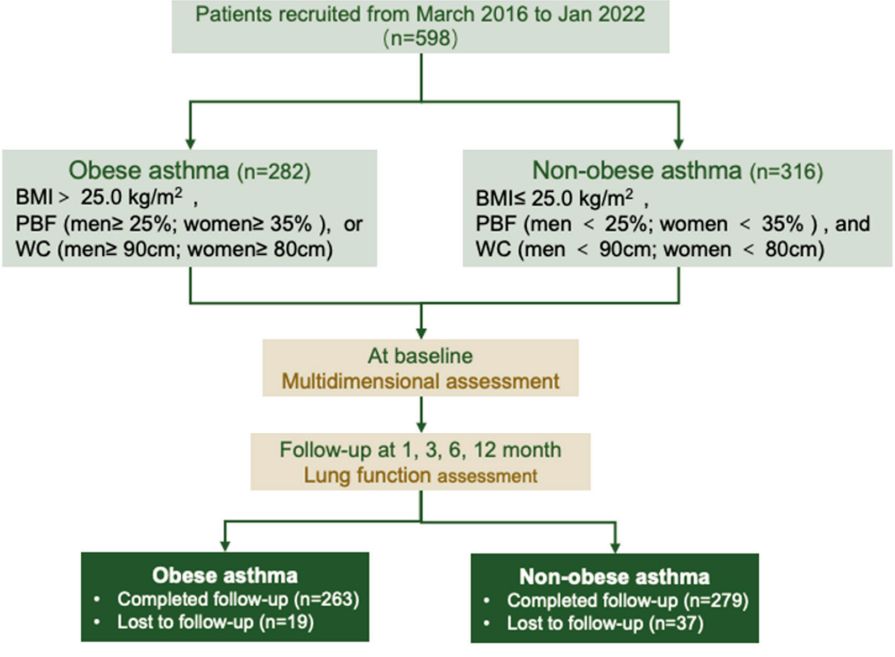

Figure 1. Flowchart of cohort included in the study. BMI: body mass index; PBF: percentage body fat; WC: waist circumference.

Table 1. Demographic and clinical characteristics of the included participants with asthma.

Variables	Obese Asthma	Non-Obese Asthma	Total	$t/U/\chi^2$	*p*-Value
n (%)	282 (47.2)	316 (52.8)	598		
Age, years, median (Q1, Q3)	48.0 (41.0, 60.00)	40.0 (31.0, 48.0)	45.0 (35.0, 55.0)	7.078 *	<0.001
Female, n (%)	183 (64.9)	207 (65.5)	390 (65.2)	0.025	0.875
Atopy, n (%)	99 (35.1)	101 (32.0)	200 (33.4)	0.616	0.433
Asthma duration, years, median (Q1, Q3)	1.0 (0, 4.0)	1.0 (1.0, 6.0)	1.0 (0, 6.0)	−0.372	0.710
Early-onset asthma, n (%)	54 (19.2)	62 (19.6)	116 (19.4)	0.008	0.930
History of family asthma, n (%)	98 (34.8)	109 (34.5)	207 (34.6)	0.001	0.971
Medications					
ICS (BDP equivalent) dose, μg/day, median (Q1, Q3)	400 (400, 1000)	400 (400, 1000)	400 (400, 1000)	−0.042	0.966
ICS/LABA, n (%)	165 (58.5)	182 (57.6)	347 (58.0)	0.065	0.789
Anti-leukotrienes, n (%)	36 (12.8)	49 (15.5)	85 (14.2)	1.068	0.301
Leukotriene, n (%)	98 (34.8)	118 (37.3)	216 (36.1)	0.365	0.546
OCS, n (%)	10 (3.5)	9 (2.8)	19 (3.2)	0.237	0.626
Asthma control					
ACQ-6, median (Q1, Q3)	0.67 (0.17, 1.50)	0.67 (0, 1.34)	0.67 (0, 1.5)	2.107 *	0.036
Uncontrolled asthma (ACQ $\geq$ 1.5)	72 (25.5)	54 (17.1)	126 (21.1)	6.388	0.011
AQLQ scores, median (Q1, Q3)	5.88 (5.09. 6.32)	5.97 (5.46, 6.50)	5.94 (5.31, 6.41)	−1.587	0.113
SAE in the past year, n (%)	86 (30.5)	78 (24.7)	164 (27.4)	2.530	0.112
Spirometry					
FEV_1, mean (SD)					
L	1.99 (0.76)	2.29 (0.81)	2.16 (0.80)	−4.647 *	<0.001
%	71.1 (20.1)	75.7 (21.5)	74.4 (20.5)	−2.382	0.018
FVC, mean (SD)					
L	3.02(0.93)	3.33(0.85)	3.20 (0.91)	−4.548	<0.001
%	89.3(17.3)	92.8 (16.4)	91.6 (16.0)	−2.542	0.011
FEV_1/FVC, %, mean (SD)	65.5 (12.3)	68.2 (14.8)	67.0 (13.1)	−2.509	0.012
Comorbidities, n (%)					
Rhinitis	166 (58.9)	191 (60.4)	357 (59.7)	0.154	0.695
Nasal polyps	26 (9.2)	33 (10.4)	59 (9.9)	0.223	0.637
Bronchiectasis	12 (4.3)	16 (5.1)	28 (4.7)	0.200	0.654
Sleep apnea	3 (1.1)	5 (1.6)	8 (1.3)	0.293	0.589
GERD	21 (7.4)	14 (4.4)	35 (5.9)	2.531	0.112
Diabetes	10 (3.5)	4 (1.3)	14 (2.3)	3.440	0.064
Eczema	46 (16.3)	54 (17.1)	100 (16.7)	0.046	0.830

* Data were normally transformed using *t*-test. ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality-of-Life Questionnaire; BDP: Beclomethasone dipropionate; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; GERD: gastroesophageal reflux disease; ICS: inhaled corticosteroid; LABA: long-acting beta-agonist; OCS: oral corticosteroid; Q1: first quartile; Q3: third quartile; SAE: severe asthma exacerbation; SD: standard deviation.

Table 2. Inflammatory characteristics of the included participants with asthma.

Variables	Obese Asthma	Non-Obese Asthma	Total	$t/U/\chi^2$	*p*-Value
n (%)	282 (47.2)	316 (52.8)	598		
Serum IgE, median (Q1, Q3), IU/mL	108.50 (42.52, 264.25)	152.19 (46.77, 323.99)	104.18 (39.30, 301.62)	−1.948	0.051
FeNO, ppb, median (Q1, Q3)	35.5 (18.0, 65.0)	42.5 (22.0,83.8)	39.0 (20.0, 73.3)	−2.546	0.011
Blood cells, median (Q1, Q3)					
Neutrophils					
%	59.11 (53.09, 64.58)	59.55 (53.19, 65.41)	59.32 (53.12, 64.99)	−1.726	0.084
$\times 10^9$/L	4.60 (3.60, 6.16)	3.24 (2.55, 4.08)	3.41 (2.70, 4.42)	−3.480	0.001
Eosinophils					
%	3.63 (2.02, 5.93)	3.76 (1.99, 6.76)	3.68 (2.0, 6.27)	−2.414	0.016
$\times 10^9$/L	0.22 (0.12, 0.35)	0.25 (0.12,0.41)	0.22 (0.11, 0.36)	−1.157	0.247
≥300 cells/μL, n (%)	102 (36.6)	128 (40.5)	230 (38.7)	0.974	0.324
Eosinophilic asthma *, n (%)	118 (41.8)	143 (45.3)	261 (43.6)	0.726	0.394
Sputum cells, median (Q1, Q3), (n = 353)					
Neutrophils, %	43.50 (17.63, 74.50)	34.5 (13.25, 64.47)	39.0 (15.19, 68.49)	−1.636	0.102
Eosinophils, %	0.25 (0, 2.75)	0.25 (0, 4.25)	0.25 (0, 3.54)	−0.770	0.442
Eosinophils, ≥3%, n (%)	48 (26.1)	51 (30.2)	99 (28.0)	0.730	0.393

* Sputum eosinophil level ≥ 3% or blood eosinophil level ≥ 300 cells/μL. FeNO: fractional exhaled nitric oxide; Q1: first quartile; Q3: third quartile.

3.2. Anthropometric, Body Composition, and BMR Characteristics

The patients with obesity had higher BMIs (25.29 vs. 21.05 kg/m^2; $p < 0.001$), FM (21.02 vs. 13.10 kg; $p < 0.001$), PBFs (32.79 vs. 24.39%; $p < 0.001$), and VFAs (95.85 vs. 55.40 cm^2; $p < 0.001$) (Table 3). Furthermore, the patients with obesity had increased muscle mass (SMM: 23.53 vs. 22.10 kg, $p < 0.001$; SMM/Height^2: 9.22 vs. 8.61 kg/m^2, $p < 0.001$; SMI: 6.98 vs. 6.49 kg/m^2, $p < 0.001$; ALM: 17.85 vs. 16.68, kg, $p < 0.001$) but decreased ALM/BMI (0.71 vs. 0.79 m^2, $p < 0.001$) (Table 3).

In our study, patients with obese asthma presented higher BMRs (1284.27 vs. 1210.08 kcal/d, $p < 0.001$) compared with patients with non-obese asthma (Table 3). The BMR is largely determined by the total lean mass, especially muscle mass [62]. A reduction in lean mass will reduce the BMR. Currently, there is no uniform definition of low muscle mass worldwide. We used five available criteria (EWGSOP, EWGSOP2, FNIH, IWGS, and AWGS) to define low muscle mass (Table 3). In this study, obese patients had significant lower proportions of low muscle mass in terms of the SMI defined by the EWGSOP (16.0 vs. 26.9%, $p = 0.001$), EWGSOP2 (22.0 vs. 44.3%, $p < 0.001$), IWGS (18.1 vs. 32.6%, $p < 0.001$), and AWGS (10.3 vs. 21.8%, $p = 0.001$). However, a higher proportion of low muscle mass in the ALM/BMI defined by the FNIH was observed (16.3 vs. 3.8%, $p < 0.001$) (Table 3).

Table 3. Anthropometric, BMR, and body composition characteristics.

Variables	Obese Asthma	Non-Obese Asthma	Total	t/χ^2	*p*-Value
n (%)	282 (47.2)	316 (52.8)	598		
Anthropometric data					
Weight, kg, mean (SD)	64.25 (11.45)	53.79 (7.72)	58.72 (10.98)	12.944	<0.001
Height, cm, mean (SD)	158.98 (8.34)	159.69 (7.01)	159.35 (7.67)	−1.126	0.261
BMI, kg/m^2, mean (SD)	25.29 (3.15)	21.05 (2.29)	23.05 (3.45)	18.676	<0.001
≥28, n (%)	59 (20.9)	0 (0)	59 (9.9)	73.35	<0.001
Waist, cm, mean (SD)	89.61 (89.61)	75.06 (6.80)	82.05 (10.42)	22.940	<0.001
Men (n = 208)	93.47 (7.94)	79.64 (7.05)	86.16 (10.18)	12.798	<0.001
≥90 cm, n (%)	62 (62.6)	0 (0)	62 (29.8)	101.899	<0.001
Women (n = 390)	87.62 (7.48)	72.56 (5.18)	79.9 (9.88)	22.28	<0.001
≥80 cm, n (%)	164 (89.6)	0 (0)	164 (42.1)	321.952	<0.001
Hip, cm, mean (SD)	97.66 (6.17)	89.73 (5.70)	93.54 (7.13)	15.837	<0.001
WHR, mean (SD)	0.92 (0.06)	0.84 (0.06)	0.88 (0.07)	15.812	<0.001
Men (n = 208)	0.95 (0.05)	0.88 (0.07)	0.91(0.07)	7.902	<0.001
Women (n = 390)	0.90 (0.06)	0.81 (0.05)	0.86 (0.07)	16.37	<0.001
BMR					
BMR, kcal/d, mean (SD)	1284.27 (235.71)	1210.08 (255.19)	1240.92 (258.49)	3.679	<0.001
BMR/BMI, mean (SD)	51.10 (8.83)	57.88 (12.22)	54.69 (11.26)	−7.836	<0.001
BMR/Height^2, kcal/m^2, mean (SD)	505.81(71.09)	473.80 (89.42)	488.89 (82.79)	4.807	<0.001
Body composition					
FM, kg, mean (SD)	21.02 (5.50)	13.10 (3.63)	16.84 (6.07)	20.524	<0.001
PBF, %, mean (SD)	32.79 (5.94)	24.39 (6.16)	28.35 (7.36)	16.916	<0.001
Men (n = 208)	28.36 (4.19)	19.30 (4.38)	23.61 (6.24)	15.198	<0.001
≥25, n (%)	81 (81.8)	0 (0)	81 (38.9)	146.062	<0.001
Women (n = 390)	35.18 (5.34)	27.07 (5.20)	30.89 (6.65)	15.168	<0.001
≥35, n (%)	106 (57.9)	0 (0)	106 (27.2)	164.65	<0.001
VFA, cm^2, mean (SD)	95.85 (30.27)	55.40 (19.22)	74.48 (32.17)	19.247	<0.001
Men (n = 208)	89.27 (25.67)	50.15 (17.98)	68.77 (29.40)	12.612	<0.001
Women (n = 390)	99.41 (31.99)	58.16 (19.31)	77.52 (33.19)	15.17	<0.001
Muscle mass					
SMM, kg, mean (SD)	23.53 (5.22)	22.10 (4.31)	22.78 (4.81)	3.645	<0.001
SMM/Height^2, kg/m^2	9.22 (1.35)	8.61 (1.20)	8.90 (1.31)	5.830	<0.001
Men (n = 208)	10.26 (1.23)	9.64 (0.91)	9.93(1.12)	4.101	<0.001
Women (n = 390)	8.66 (1.05)	8.07 (0.95)	8.34 (1.04)	5.830	<0.001
ALM, kg, mean (SD)	17.85 (4.19)	16.68 (3.50)	17.23 (3.88)	3.666	<0.001
Men (n = 208)	21.78 (3.67)	20.21 (2.70)	20.96 (3.29)	4.471	0.001
Women (n = 390)	15.72 (2.63)	14.83 (2.21)	15.25 (2.45)	3.629	<0.001
SMI (ALM/Height^2), kg/m^2, mean (SD)	6.98 (1.11)	6.49 (0.96)	6.72 (1.06)	5.823	<0.001
Men (n = 208)	7.84 (7.84)	7.34 (0.65)	7.58 (0.84)	4.354	<0.001
Women (n = 390)	6.52 (0.89)	6.04 (0.78)	6.28 (0.87)	5.73	<0.001

Table 3. *Cont.*

Variables	Obese Asthma	Non-Obese Asthma	Total	t/χ^2	*p*-Value
ALM/BMI, m^2, mean (SD)	0.71 (0.14)	0.79 (0.15)	0.75 (0.15)	−7.285	<0.001
Men (n = 208)	0.84 (0.12)	0.93 (0.11)	0.89 (0.13)	−5.905	<0.001
Women (n = 390)	0.64 (0.09)	0.72 (0.11)	0.68 (0.11)	−7.988	<0.001
Low muscle mass, n (%)					
EWGSOP					
SMI	45 (16.0)	85 (26.9)	130 (21.7)	10.485	0.001
SMM/Height^2	14 (5.0)	23 (7.3)	37 (6.2)	1.375	0.241
EWGSOP2					
ALM	3 (1.1)	4 (1.3)	7 (1.2)	0.053	0.819
SMI	62 (22.0)	140 (44.3)	202 (33.8)	33.182	<0.001
FNIH					
ALM/BMI	46 (16.3)	12 (3.8)	58 (9.7)	26.65	<0.001
IWGS					
SMI	51 (18.1)	103 (32.6)	154 (25.8)	16.408	<0.001
SMM/Height^2	8 (2.8)	13 (4.1)	21(3.5)	0.742	0.389
AWGS					
SMI	29 (10.3)	69 (21.8)	98 (16.4)	14.512	<0.001

SMI = ALM/Height^2. ALM: appendicular lean mass; AWGS: Asian Working Group for Sarcopenia; BMI: body mass index; BMR: basal metabolic rate; BSA: body surface area; EWGSOP: European Working Group on Sarcopenia in Older People; FM: fat mass; FNIH: Foundation for the National Institutes of Health Biomarkers Consortium Sarcopenia Project; IWGS: International Working Group on Sarcopenia; PBF: percentage body fat; SD: standard deviation; SMI: appendicular skeletal muscle index; SMM: skeletal muscle mass; VFA: visceral fat area; WHR: waist–hip ratio. Definitions of low muscle mass: EWGSOP: ALM/Height^2: < 7.26 kg/m^2 (men) < 5.50 kg/m^2 (women); SMM/Height^2: 8.87 kg/m^2 (men) < 6.42 kg/m^2 (women). EWGSOP2: ALM:20 kg (men) < 15 kg (women); ALM/Height^2: < 7.00 kg/m^2 (men) < 6.00 kg/m^2 (women). FNIH: ALM/BMI: < 0.789 kg/BMI (men) < 0.512 kg/BMI (women). IWGS: ALM/Height^2: $\leq$ 7.23 kg/m^2 (men) $\leq$ 5.67 kg/m^2 (women); SMM/Height^2: $\leq$ 8.50 kg/m^2 (men) $\leq$ 5.75 kg/m^2 (women). AWGS: ALM/Height^2: < 7.00 kg/m^2 (men) < 5.4 kg/m^2 (women).

3.3. Roles of BMR and Muscle Mass in Predicting Obese Asthma

ROC curves were used to assess the predictive power of the BMR and muscle mass for identifying obese asthma, with AUCs greater than 0.5. A BMR cutoff of 1209.5 was chosen for identifying obese asthma. The BMR was adjusted for the BMI and height squared (BMR divided by BMI or height squared (cm^2)) to control for the effects of weight and height. The BMR/BMI cutoff point was 19.897; the BMR/Height^2 cutoff point was 494.047. The ALM and ALM/Height^2 cutoff points were 15.59 and 6.361, respectively. The ALM/BMI cutoff point was 0.638. The SMM and SMM/Height^2 cutoff points were 20.85 and 8.165, respectively.

The cutoff values were used for the logistic regression analysis (adjusted for age and sex) to explore the association of the BMR and muscle mass with obese asthma (Figure 2). Patients with higher BMRs (Model 1: OR = 1.889; 95% CI, 1.362, 2.621; Model 2: OR = 3.198; 95% CI, 2.081, 4.915) and higher BMRs/Height^2 (Model 1: OR = 3.348; 95% CI, 2.392, 4.687; Model 2: OR = 3.924; 95% CI, 2.667, 5.773) had increased associations of obese asthma. For muscle mass, patients with higher SMM (Model 1: OR = 1.780; 95% CI, 1.280, 2.474; Model 2: OR = 3.020; 95% CI, 1.966, 4.640), higher SMM/Height^2 (Model 1: OR = 3.045; 95% CI, 2.119, 4.377; Model 2: OR = 3.836; 95% CI, 2.522, 5.837), and higher ALM/Height^2 (Model 1: OR = 2.877; 95% CI,2.053, 4.031; Model 2: OR = 4.942; 95% CI, 3.197, 7.639) had higher risks for obese asthma.

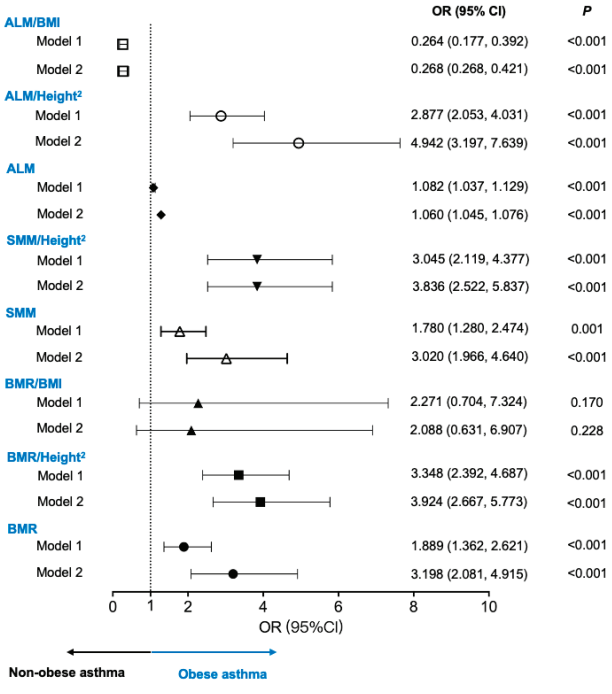

Figure 2. Associations of BMR and muscle mass with obese asthma. Model 1: unadjusted model. Model 2: adjusted for age and sex. ALM: appendicular lean mass; BMI: body mass index; BMR: basal metabolic rate; OR: odds ratio; SMM: skeletal muscle mass.

3.4. Mediation Analyses of BMR and Muscle Mass in Relationship between Obesity and Lung Function

To further explore the relationships between the BMR and muscle mass with the spirometers, we performed parallel mediation analyses (Figure 3). The models showed that the total effect of obesity (c pathway) on the dependent spirometers (FEV_1, $\%FEV_1$, FVC, %FVC, and FEV_1/FVC) was mediated by the BMR (a_1b_1 indirect pathway). Thus, obesity was positively associated with the BMR (path a_1, $\beta = 75.161$, $p < 0.001$). Additionally, the BMR was positively associated with the spirometers (path b_1, all $p < 0.05$). Bootstrapping analysis revealed significant indirect effects of obesity on the measurements of spirometry through the BMR (all $p < 0.05$). Mediation analyses of the BMR/BMI, BMR/Height^2, ALM/BMI, ALM/Height^2, and SMM/Height^2 in the relationship between obesity and the spirometers are shown in Figures S1–S5.

3.5. BMR and Muscle Mass Associated with Future Lung Function

The spirometry was conducted at baseline, the first month, the third month, the sixth month, and the twelfth month. A total of 542 subjects with asthma completed the 12-month follow-up (Figure 1). The differences in the spirometers in patients with different BMR and muscle mass levels (by the cutoff values shown above) were compared (Figures 4 and S6–S8). An age–sex-adjusted ANOVA performed on the BMR and muscle mass revealed that, from the baseline to the twelfth month at the end of the follow-up, patients with higher BMRs (all $p < 0.05$), BMRs/Height^2 (all $p < 0.05$), SMM (all $p < 0.05$), SMM/Height^2 (all $p < 0.05$), ALM (all $p < 0.05$), and ALM/BMIs (all $p < 0.05$) had consistently increased FEV_1 values compared with patients with lower BMRs (Figure 4). Similar results were also found for the spirometers, including the $\%FEV_1$ (Figure S6), FVC (Figure S7), and %FVC (Figure S8).

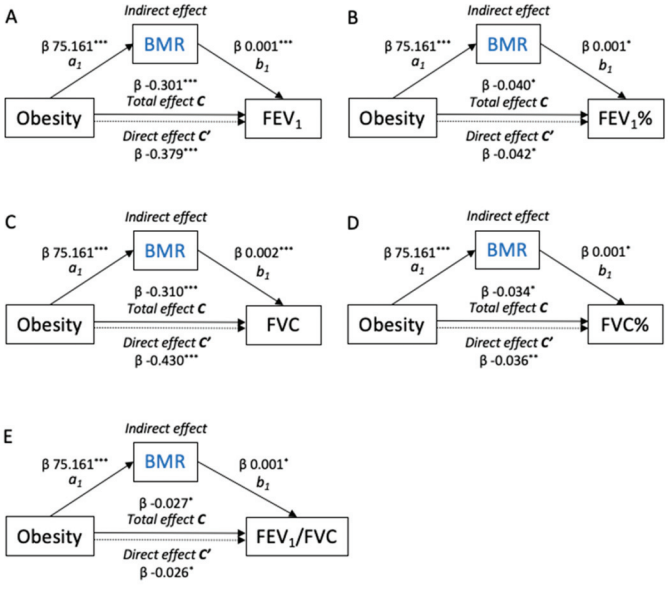

Figure 3. Path model diagrams with the results of the mediation analysis. (**A**) FEV_1, (**B**) FEV_1%, (**C**) FVC, (**D**) FVC%, and (**E**) FEV_1/FVC. Path model showing the effect of obesity on the measurements of the lung function as mediated by the BMR. Total β effect (path c) represents the effect of obesity on the lung function with no mediators in the model. Direct β effect (path c') represents the effect of obesity on the lung function when the BMR was included in the model. Indirect effects (path a_1b_1) represent the effect of obesity on the lung function through the BMR. These models were adjusted for age, sex, ICS dosage, and SAE in the past year. The figures show unstandardized β regression coefficients (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

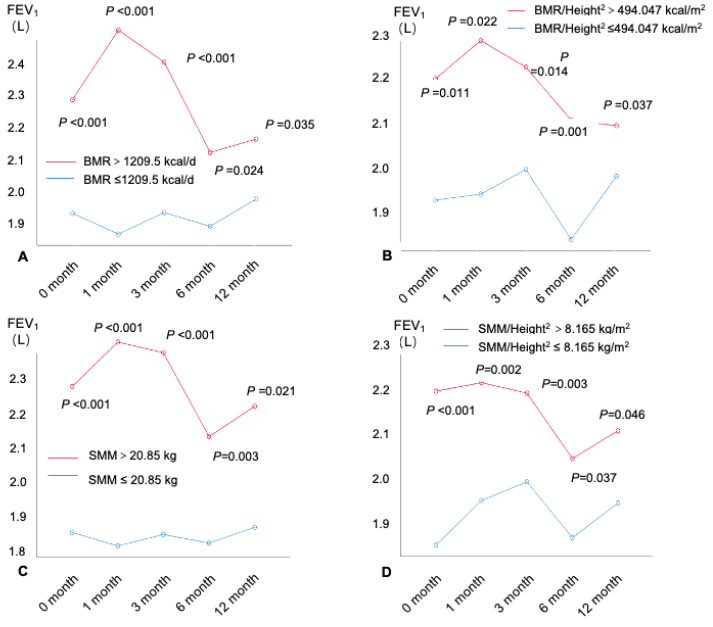

Figure 4. *Cont.*

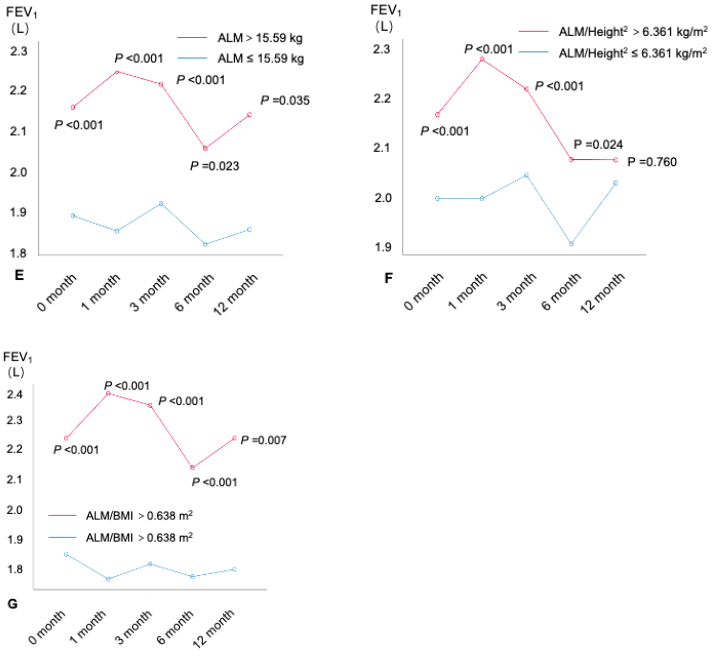

Figure 4. FEV_1(L) at each visit within the 12-month follow-up by BMR and muscle mass. (**A**) BMR, (**B**) BMR/Height^2, (**C**) SMM, (**D**) SMM/ Height^2, (**E**) ALM, (**F**) ALM/Height^2, and (**G**) ALM/BMI. FEV_1: forced expiratory volume in 1 s; SMI = ALM/Height^2. ALM: appendicular lean mass; BMI: body mass index; BMR: basal metabolic rate; SMI: appendicular skeletal muscle index; SMM: skeletal muscle mass.

4. Discussion

To the best of our knowledge, our study is the first to explore the BMR in obese asthma and to further explore the relationship between the BMR and muscle mass with the future lung function in patients with asthma. Our study redefined obese asthma through the combined use of three indicators: the BMI, PBF, and WC. Our study found that patients with obese asthma had higher BMRs and higher muscle mass compared to patients with non-obese asthma. Moreover, patients with obese asthma had lower proportions of low muscle mass. Furthermore, we found that higher BMRs and higher muscle mass were associated with obese asthma, which suggested that some patients with obese asthma do not only have higher fat mass but also higher muscle mass, presenting higher BMRs. Mediation analysis further indicated that obesity was directly and significantly correlated with the lung function in the patients with asthma, partially mediated by the BMR or muscle mass. At the next 12-month follow-up, patients with higher BMRs or muscle mass had significantly better lung function.

To date, the GINA has not provided clear recommendations on the assessment criteria or tools for the obese status in asthma. The diagnosis of obesity in asthma is often based on the BMI, an inaccurate measure of the body fat content. Although vast evidence of the adverse effects of obesity on diseases exists, some studies have concluded that obesity improves disease and promotes survival for some diseases [63–65]. For instance, Nadi et al. observed a negative association between the BMI and asthma severity [66]. These controversial conclusions can be attributed to the deficiency of the BMI: it does not take into account the muscle mass (lean tissue), body fat content, overall body composition, and racial and sex differences [67,68]. Describing obesity by the BMI can result in an inaccurate assessment of the adiposity. In contrast, adiposity measurements such as the waist: WC or hip ratio (WHR) are strongly associated with disease events. Therefore, this

study attempted to perform the multidimensional assessment of the obesity status by three anthropometric indicators and body composition analysis. In our population, if obesity had been defined by the BMI alone, the patients with obesity would have accounted for only 9.9% of the total subjects. However, when the BMI, WC, and PBF were considered, the proportion of patients with obesity increased to 47.2%. We believe that using the BMI alone to define obese asthma may incorrectly estimate the proportion of obesity in the Asian asthma population. Other adiposity measures, alone or in combination with the BMI, may be more appropriate to measure the obesity status, which can better predict asthma-related outcomes and prognoses.

It is intriguing to note that while asthma and obesity exhibit certain overlapping characteristics, there are distinct molecular entities, such as microRNAs, that exert disparate effects on their respective pathologies. For example, a study [69] identified specific microRNAs that exhibit differential expression profiles in asthma and obesity, suggesting unique regulatory roles in the pathogenesis of asthma and obesity. Moreover, our study findings indicate that patients with obese asthma exhibit lower levels of serum IgE and F_ENO compared to patients with non-obese asthma. This contrasts with the commonly observed higher levels of IgE and FENO in patients with asthma, which are typically associated with increased airway hyper-responsiveness and inflammation [70,71]. The lower levels of IgE and F_ENO in patients with obese asthma may be attributed to the impact of obesity on the pathophysiology of asthma. Obesity can alter immune responses, potentially leading to a decrease in the production of certain eosinophilic inflammation mediators [72]. Moreover, obesity may result in restricted lung function, which could influence the F_ENO levels [73]. It is important to note that the reduction in the IgE levels does not necessarily imply milder symptoms in patients with obese asthma, as obesity itself may exacerbate asthma symptoms and the risk of exacerbation [74]. However, the current study's sample size was limited, and the *p*-value for IgE was close to the conventional threshold of 0.05, indicating that the findings are near the border of statistical significance. Therefore, this observation should be validated in larger sample sizes and with consideration of other potential confounding factors to ensure the stability and replicability of the results.

The BMR serves as a proxy for the overall metabolic activity of the body and is regarded as a determinant of metabolic health that is independent of body fatness [75,76]. It is characterized as the energy expenditure necessary to preserve the body's structural and functional equilibrium in a state of rest, fasting, and thermal neutrality. In this study, the patients with obese asthma had higher BMRs than those with non-obese asthma. A significant correlation in the BMR was also found in relation to obesity in patients with asthma. Our results are consistent with a study including a healthy Chinese population, in that the BMRs of adults with obesity were significantly higher than those of adults with normal BMIs [77]. Previous studies have demonstrated that the BMR not only depends on age [78], sex, race/ethnicity [79], height [78], weight [78], BMI [79], WC [78], and FFM [80] but is also influenced by the FM and PBF [78]. These previous findings support our results that patients with obese asthma identified by their BMIs, WCs, and PBFs have higher adiposity and increased muscle mass and BMRs.

It has been concluded that obesity is associated with a deterioration of the lung function and increased bronchial hyper-responsiveness [81,82]. We also found that obesity causes reductions in the FEV_1 and FVC. The BMR has been identified as the principal focus of the development and treatment of obesity [18,83]. However, it is still unclear whether the effects of the BMR exist in the relationship between obesity and spirometers in asthma. Our study found that the BMR was positively associated with the spirometers, which implies that the higher the BMR, the higher the FEV_1, $\%FEV_1$, FVC, %FVC, and FEV_1/FVC levels in subjects with asthma. These findings corroborate previous data from the study by Itagi et al. in which an analysis was undertaken on healthy middle-aged individuals [27]. In addition, previous studies have shown that patients with asthma have increased BMRs [28,84]. Interestingly, our study further confirms the important mediation role of a decreased BMR in obesity-induced deterioration in the lung function, which may be one of

the key mechanisms for the worsening lung function in obese asthma. Additionally, bootstrapping, a non-parametric resampling technique used in mediation analysis, enabled us to robustly assess the significance of the indirect effects by generating confidence intervals with increased accuracy [85–88]. To ensure the reliability of our bootstrapping analysis, we addressed its key assumptions, including the independence of the observations and the representativeness of the sample [85,89]. We treated each participant's measurement as an independent observation, supported by our data collection process, and adhered to comparatively stringent inclusion and exclusion criteria to enhance the sample representativeness. Furthermore, we conducted 5000 bootstrap resamples to ensure stable confidence intervals [90–92]. By employing these robust methodological practices, we strengthened the validity of our mediation analysis findings. The bootstrapping analysis provides a precise measure of the uncertainty, enhancing the confidence in our conclusions. The stability of the resampling process and the comparatively large sample size further enhance the reliability of our results.

We conducted additional analyses to further explore the impact of SMM on airflow obstruction in individuals with non-obese and obese asthma. In the participants with non-obesity, those with higher SMM exhibited significantly improved lung function, as indicated by their higher FEV_1 (2.42 L vs. 2.13 L, $p = 0.007$; 0.93 vs. 0.70, $p < 0.001$) and FVC (3.42 L vs. 2.96 L, $p < 0.001$; 1.06 vs. 0.87, $p < 0.001$) values, compared to those with lower SMM. In the participants with obesity, those with higher SMM demonstrated significantly better lung function, with higher median FEV_1 (2.38 L vs. 1.47 L, $p < 0.001$; 0.87 vs. 0.63, $p < 0.001$) and FVC (3.44 L vs. 2.41 L, $p < 0.001$; 0.99 vs. 0.87, $p = 0.006$) values, compared to those with lower SMM. Moreover, a significant difference in the FEV_1/FVC ratio was observed between the groups (0.73 vs. 0.62, $p < 0.001$), with higher SMM associated with a higher ratio. Therefore, regardless of whether they are obese or non-obese, patients with asthma with higher SMM exhibit better pulmonary ventilation function. The significant difference in the FEV_1/FVC ratio in the participants with obesity is clinically significant, indicating that higher SMM is associated with a lower risk of airflow obstruction in individuals with obese asthma. This suggests that strategies to increase muscle mass may have potential benefits in improving the lung function in individuals with obese asthma. The differential impacts of SMM on the FEV_1/FVC ratio between the participants with non-obesity and those with obesity highlights the importance of considering the obesity status in mitigating asthma-related airflow limitations.

To further explore the effects of the BMR and muscle mass on the long-term lung function in patients with asthma, we assessed the relationship between the different BMR and muscle mass levels with the lung function in the following year. The results found that the higher the BMR and muscle mass, the better the lung function sustained in the next year. Therefore, we hypothesize that increasing the BMR and muscle mass may be potential therapeutic targets for improving and maintaining long-term lung function for patients with asthma. As mentioned above, previous studies have confirmed that the BMR is elevated in patients with obesity or asthma. Our study further confirms that the BMR is elevated in patients with obese asthma, and that an elevated BMR has a protective effect on the lung function in patients with asthma. In clinical practice, we usually advise patients with obese asthma to lose weight to improve their asthma control and lung function. However, our findings suggest that weight loss alone may lead to deterioration in the lung function because a reduction in the BMI is accompanied by a reduction in the BMR. We therefore recommend that the interventions for obese asthma target both the reduction in adiposity and the maintenance of a high BMR and high muscle mass. For example, exercise training, known for its benefits in improving cardiopulmonary fitness and reducing obesity, emerges as a promising approach to elevate the BMR and SMM. Furthermore, dietary interventions focusing on high protein intake and resistance training have demonstrated effectiveness in increasing the muscle mass and improving the lung function in individuals with obesity [93–95]. Additionally, pharmacological interventions, such as β2-agonists and vitamin D supplementation, have been suggested to enhance muscle strength and

mitigate obesity-induced asthma exacerbations [93,96]. Future research should investigate the efficacy of these interventions in improving the BMR, SMM, and lung function and reducing the airway obstruction in patients with obese asthma.

There are several limitations to our study. Firstly, the BMI has been used to assess the obesity status in patients with asthma and to define obese asthma for many years. However, in order to assess the obesity status multidimensionally, based on previous nutritional guidelines and studies, although we used multiple indicators simultaneously to define obese asthma, there still may have been an inaccurate assessment of obesity. Future studies could explore multidimensional approaches to assessing the obesity status in patients with asthma. Secondly, our study included only patients from the Chinese population. The results may not be applicable to other asthmatic populations. Studies conducted on healthy Chinese populations have confirmed that an increased BMI is accompanied by not only increased adiposity but also increased muscle mass [97,98]. Additional research is required to ascertain the generalizability of our findings to broader groups of patients with asthma. Thirdly, BIA and dual-energy X-ray absorptiometry (DXA) are regarded as valid techniques for assessing the BC. In this study, the BC was evaluated solely through the use of BIA. Previous reports support the notion that multifrequency BIA yields fat mass and muscle mass measurements that are consistent with those obtained via DXA across diverse cohorts [99,100]. Fourthly, while we acknowledge that a decreased BMR could result from a deteriorating pulmonary function, which, in turn, leads to reduced physical activity and impacts muscle mass and nutritional health, creating a vicious cycle, our longitudinal cohort study was designed to establish the directionality of the relationship, considering the BMR and muscle mass as the exposure variables and the lung function as the dependent variable.

5. Conclusions

In summary, our study establishes a significant link between the BMR and muscle mass and obesity and the lung function in individuals with asthma. The BMR and muscle mass not only play a role in mediating the relationship between obesity and the lung function, but they also determine the long-term lung function. Consequently, it is crucial to incorporate these previously overlooked nutritional indicators, particularly the BMR, into asthma assessments. Our findings pave the way for future clinical studies to adopt a more comprehensive approach to understand the intricate relationship between the nutritional status and lung function in chronic respiratory diseases. We propose that the therapeutic interventions for patients with obese asthma should not solely focus on reducing adiposity but should also prioritize maintaining a high BMR and high muscle mass.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/nu16121809/s1, Figure S1.Path model diagrams with the results of mediation analysis. Path model showing the effect of obesity on the measurements of lung function as mediated by BMR/BMI. Total β effect (path c) represents the effect of obesity on lung function with no mediators in the model. Direct β effect (path c') represents the effect of obesity on lung function when BMR/BMI is included in the model. Indirect effects (path a_1b_1) represent the effect of obesity on lung function through BMR/BMI. These models were adjusted for age, sex, ICS dosage, and SAE in the past year. The figures show unstandardized β regression coefficients (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Figure S2. Path model diagrams with the results of mediation analysis. Path model showing the effect of obesity on the measurements of lung function as mediated by BMR/Height^2. Total β effect (path c) represents the effect of obesity on lung function with no mediators in the model. Direct β effect (path c') represents the effect of obesity on lung function when BMR/Height^2 is included in the model. Indirect effects (path a_1b_1) represent the effect of obesity on lung function through BMR/Height^2. These models were adjusted for age, sex, ICS dosage, and SAE in the past year. The figures show unstandardized β regression coefficients (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Figure S3. Path model diagrams with the results of mediation analysis. Path model showing the effect of obesity on the measurements of lung function as mediated by ALM/BMI. Total β effect (path c) represents the effect of obesity on lung function with no mediators in the model. Direct β effect (path

c') represents the effect of obesity on lung function when ALM/BMI is included in the model. Indirect effects (path a_1b_1) represent the effect of obesity on lung function through ALM/BMI. These models were adjusted for age, sex, ICS dosage, and SAE in the past year. The figures show unstandardized β regression coefficients (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Figure S4. Path model diagrams with the results of mediation analysis. Path model showing the effect of obesity on the measurements of lung function as mediated by ALM/Height^2. Total β effect (path c) represents the effect of obesity on lung function with no mediators in the model. Direct β effect (path c') represents the effect of obesity on lung function when ALM/Height^2 is included in the model. Indirect effects (path a_1b_1) represent the effect of obesity on lung function through ALM/Height^2. These models were adjusted for age, sex, ICS dosage, and SAE in the past year. The figures show unstandardized β regression coefficients (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Figure S5. Path model diagrams with the results of mediation analysis. Path model showing the effect of obesity on the measurements of lung function as mediated by SMM/Height^2. Total β effect (path c) represents the effect of obesity on lung function with no mediators in the model. Direct β effect (path c') represents the effect of obesity on lung function when SMM/Height^2 is included in the model. Indirect effects (path a_1b_1) represent the effect of obesity on lung function through SMM/Height^2. These models were adjusted for age, sex, ICS dosage, and SAE in the past year. The figures show unstandardized β regression coefficients (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Figure S6. Pre-FEV_1(%) at each visit within the 12-month follow-up by BMR and muscle mass. FEV_1: forced expiratory volume in 1 s; SMI = ALM/Height^2. ALM: appendicular lean mass; BMI: body mass index; BMR: basal metabolic rate; SMI: appendicular skeletal muscle index; SMM: skeletal muscle mass. Figure S7. Pre-FVC(L) at each visit within the 12-month follow-up by BMR and muscle mass. FVC: forced vital capacity; SMI = ALM/Height^2. ALM: appendicular lean mass; BMI: body mass index; BMR: basal metabolic rate; SMI: appendicular skeletal muscle index; SMM: skeletal muscle mass. Figure S8. Pre-FVC(%) at each visit within the 12-month follow-up by BMR and muscle mass. FVC: forced vital capacity; SMI = ALM/Height^2. ALM: appendicular lean mass; BMI: body mass index; BMR: basal metabolic rate; SMI: appendicular skeletal muscle index; SMM: skeletal muscle mass.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board (or Ethics Committee) of West China Hospital, Sichuan University (Chengdu, China) (NO. 2014–30, approved on 24 April 2014).

Informed Consent Statement: Informed consent was obtained from all subjects involved in this study.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request. The data are not publicly available due to participant privacy and consent concerns.

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Both Maternal High-Fat and Post-Weaning High-Carbohydrate Diets Increase Rates of Spontaneous Hepatocellular Carcinoma in Aged-Mouse Offspring

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Abstract: Both maternal obesity and postnatal consumption of obesogenic diets contribute to the development of metabolic dysfunction-associated steatotic liver disease (MASLD) and hepatocellular carcinoma (HCC). However, there is no consensus as to whether diets that are high in fat or carbohydrates/sugars differentially influence the development of HCC. Moreover, the long-term effects of prenatal HF exposure on HCC and whether this is influenced by postnatal diet has not yet been evaluated. C57BL/6 dams were fed either a low-fat, high-carbohydrate control (C) or low-carbohydrate, high-fat (HF) diet. At weaning, male and female offspring were fed the C or HF diet, generating four diet groups: C/C, C/HF, HF/C and HF/HF. Tissues were collected at 16 months of age and livers were assessed for MASLD and HCC. Glucose regulation and pancreatic morphology were also evaluated. Liver tissues were assessed for markers of glycolysis and fatty acid metabolism and validated using a human HCC bioinformatic database. Both C/HF and HF/HF mice developed obesity, hyperinsulinemia and a greater degree of MASLD than C/C and HF/C offspring. However, despite significant liver and pancreas pathology, C/HF mice had the lowest incidence of HCC while tumour burden was highest in HF/C male offspring. The molecular profile of HCC mouse samples suggested an upregulation of the pentose phosphate pathway and a downregulation of fatty acid synthesis and oxidation, which was largely validated in the human dataset. Both pre-weaning HF diet exposure and post-weaning consumption of a high-carbohydrate diet increased the risk of developing spontaneous HCC in aged mice. However, the influence of pre-weaning HF feeding on HCC development appeared to be stronger in the context of post-weaning obesity. As rates of maternal obesity continue to rise, this has implications for the future incidence of HCC and possible dietary manipulation of offspring carbohydrate intake to counteract this risk.

Keywords: maternal obesity; metabolic dysfunction-associated steatotic liver disease; hepatocellular carcinoma; glycolysis; fatty acid oxidation; high fat; high carbohydrate

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1. Introduction

Hepatocellular carcinoma (HCC) is currently the fourth leading cause of cancer-related deaths worldwide, with a 5-year survival rate of less than 20% [1]. The majority of HCC cases are diagnosed in aged individuals [2] and at rates 3–4 times higher in men than women [3]. Chronic liver disease arising from viral infections including hepatitis B and C is

a main contributor to the development of HCC [4]. In addition, in countries with high rates of obesity, metabolic dysfunction-associated steatotic liver disease (MASLD) and metabolic dysfunction-associated steatohepatitis (MASH) account for a rapid rise in the prevalence of HCC [5].

Global rates of obesity have more than doubled in the past 40 years, and around 30% of women of childbearing age are currently estimated to be obese [6]. Among the long-term conditions associated with maternal obesity, the risk of developing MASLD is higher in adult offspring born to obese mothers [7–10]. Recently, using a combination of chemotoxic exposure and maternal high-fat (HF) feeding, Sun et al. reported that the incidence of HCC was higher in young adult mice born to HF-fed dams and that tumour burden increased across multiple generations [11]. However, whether these findings are relevant in older offspring and/or in the absence of chemotoxic manipulation is unclear. Moreover, a possible interaction between pre- and postnatal HF diet on risk of HCC has not yet been investigated.

Overconsumption of saturated fats, carbohydrates and monosaccharides, including sucrose and fructose, contributes to the development of obesity, glucose intolerance and MASLD/MASH. Many preclinical models use HF and/or high-sucrose diets to study HCC in the context of obesity [12]. By contrast, caloric restriction of up to 40% has been reported to significantly reduce liver inflammation and fibrosis and decrease HCC-tumour burden, incidence and progression [13]. Moreover, an early study found that caloric restriction was most effective in slowing the growth of hepatocellular adenomas and adenocarcinomas in mice that underwent food restriction from weaning [14]. There is currently no consensus about the relative contribution of dietary sugar/carbohydrates and fat on the risk of developing HCC, as positive, neutral and negative associations have been reported between consumption sucrose or fatty acids (FAs) and the frequency of HCC [15–17]. Recent evidence also suggests that long-term consumption of low-fat, high-carbohydrate diets increase cholesterol deposition in the liver [18] and promote HCC [19]. In addition, several groups have reported significantly lower tumour burden in diethylnitrosamine (DEN)-treated mice fed an HF + low-sucrose diet compared to mice fed an HF + high-sucrose or high-sucrose-only diet [20,21]. This suggests that although dietary fats, carbohydrates and sugars can all induce metabolic disease and MASLD, they may contribute differentially to the development of HCC.

Under physiological conditions, metabolism of glucose and fat via glycolysis and β-oxidation of FAs, respectively, are mutually regulated by the glucose–FA cycle in the liver (Supplementary Figure S1). Many cases of MASLD-associated HCC are characterised by a downregulation of FA oxidation and concurrent stimulation of glycolysis, alongside the shunting of glycolytic metabolites towards anaerobic pathways [22]. By contrast, some β-catenin-activated HCCs display the opposite pattern of enhanced FA oxidation and reduced glycolysis [23]. Increasing evidence also suggests that HCC cells shuttle glucose into the pentose phosphate pathway (PPP) to sustain FA synthesis and maintain redox homeostasis [24]. In HCC cases associated with obesity and MASLD/MASH, there is evidence that both glucose consumption and FA synthesis are upregulated, while FA β-oxidation and acetyl-coA production are decreased [22]. This highlights both the heterogeneity of metabolic reprogramming in HCC and a need to disentangle the contribution of glycolysis and FA metabolism in obesity-related HCC. Moreover, as maternal HF feeding is associated with alterations in offspring hepatic lipid metabolism and glucose metabolism [25,26], such studies may provide additional insight into the role of maternal obesity on offspring risk of HCC.

This study investigated the impact of combinations of pre- and post-weaning HF or high-carbohydrate feeding on liver and pancreas pathology, as well as the development of HCC in 16-month-old male and female offspring. Assessment of molecular pathways related to liver glycolysis and FA β-oxidation were also evaluated. We found that the incidence of HCC was highest in lean males born to mothers fed an HF diet, despite the fact that these mice had less liver and pancreas pathology than offspring fed the HF diet

post weaning. In non-cancerous liver tissues, there was an upregulation of genes associated with FA synthesis, while the molecular profile in tumours suggested an upregulation of the PPP and a downregulation of FA synthesis and oxidation.

2. Materials and Methods

Mouse model of pre- and postnatal feeding: Female C57BL/6 dams were randomly fed either a control (C) or high-fat (HF) diet (Special Diet Services, Witham, UK) as previously described [27,28]. The C diet (3.68 kcal/g) was composed of Atwater Fuel Energy (AFE) 10% fat, 20% protein and 70% carbohydrates. The HF diet (4.57 kcal/g) contained AFE 45% fat, 20% protein and 35% carbohydrates. Dams were fed the C or HF diets for 4 weeks before mating (at 18–27 weeks old) and kept on the diet during gestation and lactation. Stud C57BL/6 mice were fed the C diet throughout the experiment. Macro minerals, vitamins, and amino acid composition was matched between diets and detailed dietary information is provided in Supplementary Table S1. At weaning, male and female offspring were fed either the C or HF diet, generating four diet groups, C/C, C/HF, HF/C, and HF/HF, representing the pre- and post-weaning diet, respectively. Offspring were group-housed (average n = 4 mice/cage) by litter and maintained on the diet until sacrifice at 16 months of age. Offspring were assigned a unique numerical identifier so that the experimenters were blinded to maternal diet throughout the experiment until statistical analyses. Weekly *ad libitum* food intake was recorded for the first 6 weeks post weaning and daily food intake per mouse was calculated by dividing the total amount of food consumed by the number of mice per cage per day and values were then averaged across the *ab libitum* period. Mice underwent food restriction for 3 months at 6- and 12-months of age as part of a separate behavioural study [27]. During this time, mice were provided daily with sufficient food to maintain approximately 90% of their free-feeding weight. Food and caloric intake were also calculated as an average of values from the start and end of the restriction time period. All experiments were reviewed and approved by the Open University Animal Welfare and Ethics Review Board and the Home Office as per the UK Animal (Scientific Procedures) Act 1986 Amendment Regulations 2012 (PPL 70/8507). Experiments were carried out in accordance with ARRIVE guidelines.

Glucose (GTT) and insulin (ITT) tolerance tests: Offspring underwent GTT (n = 10–20/group) and ITT (n = 6–16/group) before sacrifice. For GTT, mice were fasted overnight, topical anaesthetic was applied to tails, and animals were injected i.p. with glucose (2 mg/g, dissolved in 0.9% sterile saline). Blood glucose concentrations were measured from the tail vein at baseline and 15, 30, 60 and 120 min post injection using an Accu-check blood glucose monitor (Roche, Welwyn Garden City, UK). In a subset of animals, blood samples were also collected at 0, 5, 15 and 30 min after glucose injection and plasma was processed for insulin concentrations using the Ultra Sensitive Mouse Insulin ELISA kit according to manufacturer's instructions (Crystal Chem, Zaandam, The Netherlands). For ITT, mice were fasted for 3 h, injected i.p. with 0.35 U/kg recombinant human insulin, (Merck Life Science UK Limited, Gillingham, UK) and blood glucose was measured as in the GTT.

Tissue collection: Mice were given an overdose of sodium pentobarbital and perfused intracardially with 0.01 M phosphate-buffered saline (PBS). Blood was collected into heparinized tubes, spun down (2000 g for 5 min), and plasma was collected and stored at −80 °C. For frozen tissues, liver and pancreas were snap-frozen in isopentane on dry ice and stored at −80 °C until use. For fixed tissues, mice were additionally perfused with 4% paraformaldehyde (PFA), tissues were kept overnight in PFA at 4 °C, rinsed in PBS and stored in 30% sucrose. Tissues were then sliced on a cryostat (20 µm thickness), collected onto Superfrost microscope slides (Fisher Scientific, Loughborough, UK) and stored at −20 °C.

Liver histology and tumour identification: Liver sections were processed for H&E (Abcam, Cambridge, UK) and Picrosirius Red (Pioneer Research Chemicals, Essex, UK) staining. Images were captured on a Nikon Eclipse 80 Brightfield microscope (Nikon, Milton Keynes,

UK). HCC was confirmed manually from H&E-stained tumours. Macrosteatosis was quantified from H & E images (3 images/animal, n = 5/sex/group) using Fiji software version 2.9.0 (NIH, MD, USA) by assessing the % area of liver covered by circular white spaces (circularity 0.1–0.54). Microsteatosis was determined by manually scoring images according to the total hepatic area affected (0 (<5%), 1 (5–33%), 2 (34–66%) and 3 (>66%), as per a published protocol [29]. Degree of liver fibrosis was quantified by calculating % area of liver positive for Picrosirius Red staining (3 images/animal, n = 5/sex/group) using Fiji software.

Plasma cholesterol: Plasma samples from non-fasted mice (n = 5/group) were processed in duplicate for high-density (HDL) and very-and low-density lipoproteins (v/LDL) using a Cholesterol Assay Kit, as per manufacturer's instructions (Abcam, Cambridge, UK).

Immunohistochemistry: Liver and pancreas tissues were processed as described previously [28]. Tissues were washed in 0.01 M PBS, incubated with 3% H_2O_2 in methanol for 10 min and blocked with 15% goat serum. For insulin staining, sections underwent antigen retrieval by heating the slides in 10 mM sodium citrate + 0.01% Tween 20 at 80 °C in a water bath for 20 min. For CD45 and F4/80 staining, tissues were treated for 15 min with boiling 1 mM EDTA. Tissues were incubated overnight at 4 °C with anti-glucagon (1:350, Abcam, Cambridge, UK), anti-insulin (1:150, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-CD45 (1:150, Fisher Scientific, Loughborough, UK) or anti-F4/80 (1:200, Cell Signalling Technology, Leiden, The Netherlands) diluted in 0.1% Triton X-100. The next day, sections were washed with PBS, incubated for 1 h at room temperature with biotinylated anti-rabbit or anti-mouse (1:400) and ABC kit (1:200, Vector Labs, Newark, USA) and developed using the glucose oxidase DAB method. For pancreas sections labelled with anti-CD45, sections were counterstained with Nuclear Fast Red (Merck, UK). Images were captured on a Nikon Eclipse 80 Brightfield microscope (Nikon, Normanton, UK). For pancreas images, islet number/mm^2 tissue and islet area/mm^2 tissue were quantified manually using Fiji (3 sections/animal, n = 5 mice/sex/group). For liver and pancreas sections, the % area of liver positive for CD45 was quantified using Fiji (3 sections/animal, n = 5 mice/group).

RT-qPCR: Frozen liver samples from normal liver samples from male offspring that did not develop HCC (n = 6/group) and tumours from male C/C (n = 6) and HF/C mice (n = 8) were incubated for at least 24 h in RNAlater-ICE (Fisher Scientific, Loughborough, UK), and mRNA was isolated using an RNeasy Plus Micro Kit using a TissueLyser LT (Qiagen, Manchester, UK). cDNA was synthesised using the Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit (Fisher Scientific). KiCqStart® SYBR® Green Primers (Merck, Gillingham, UK) were used to quantify levels of hexokinase 2 (*Hk2*), phosphofructokinase, liver type (*Pfkl*), pyruvate kinase M2 (*Pkm2*), glucose-6-phosphate dehydrogenase (*G6pdx*), acyl-CoA synthetase long-chain family member 4 (*Acsl4*), carnitine palmitoyltransferase 1A (*Cpt1a*), acyl-CoA dehydrogenase family member 11 (*Acad11*), sterol regulatory element-binding transcription factor 1c (*Srebp1c*) and b-actin (*Actb*) using the QuantiTect SYBR Green PCR Kit (Qiagen). For comparison between diet groups, C/HF was used as the reference group. For comparison between tumour and non-tumour tissues, the non-tumour values from the corresponding diet group (C/C or HF/C) were used as the reference value. Primer sequences are provided in Supplementary Table S2.

Western blot: Frozen liver samples of non-tumour liver samples from male offspring diet groups (n = 6/group) and tumours from male C/C (n = 6) and HF/C mice (n = 8) were sonicated in Ripa lysis buffer, centrifuged (10,000× g for 10 min at 4 °C), and the supernatant was stored at −80 °C until use. A total of 35–50 µg of protein was separated on 4–20% Tris-Glycine gels (Fisher Scientific, Loughborough, UK) and transferred onto nitrocellulose membranes that were blocked for 1 h with 8% non-fat milk. Blots were incubated overnight at 4 °C with anti-glucose-6-phosphate dehydrogenase (1:1500, Abcam), anti-ascl4 (1:500, Fisher Scientific UK), anti-glutathione peroxidase 4 (GPX4, 1:500, Cell Signalling Technology, Leiden, The Netherlands) or anti-hyperoxidized peroxiredoxin-3 (PRX3-$SO_{2/3,}$ 1:500, Cambridge Biosciences, Cambridge, UK). Blots were subsequently

developed with an ECL kit (Fisher Scientific UK), stripped and reprobed with anti-GAPDH (1:50,000, Merck) to ensure equal protein loading. Blots were performed in duplicate, the optic densities of the bands were quantified using Fiji, and final values were calculated by dividing the optic density of the protein of interest by the corresponding GAPDH value.

Bioinformatic analyses: mRNA levels of *Hk2*, *Pfkl*, *Pkm2*, *G6pd*, *Acsl4*, *Cpt1a*, *Acad11* and *Srebp1* in normal human liver (n = 50) and HCC tissues (n = 371) were investigated using the University of Alabama at Birmingham Cancer (UALCAN) interactive database, which is curated using The Cancer Genome Atlas, MET500 and Clinical Proteomic Tumor Analysis Consortium data portal [30]. Box and whisker plots including interquartile ranges and Welch's *t*-tests were used to estimate differences in expression levels between normal and primary tumours [30,31].

Statistical analysis: All analyses were carried out using GraphPad Prizm (Boston, MA, USA). Data were checked for normality using the Shapiro–Wilk test. The ROUT test was used to determine and exclude statistical outliers. Offspring tumour incidence was calculated using Fisher's exact test. Comparison of diet x time effects was calculated using a repeated measures two-way ANOVA, while sex x diet effects were analysed using two-way ANOVA with the Sidak post hoc test. A $p < 0.05$ was considered to be statistically significant. Data are presented as mean ± SEM unless stated otherwise.

3. Results

3.1. Postnatal HF Diet Induced Weight Gain, While Postnatal C Diet Increased HCC Incidence

Data regarding dam and offspring weight, as well as offspring adiposity, food and calorie consumption, have been published previously [27,28]. In brief, dams fed the HF diet weighed significantly more than *C*-fed females at mating and throughout gestation and weaning. Male and female offspring fed the HF diet postnatally (C/HF and HF/HF) also weighed significantly more than offspring fed the C diet (C/C and HF/C) from week 3 post weaning onwards (Figure 1a,b). No weight differences were observed between C/C and HF/C or between C/HF and HF/HF mice at weaning, over the life course or during periods of food restriction (Figure 1a,b). Total food intake was similar among all diet groups during periods of *ad lib* and restricted feeding (Figure 1c,e) and all offspring lost a similar amount of body weight (proportional to their free-feeding weight) during food restriction (Figure 1d,f). Male C/C, C/HF, HF/C and HF/HF mice consumed the same number of total calories during *ad lib* feeding (Figure 1g) and this pattern was maintained when adjusted by mouse body weight (Figure 1i). Intake of absolute calories was higher in HF-fed male mice than *C*-fed mice during food restriction, but not when adjusted by body weight (Figure 1g,i). HF-fed females ate more total calories than *C*-fed females during periods of both *ad lib* and restricted food intake (Figure 1h). However, when adjusted for body weight, absolute *ad lib* kcal consumption was the same between diet groups but decreased in C/HF and HF/HF females during periods of food restriction (Figure 1j).

During tissue collection from 16-month-old offspring, we observed that approximately 9% of offspring had developed macroscopic liver tumours (Figure 2a), which was more than 3-times higher than reported rates of spontaneous tumour development across the lifespan of C57BL/6 mice [32]. Pathological characterization confirmed the majority of these tumours as HCC (Figure 2a). Analysis of tumour incidence by sex revealed that tumour rate was almost twice as high in male (10%) versus female (5%) offspring (p = 0.03, 95% CI [0.87, 9.89]) (Figure 2a). In addition, of the combined male- and female-offspring diet groups, HF/C mice had the highest tumour incidence (13%), followed by C/C (11%), HF/HF (8%) and C/HF mice (3%) (p = 0.01; Figure 2b). Notably, C/HF mice had significantly lower tumour incidence than both HF/C (p = 0.007, 95% CI [3.78, 17.6]) and C/C offspring (p = 0.0045, 95% CI [2.18, 15.4]). A similar pattern of tumour incidence between diet groups was also observed in male offspring (p = 0.04, Figure 2c), while rates of HCC in female offspring were lower in HF/C and HF/HF mice compared to C/C, although this difference was not statistically significant (p = 0.10, Figure 2d).

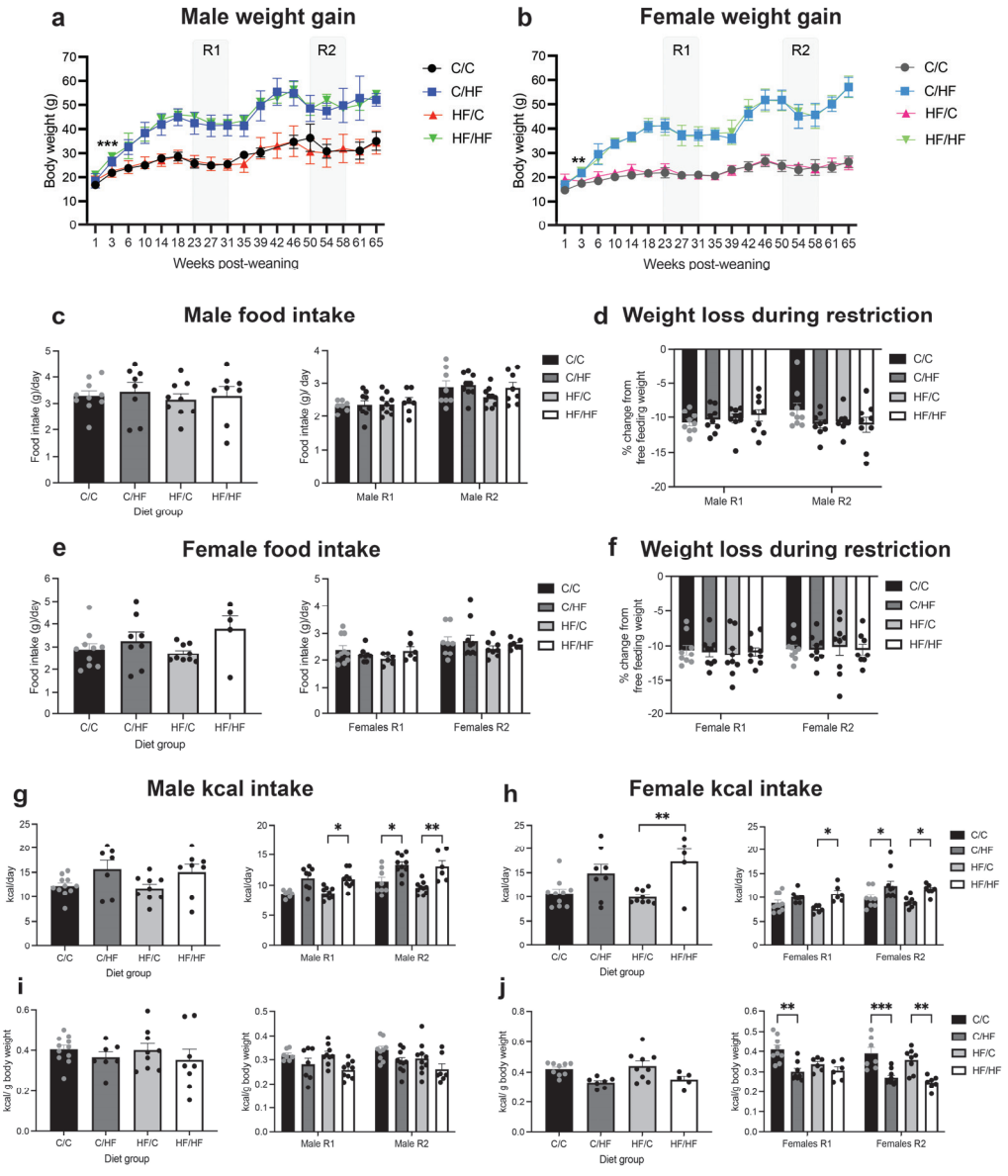

Figure 1. Body weight of male (**a**) and female (**b**) C/C (males = 9, females = 12), C/HF (males = 15, females = 9), HF/C (males = 9, females = 10) and HF/HF (males = 9, females = 9) offspring across their lifespan. Error bars show standard deviations. Gray areas indicate periods of food restriction (R1 and R2). ** $p < 0.01$, *** $p < 0.001$, C/HF and HF/HF mice vs. C/C and HF/C mice, two-way repeated measures ANOVA with Sidak's post hoc. (**c**–**j**), Total food intake/day (**c**,**e**), weight loss during periods of food restriction (**d**,**f**), total kcal intake/day (**g**,**h**) and kcal/g body weight (**i**,**j**) of male (**c**,**d**,**g**,**i**) and female (**e**,**f**,**h**,**j**) offspring during *ad libitum* and restricted food intake. C/C (males = 10, females = 10), C/HF (males = 8, females = 8), HF/C (males = 9, females = 9) and HF/HF (males = 8, females = 5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, two-way ANOVA with Sidak's post hoc test. Figure adapted from [25,26].

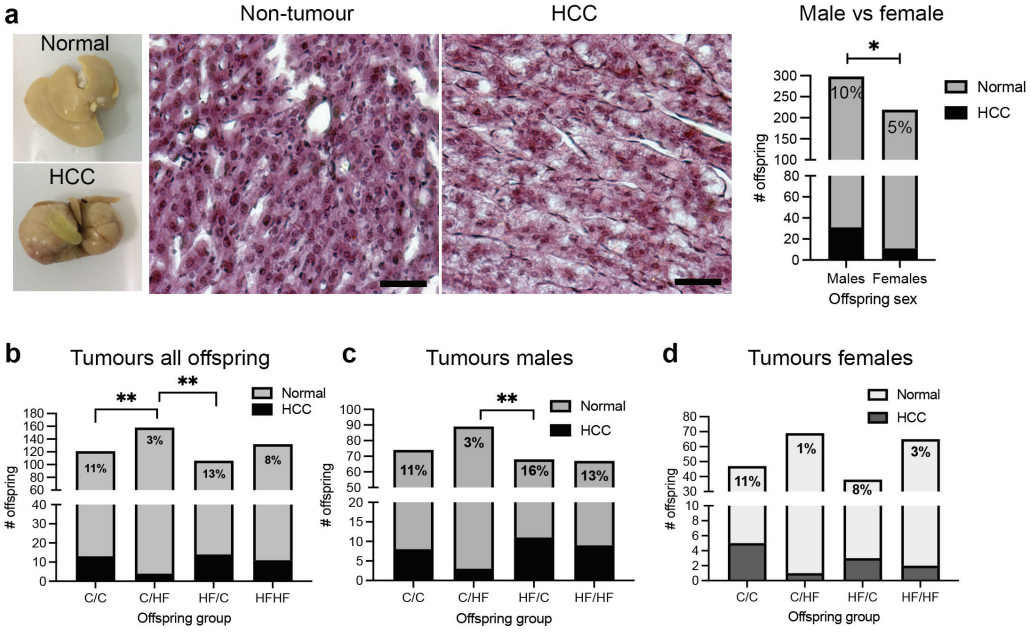

Figure 2. (**a**) Gross morphology of a liver without any abnormalities (normal) and with large, macroscopic tumours (HCC). H & E staining showing normal cellular architecture in non-tumour tissue and enlarged trabeculae with steatosis in an HCC tumour. Quantification of the total number of male and female offspring with and without HCC. (**b**–**d**), quantification of HCC by diet group (**b**) and within male-offspring (**c**) and female-offspring (**d**) diet groups. Inset numbers represent the percentage of animals with HCC in each group. Scale bars = 20 µm. * $p < 0.05$, ** $p < 0.05$, Fisher's exact test.

3.2. Measures of MASLD Were Higher in HF-Fed Animals

To determine if the incidence of HCC was related to the effect of pre- and/or post-weaning HF diet on liver pathology, markers of liver steatosis, fibrosis and inflammation were assessed. As shown in Figure 3, macrosteatosis was significantly higher in male C/HF vs. C/C animals and in female HF/HF vs. C/HF and HF/C female offspring (Figure 3a,b). Male C/HF mice also had significantly greater macrosteatosis than female counterparts, while macrosteatosis was higher in HF/HF females than male animals (Supplementary Figure S2a). Analysis of microsteatosis found that both C/HF and HF/C male offspring had significantly greater microsteatosis compared to C/C males (Figure 3d). A similar, non-significant pattern was observed in female mice (Figure 3e), while no significant differences in degree of microsteatosis were observed between males and females (Supplementary Figure S2b). Average hepatocyte area was also significantly greater in C/HF vs. C/C males (Figure 3f) and in HF/HF females vs. C/HF and HF/C females (Figure 3g). Male C/HF mice had significantly higher hepatocyte area than females in the same diet group (Supplementary Figure S2c).

Liver fibrosis did not differ significantly between male diet groups (Figure 4a). In female mice, the HF/C group had significantly less fibrosis than C/C animals (Figure 4a) and C/HF females also showed significantly less fibrosis than male C/HF animals (Supplementary Figure S2d). Quantification of CD45 (Figure 4b and Supplementary Figure S2e) and F4/80 (Figure 4c) found no significant differences between male or female diet groups, although F4/80 staining was significantly higher in female C/HF and HF/C mice vs. males in the same diet group (Supplementary Figure S2f).

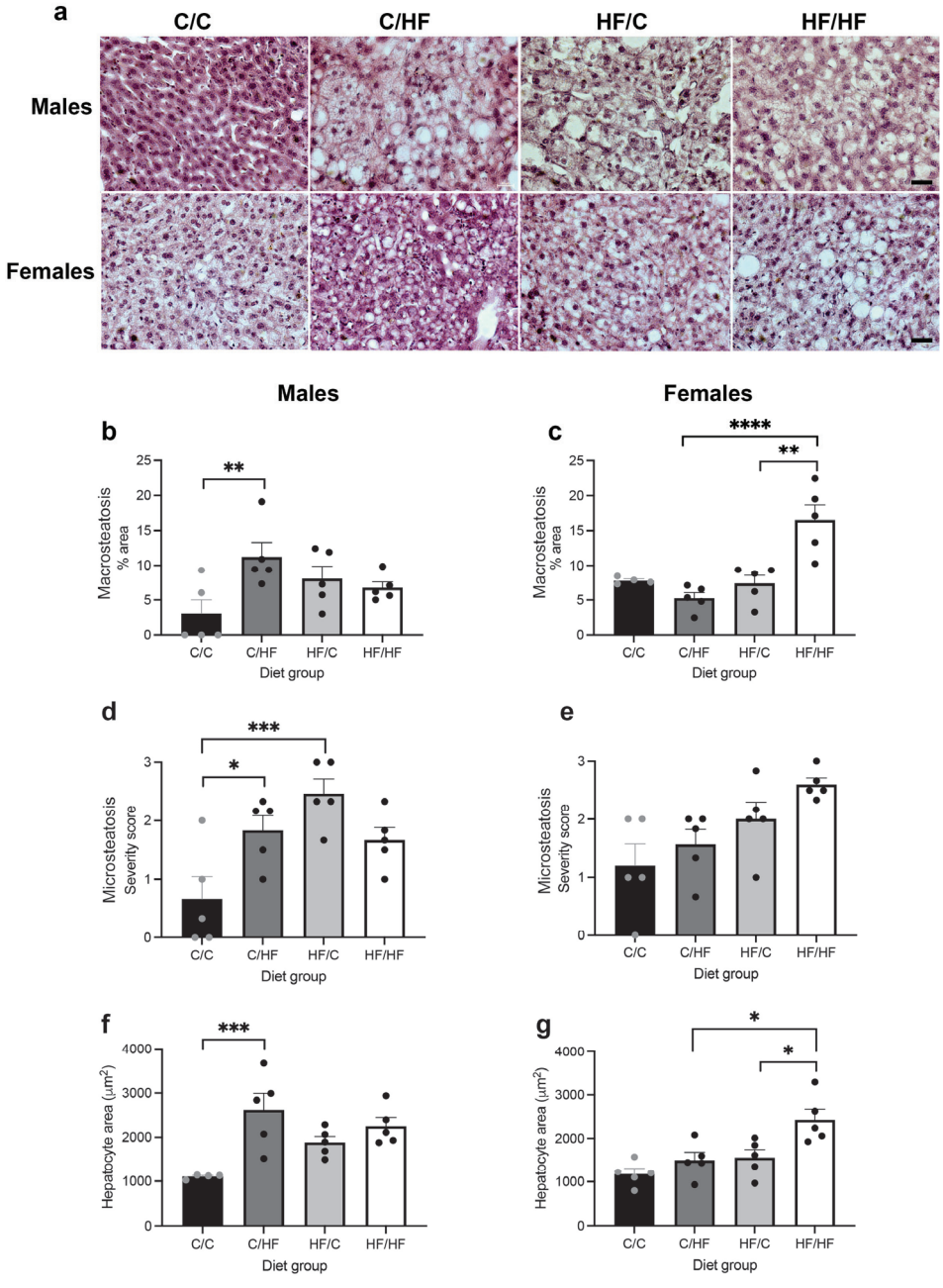

Figure 3. (**a**) H & E staining of liver tissues from male and female C/C, C/HF, HF/C and HF/HF offspring. Quantification of % area liver positive for macrosteatosis within male (**b**) and female (**c**) diet groups. Microsteatosis severity score between diets in males (**d**) and females (**e**). Quantification of hepatocyte area in male (**f**) and female diet groups (**g**). Scale bars = 50 µm. C/C (males = 4–5, females = 5), C/HF (males = 5, females = 5), HF/C (males = 5, females = 5) and HF/HF (males = 5, females = 5). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, two-way ANOVA with Sidak's post hoc test.

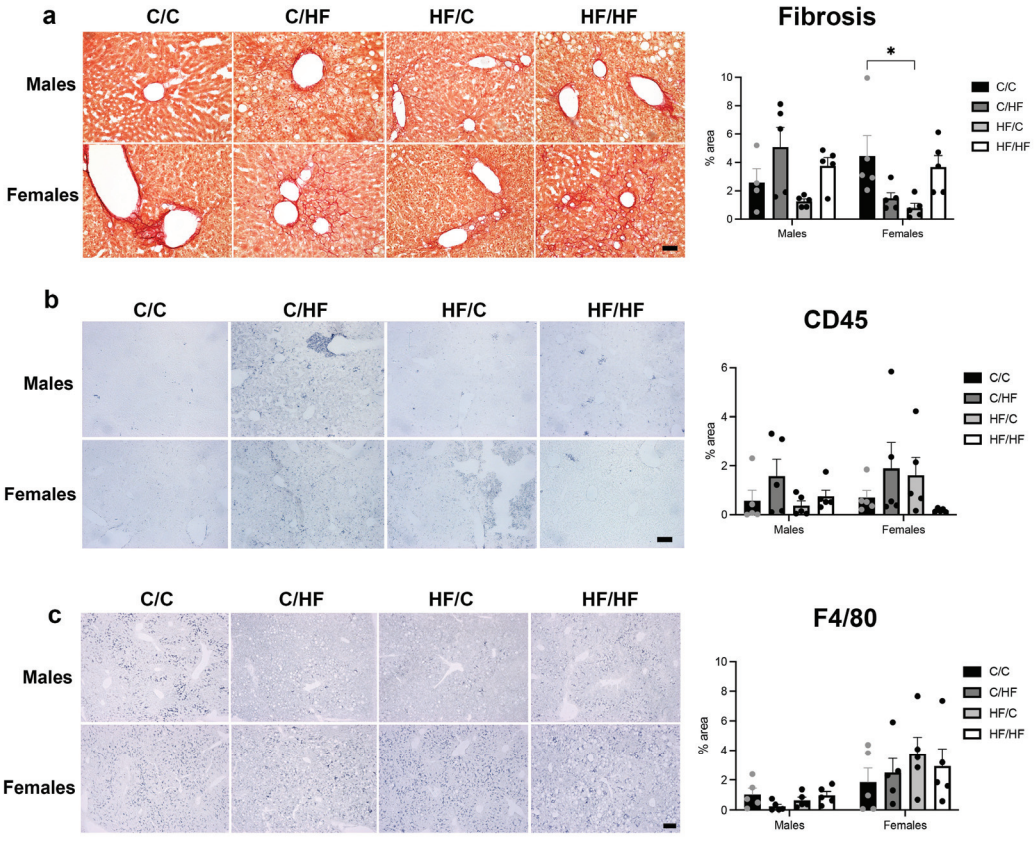

Figure 4. (**a**) Picrosirius Red staining of liver tissues from C/C, C/HF, HF/C and HF/HF offspring and quantification of % area liver positive for fibrosis between diet groups. (**b**) CD45 staining and quantification of liver tissues in different diet groups. (**c**) Images of F4/80-positive macrophages and quantification of % area liver positive for F4/80 staining of livers from different diet groups. Scale bar: (**a**) = 50 μm, (**b**,**c**) = 200 μm. C/C (males = 4–5, females = 5), C/HF (males = 5, females = 5), HF/C (males = 5, females = 5) and HF/HF (males = 5, females = 5). * $p < 0.05$, two-way ANOVA with Sidak's post hoc test.

Finally, analysis of non-fasted plasma HDL concentrations found no significant differences between diet groups (Supplementary Figure S3a,b). In male offspring, HF/C mice had significantly lower v/LDL compared to C/C and HF/HF animals, while no differences were noted between female diet groups (Supplementary Figure S3c,d). In general, female mice showed lower cholesterol levels than male animals, except for v/LDL concentrations in HF/C animals, which did not differ between sexes.

3.3. Postnatal HF, but Not C Diet, Induced Glucose Intolerance and Hyperinsulinemia

Previous reports have suggested a correlation between hyperinsulinemia and increased risk of HCC [33]. Blood glucose concentrations were similar between all male-offspring diet groups during the GTT (Figure 5a,b). However, glucose levels were significantly higher in C/HF and HF/HF mice during the ITT (Figure 5c,d). Female C/HF and HF/HF mice demonstrated significantly higher blood glucose concentrations compared to C/C and HF/C animals, respectively, during the GTT (Figure 5a,b). Female HF/HF mice also had significantly higher blood glucose levels than HF/C females in the ITT. During GTT and ITT, blood glucose concentrations were significantly lower in C/C, C/HF and

HF/C female mice compared to their male counterparts (Supplementary Figure S4a,b). Analysis of plasma insulin concentrations during the GTT showed that male C/HF and HF/HF mice and female C/HF animals were hyperinsulinemic (Figure 5e). Similarly, male C/HF and HF/HF mice had higher insulin levels following glucose administration compared to female offspring (Supplementary Figure S4c).

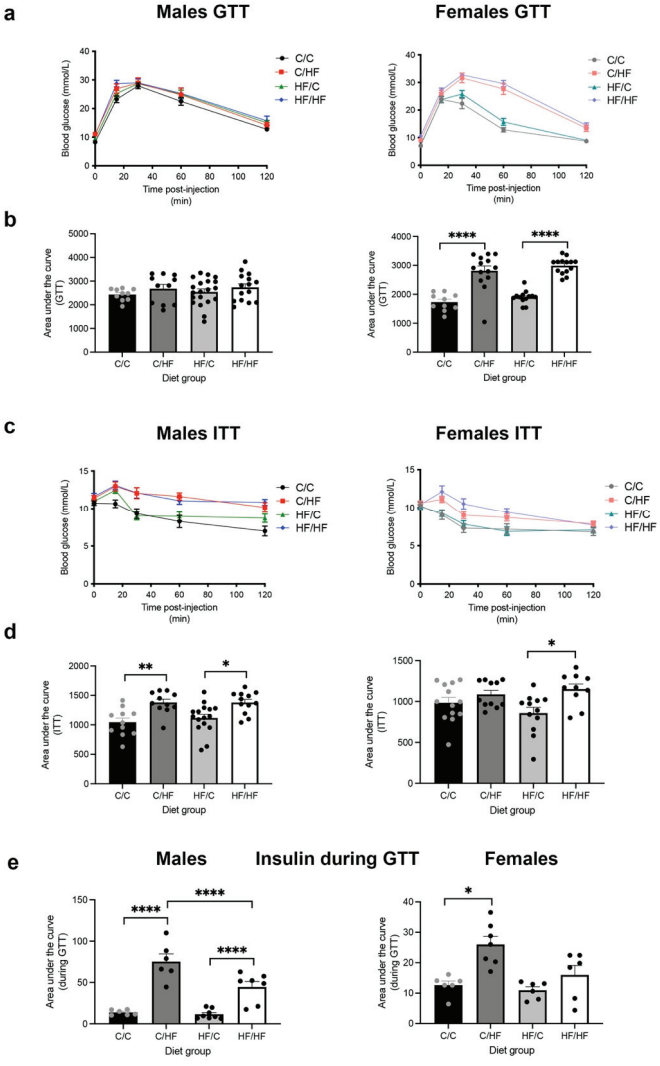

Figure 5. Time course (**a**) and area under the curve (**b**) of plasma glucose concentrations during the glucose tolerance test (GTT) test in 16-month-old C/C, C/HF, HF/C and HF/HF male and female offspring. C/C (males = 11, females = 10), C/HF (males = 11, females = 14), HF/C (males = 20, females = 13) and HF/HF (males = 15, females = 14). Time course (**c**) and area under the curve (**d**) during insulin tolerance test (ITT). C/C (males = 11, females = 13), C/HF (males = 11, females = 11), HF/C (males = 16, females = 12) and HF/HF (males = 12, females = 10). (**e**) Area under the curve of plasma insulin concentrations released during the GTT. C/C (males = 6, females = 6), C/HF (males = 6, females = 7), HF/C (males = 8, females = 6) and HF/HF (males = 7, females = 6). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, two-way ANOVA with Sidak's post hoc test.

In the pancreas, the average area of insulin-positive islets was significantly higher in male HF/HF mice vs. both C/HF and HF/C males (Figure 6a) and compared to HF/HF females (Supplementary Figure S4d). No differences were noted between diet groups or sex in the density of insulin-positive islets (Supplementary Figure S4e,f). A similar pattern was observed for glucagon staining, with larger glucagon-positive islets in male HF/HF mice vs. C/HF and HF/C males and in C/HF vs. C/C female offspring (Figure 6b and Supplementary Figure S4g–i). No differences in CD45 staining were seen between either male or female diet groups or between male and female offspring (Figure 6c and Supplementary Figure S4i). Similarly, F4/80 expression was unaltered between male diet groups, but significantly higher in female HF/HF mice vs. C/HF and HF/C animals (Figure 6c). Levels of F4/80 were also higher in the pancreas of female HF/C and HF/HF mice than male offspring (Supplementary Figure S4k).

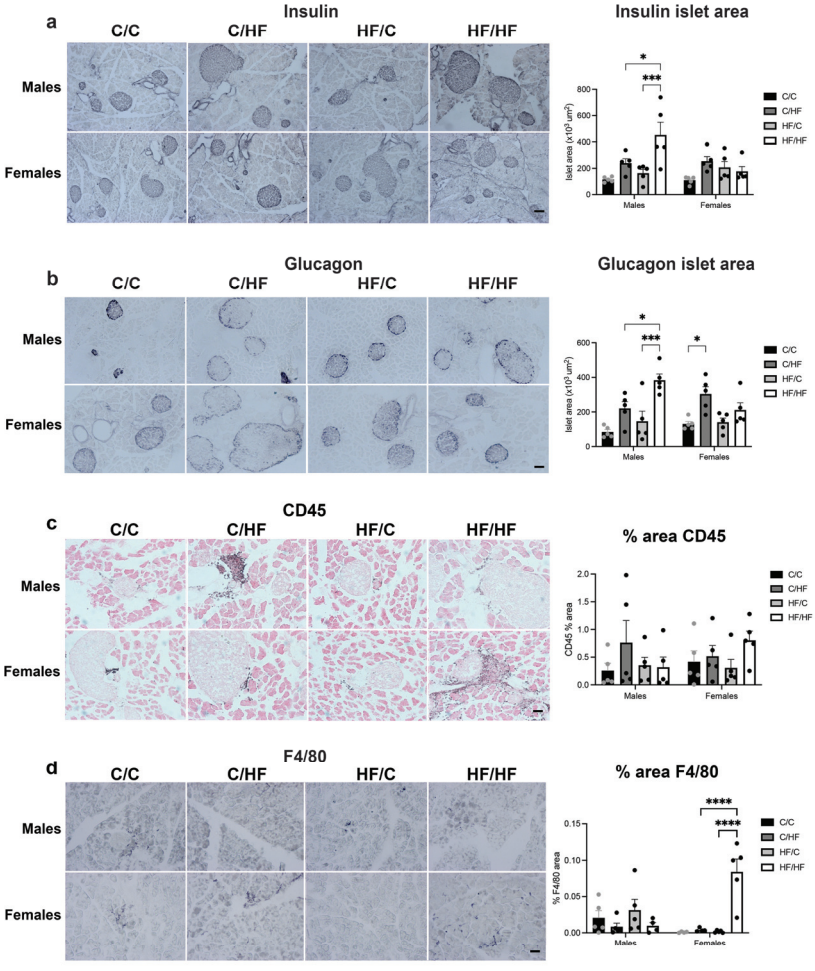

Figure 6. Photomicrographs and quantification of islet area in pancreases of 16-month-old male and female C/C, C/HF, HF/C and HF/HF offspring stained with insulin (**a**), glucagon (**b**), CD45 (**c**) and F4/80 (**d**). $n = 5$ for all groups. Scale bars = 100 µm. * $p < 0.05$, *** $p < 0.001$,**** $p < 0.0001$, two-way ANOVA with Sidak's post hoc test.

3.4. Markers of Glycolysis and Fatty Acid Oxidation Were Altered in Both Diet Groups and in HCC

To determine the effect of dietary manipulation on markers of glycolysis and the PPP, mRNA levels of the rate-limiting enzymes *Hexokinase* 2 (*Hk2*), *Phosphofructokinase*, *liver type* (*Pfkl*), *Pyruvate kinase M2 (Pkm2*) and *Glucose-6-phosphate dehydrogenase* (*G6pdx*), were assessed in non-tumour livers from male offspring that did not develop HCC. mRNA levels of *Hk2* did not differ significantly between diet groups (Figure 7a). Levels of *Pfkl* and *Pkm2* were also similar between diet groups (Figure 7b,c), while *G6pdx* expression in HF/C animals was approximately 4-fold higher than in C/HF and HF/HF animals (Figure 7d). Gene expression of three enzymes involved in various steps of FA activation (*Acyl-CoA synthetase long-chain family member 4*, *Ascl4*), transport across the mitochondrial membrane (*Carnitine palmitoyltransferase 1A*, *Cpt1a*) and β-oxidation (*Acyl-CoA dehydrogenase family, member 11*, *Acad11*) was also evaluated in non-tumour tissues. mRNA levels of *Ascl4* were significantly upregulated in C/C and HF/C offspring compared to C/HF animals (Figure 7e), while expression of *Cpt1a* and *Acad11* was similar between diet groups (Figure 7f,g). Given that ACSL4 can regulate the expression of Sterol Regulatory Element-Binding Protein 1c (SREBP-1c), a master regulator of genes required for FA synthesis [34], *Srebp1c* expression was also evaluated and found to be significantly higher in C/C mice compared to C/HF animals (Figure 7h).

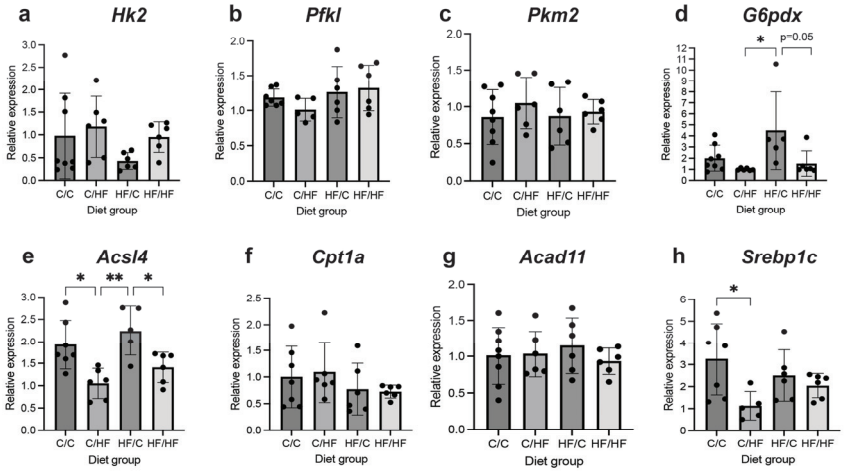

Figure 7. mRNA levels of *Hk2* (**a**), *Pfkl* (**b**), *Pkm2* (**c**), *G6pdx* (**d**), *Acsl4* (**e**), *Cpt1a* (**f**), *Acad11* (**g**) and *Srebp1c* (**h**) in non-cancerous liver tissues from 16-month-old male C/C (n = 7–8), C/HF (n = 6), HF/C (n = 6) and HF/HF (n = 6) offspring. * $p < 0.05$, ** $p < 0.01$, one-way ANOVA with Sidak's post hoc test.

To determine if patterns of gene expression were similar or opposite in HCC tissues, markers of glycolysis and FA oxidation were also determined in tissues from tumours collected from male C/C and HF/C mice and compared to normal liver tissues from C/C and HF/C mice that did not develop HCC. Gene expression from biometric data obtained from human HCC samples in the UALCAN database was also evaluated. In mouse tis-sues, *Hk2* expression was significantly increased in tumours from HF/C mice, while *Pfkl* expression was significantly decreased in tumours from both C/C and HF/C mice (Figure 8a,b). *Pkm2* and *G6pdx* levels were unchanged relative to levels in the corresponding non-tumour diet group (Figure 8c,d). mRNA levels of *Ascl4* were not significantly different between groups, although there was a pattern of increased expression in tumours from C/C and HF/C animals (Figure 8e), while levels of *Cpt1a*, *Acad11* and *Srebp1c* were all significantly decreased in C/C and HF/C tumours relative to non-tumour tissues (Figure 8f–h). In

human HCC samples, mRNA levels of *HK2*, *PFKL*, *PKM2* and *G6PD* were all significantly increased in HCC compared to normal liver tissues (Figure 8i–l). *ACSL4* expression was also significantly higher in HCC (Figure 8m), while *CPT1A* levels were unaltered (Figure 8n) and *ACAD11* was significantly decreased in HCC tissues (Figure 8o). mRNA levels of the human sterol regulatory element-binding transcription factor 1 (*SREBF1*) gene were also unchanged between normal and HCC samples (Figure 8p).

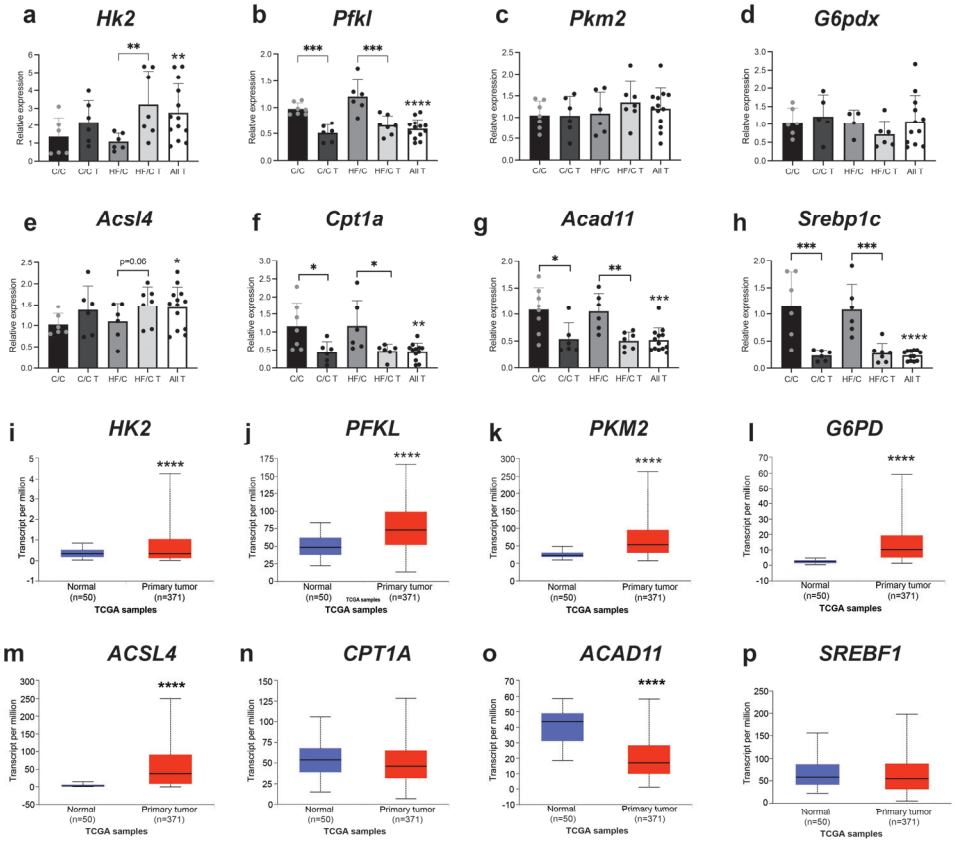

Figure 8. mRNA levels of *Hk2* (**a**), *Pfkl* (**b**), *Pkm2* (**c**) and *G6pdx* (**d**), *Acsl4* (**e**), *Cpt1a* (**f**), *Acad11* (**g**) and *Srebp1c* (**h**) in non-cancerous liver and tumours (T) from male C/C and HF/C mice. C/C (normal = 7, HCC = 6), HF/C (normal = 6, HCC = 7). The relative expression of genes in pooled tumours from both C/C and HF/C mice is represented in the all-tumours (All T) group. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, two-tailed Student's t-test. mRNA levels of *HK2* (**i**), *PFKL* (**j**), *PKM2* (**k**), *G6PD* (**l**), *ACSL4* (**m**), *CPT1A* (**n**), *ACAD11* (**o**) and *SREBF1* (**p**) in normal human liver (blue) and HCC (red) tissues. Box and whisker plots were downloaded from the UALCAN website. **** $p <$ 0.0001, Welch's *t*-tests.

Finally, to confirm if protein levels of genes that were differentially expressed between mouse diet groups and/or tumours were similarly altered, Western blots of mouse non-tumour and tumour tissues were analysed for expression of G6PD, ASCL4 and SREBP1. G6PD levels were significantly increased in HCC tissues vs. C/HF mice (Figure 9a). ASCL4 protein levels were significantly higher in C/C vs. C/HF mice and in tumours, compared to normal tissues in all other diet groups (Figure 9b). SREBP1 proteins were not detected in cytosolic liver fractions. In addition, because of the inter-relationship between the glycolysis-FA cycle and ferroptosis (Supplementary Figure S1), an iron-dependent mecha-

nism of cell death that is thought to exacerbate liver inflammation and fibrosis preceding HCC development [35], expression of the GPX4 liver enzyme and the ferroptosis marker PRX3-$SO_{2/3}$ [36], was also analysed. Neither GPX4 nor PRX3-$SO_{2/3}$ was significantly different between diet groups in normal liver tissues, although both were significantly lower in HCC samples (Figure 9c,d).

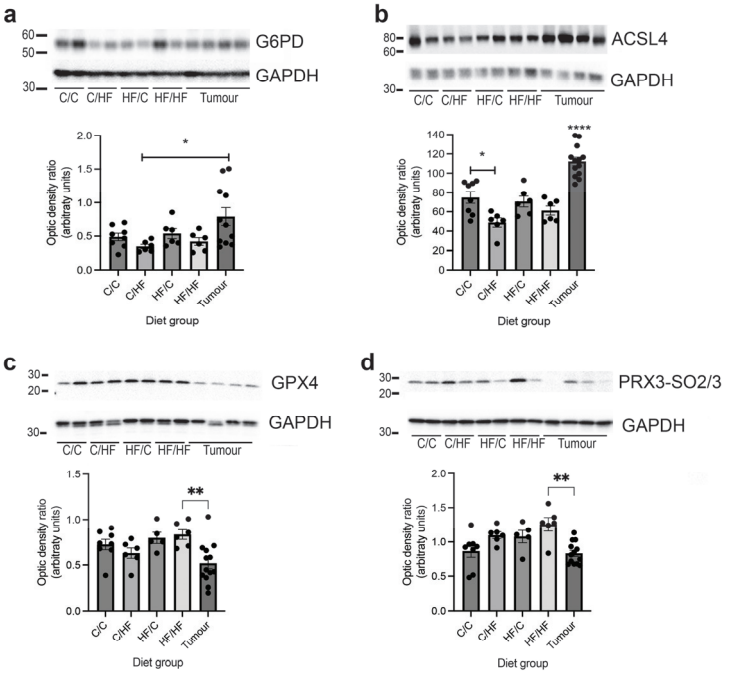

Figure 9. Western blots of G6PD (**a**), ASCL4 (**b**), GPX4 (**c**) and PRX3-$SO_{2/3}$ (**d**) expression in non-cancerous and HCC liver tissues from male mice. C/C = 7, C/HF = 6, HF/C = 5, HF/HF = 6; tumour = 13. * $p < 0.05$, ** $p < 0.01$, one-way ANOVA with Sidak's post hoc test. For panel (**b**), **** $p < 0.0001$ represents the fact that ASCL4 expression in the tumour samples is significantly different from all other groups.

4. Discussion

Both maternal obesity and postnatal consumption of high-fat or high-carbohydrate diets are associated with increased risk of MASLD/MASH, but their combined effects on the risk of spontaneous HCC have not yet been evaluated. In the current study, post-weaning HF feeding resulted in higher body weight, hyperinsulinemia and related pancreatic pathology, and a greater degree of MASLD than *C*-fed offspring. Despite greater liver pathology in HF-fed mice, the incidence of HCC was lowest in C/HF males and highest in HF/C animals. The molecular profile of tumours that developed in C/C and HF/C offspring suggested an upregulation of the PPP and a downregulation of FA synthesis and oxidation.

Numerous studies suggest a role for developmental priming of MASLD and MASH in offspring exposed to an HF diet during gestation and/or lactation [7–9,37]. A positive association between maternal pre-pregnant BMI and risk of MASLD in children has also been reported clinically [38]. Maternal HF feeding has recently been directly implicated in the development of spontaneous HCC [39] and following chemotoxic pre-treatment in mouse offspring [11]. Thus, the current observation of increased microsteatosis and development of HCC in 16-month-old HF/C offspring is consistent with previous reports. Interestingly, the influence of maternal HF feeding on HCC development appeared to

be stronger in the context of post-weaning obesity, as overall rates of HCC were similar between C/C and HF/C mice but ~3-fold higher in HF/HF vs. C/HF offspring. Relative to C/HF mice, HF/HF animals had a greater degree of pancreas-islet hypertrophy and lower levels of hyperinsulinemia, suggesting that HF/HF mice had more advanced glucose intolerance, which may have increased the risk of HCC [40]. However, additional work is needed to determine the mechanisms underlying the influence of maternal diet on HCC risk in the context of a lean and obese postnatal environment.

Rates of HCC were 2x higher in male HF/C vs. HF/C females, similar to the sex imbalance observed in human HCC [3]. This is also congruent with findings that male offspring of HF-fed mothers develop greater liver steatosis than female mice [41] and with our observations that, across all diet groups, female offspring had less liver and pancreas pathology and delayed onset of glucose intolerance compared to their male littermates. Intriguingly, in *C*-diet-fed mice, the development of HCC occurred in the absence of altered glucose metabolism or significant liver fibrosis and inflammation. Experimental evidence suggests that the relative ratio of dietary fat and sucrose are important in the initiation and propagation of HCC. For example, Healy et al. found that tumour burden and size was highest in DEN-treated mice fed a 23% fat + 21% sucrose or 31% sucrose + 15% fructose diet, while those fed a 71% fat + low-sucrose diet had the lowest tumour incidence [20,21]. Duan et al. [42] reported that DEN-treated rats fed with a diet high in saturated FAs developed fewer and smaller HCC tumours than *C*-fed animals. Moreover, there is some evidence that consumption of ketogenic diets where the fat:carbohydrate ratio is 4:1 or 3:1 can delay tumour growth and prolong survival time, although clinical trials in HCC are limited [43]. In the current study, the C diet had lower fat composition, but higher carbohydrate and sucrose content compared to the HF diet (Supplementary Table S1). As sustained intake of high-carbohydrate diets is associated with increased hepatic glucose [44], and has been associated with development of HCC in aged mice [19], this may account in part for the increased HCC observed in C/C and HF/C animals. In addition, elevated consumption of fructose, which is an intermediary in glucose metabolism, is associated with the development of MASLD and increased risk of HCC [45,46]. Moreover, fructose is one of the primary metabolites used for *de novo* lipogenesis, including synthesis of triglycerides (TG) [45]. Inhibition of TG secretion packaged as vLDL is associated with steatosis in the absence of hepatic inflammation or insulin resistance [47]. Thus, the observed decrease in plasma v/LDL in HF/C males suggests that either impaired vLDL secretion and/or TG accumulation may have contributed to HCC development in these animals. However, these results must be interpreted with caution because the effect of the post-prandial interval on cholesterol concentrations was not controlled for in the non-fasted mice. Whether this is sufficient to drive HCC in the absence of other liver pathologies requires further investigation, although, notably, low-serum-LDL concentrations are independent predictors of HCC in humans [48].

In offspring fed the HF diet, there was a negative association between liver and pancreas pathology and prevalence of HCC. This was unexpected, considering the relationship between MASLD/MASH, glucose intolerance and increased risk of HCC [49]. A recent study by Pedersen et al. found that HCC incidence was higher in DEN-treated mice fed a diet enriched with saturated FAs compared to those containing monosaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs) [50]. Here, 19% of the fat in the C diet was derived from saturated FAs, while MUFAs and PUFAs contributed 27% and 54%, respectively, of the fat content. In the HF diet, saturated FAs and MUFAs both made up 38% of the fat content, while the remaining 24% was contributed from PUFAs. This differential FA composition is interesting, in light of the relative contribution of MUFAs and PUFAs to hepatic ferroptosis. In ferroptosis, PUFAs that are produced via the activity of ASCL4 undergo peroxidation, leading to the generation of reactive oxygen species and cell death [51]. However, the ability of PUFAs to stimulate ferroptosis is competitively blocked by exogenous MUFAs [52] and by the actions of GPX4, which converts lipid peroxidases back to their respective alcohols using NADP+ that is generated in the PPP [51]. GPX4 activity is directly inhibited by

ASCL4 [51]. In HCC itself, hepatocytes upregulate protective mechanisms to counteract ferroptosis and evade death [53]. Therefore, the relatively high concentrations of MUFAs in the HF diet, in conjunction with elevated concentrations of saturated FAs that are converted to MUFAs in the liver [54], may have inhibited ferroptosis and delayed or counteracted the onset of HCC in the C/HF mice. However, while levels of GPX4 and the ferroptosis marker PRX3-$SO_{2/3}$ were downregulated in mouse HCC samples, neither protein was altered in livers from non-cancerous C/HF mice. It may be that ferroptosis did not play a significant role in the induction of HCC in this mouse model or that the liver samples in the animals that did not develop HCC were resistant to this pathway. More work is needed to determine why most C/HF mice did not develop HCC despite having a high degree of MASLD.

Although changes to both glycolysis and FA metabolism have been implicated in HCC, there is significant heterogeneity in the metabolic reprogramming of each pathway, depending on the metabolic background in which the HCC develops. In the current study, *Gpdx* mRNA levels were significantly increased in non-cancerous liver tissues of HF/C mice, relative to C/HF and HF/HF animals. No differences were observed between diet groups on other markers of glycolysis. However, in mouse HCC samples, levels of *Hk2* mRNA and G6PD protein were increased. These changes match those observed in the human HCC samples. This suggests that the HCC that developed in C/C and HF/C animals may have been related to increased glucose shunting to the PPP, which is consistent with reports that the PPP is a key pathway by which HCC cells sustain FA synthesis and maintain redox homeostasis [24].

In the FA pathway, expression of *Acsl4* and *Srebp1c* was higher in normal tissues from diet groups that were more likely to develop HCC. This is consistent with previous work showing that a high-carbohydrate, low-fat diet induces FA synthesis in hepatocytes [55]. Notably, *Acsl4* mRNA and protein levels were also significantly upregulated in HCC tissues, suggesting that this may be an early alteration in preneoplastic lesions. However, in contrast with non-tumour tissues, mRNA levels of *Cpt1a*, *Acad11* and *Srebp1c* were downregulated in mouse HCC. Again, these results mirror the changes in *ACSL4* and *ACAD11* expression in human HCC tissues. Although decreased FA β-oxidation has been previously reported in HCC associated with obesity [22], most studies report a positive association between SREBP1c expression and increased risk of HCC [56]. Additional experiments are needed to determine the impact of *Srebp1c* downregulation and to disentangle the seemingly paradoxical relationship between elevated levels of ACSL4 and poor HCC prognosis [57], alongside the role for ACSL4 in PUFA activation and increased sensitization to ferroptosis [58].

Although this is the first study to characterize the combined effect of pre- and post-weaning diet on rates of HCC in aged animals, it does have several limitations. Due to the longitudinal nature of the study, pathological examination of HCC could only be carried out at one time point across the offspring lifespan. Consequently, it is not clear when the HCC began or if the non-cancerous tissues would have become cancerous with more advanced aged. In addition, all mice underwent two periods of food restriction (at 6 and 12 months of age), during which the animals were maintained at 90% of their free-feeding body weight [28]. A recent systematic review of animal models of hepatic cancer concluded that caloric restriction of 20–30% delayed tumour development and reduced tumour burden and metastasis [13]. In this study, all male offspring consumed the same amount of total food/day and kcal/body weight across their lifespan. Nevertheless, we cannot discount the fact that this restriction and re-feeding paradigm may have differentially affected the metabolism and relative risk of developing HCC in lean vs. obese animals. An early study by Gumaa and Mclean [59] found that rats fasted for 72 h had decreased concentrations of liver pentose phosphate and that levels were restored in animals re-fed a high-carbohydrate diet but not animals who were re-fed with an HF diet. Whether a similar effect is observed in food-restricted mice and in the context of obesity remains unknown. Additional experiments without food restriction are needed to confirm the role of pre- and

post-weaning carbohydrate and HF diets on the risk of developing spontaneous HCC. Finally, due to insufficient numbers of tumours in HF-fed mice, only HCC samples from C/C and HF/C mice were analysed. Future comparative analysis of FA β-oxidation and glycolysis in tumours that arose in lean vs. obese mice may provide additional information about the pre- and postnatal factors that contribute to HCC heterogeneity.

5. Conclusions

In summary, this study found that both maternal HF and post-weaning C diet increased the risk of developing spontaneous, age-related HCC in the absence of chemotoxic exposure or genetic predisposition. This may have been due to early alterations in genes related to the PPP and FA synthesis and/or oxidation, which were exacerbated by prolonged intake of a high-carbohydrate diet and attenuated by a high dietary ratio of MUFAs to PUFAs. As rates of maternal obesity continue to rise globally, this has possible implications for the future incidence of HCC, and suggests that reducing carbohydrate intake in offspring may successfully counteract this risk.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu16162805/s1, Figure S1: Simplified schematic of the inter-relationship between glycolysis, the pentose phosphate pathway and fatty acid β-oxidation in hepatocytes; Figure S2: Assessment of liver steatosis and inflammation between male and female offspring; Figure S3: Plasma cholesterol concentrations; Figure S4: Measures of plasma glucose and insulin concentrations and pancreatic pathology in offspring; Table S1: Diet Composition; Table S2: Primer sequences used for RT-qPCR.

Author Contributions: Conceptualization, C.A.H. and F.C.; methodology, D.H., L.C., A.W., H.C., I.A., A.C.I. and C.A.H.; formal analysis, D.H., A.W., H.C. and C.A.H.; writing—original draft preparation, C.A.H. and F.C.; writing—review and editing, D.H., L.C., A.W., H.C., I.A., A.C.I., F.C. and C.A.H.; supervision, C.A.H. and F.C. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Human HCC bioinformatics datasets used in this study are publicly available and can be found at https://ualcan.path.uab.edu/index.html (accessed on 5 July 2024). Further enquiries can be directed to the corresponding author.

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Disparities in the Cardiometabolic Impact of Adiposity among African American and Hispanic Adolescents

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Abstract: As adiposity increases in youth, so does the prevalence of cardiometabolic risk factors (CMRFs). The etiology of adiposity-based chronic disease and CMRFs includes ethnoracial disparities that are rarely considered in current treatment approaches. Precision interventions require further characterization of these disparities among high-risk youth. The objective of this study was to characterize differences in CMRF among African American (AA) and Hispanic (H) adolescents with varying levels of adiposity. A cross-sectional analysis of 2284 adolescents aged 12–17 was conducted using 3-year clinical data from Lifedoc Health. CMRF prevalence were compared using χ^2, with logistic regression models (LRM) applied to explore the relationships between exposures (age, sex, ethnoracial group, adiposity) and CMRF outcomes. Prevalence of CMRF rose with increasing adiposity, which was the strongest determinant of risk overall. However, individual risk profiles differed between the two groups, with H having higher prevalence of metabolic syndrome (MetS), higher triglycerides and liver enzymes, and low high-density lipoprotein cholesterol (HDL-c). Meanwhile, AA had higher prevalence of elevated blood pressure (BP) in the overweight category, prediabetes in overweight to severe obesity, and type 2 diabetes in obesity. LRM showed 3.0-fold greater chance of impaired glucose metabolism in AA than H, who were 1.7, 5.9, and 8.3 times more likely to have low HDL-c, high liver enzymes, and high triglycerides, respectively. Overweight/obesity prevalence was very high among AA and H adolescents. Excess adiposity was associated with an increased prevalence of CMRF, with individual risk factors differing between groups as adiposity increased. Research within routine clinical settings is required to better characterize these discrepancies and ameliorate their adverse impact on health in the transition to adulthood.

Keywords: pediatric; obesity; cardiometabolic; risk factors; adiposity; racial disparities; chronic disease

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1. Introduction

The rate of obesity among children and adolescents aged 10–17 years in the US was reported to be 16.2% in the 2019–2020 National Survey of Children's Health [1]. The prevalence of overweight/obesity varies between ethnoracial groups. Data from the National Health and Nutrition Survey (NHANES) (2015–2016) show that rates in African

American and Hispanic children/adolescents aged 2–19 years exceed the national average and are higher than for other ethnoracial groups [2].

As adiposity increases in children and adolescents, so does the prevalence of cardiometabolic risk factors (CMRFs), including hypertension, dyslipidemia, and type 2 diabetes (T2D), all of which collectively make up metabolic syndrome [3]. The etiology of adiposity-based chronic disease and CMRFs includes ethnoracial disparities but these are rarely considered in current treatment approaches. As such, describing the prevalence, distribution, and factors associated with childhood obesity in more vulnerable groups may help to design effective interventions. Pragmatic real-world data from clinical settings that assess differences in the impact of adiposity on CMRFs in ethnoracially diverse adolescents are needed.

In Tennessee, rates of obesity among adolescents (20.8%) exceeded the national average [1], while 35% of adults were living with obesity in 2021 [4]. Compared with other US cities, Memphis, TN has the seventh highest proportion of African Americans (64.6%) but under-representation of Hispanics (7.7%) [3,5]. Lifedoc Health (LDH), a multidisciplinary healthcare organization, successfully implemented a data-driven model and clinical protocols to attenuate the burden of cardiometabolic-based chronic disease in children and adolescents in the Greater Memphis area [6]. However, the implementation of a more precise transcultural model requires the characterization of different CMRF phenotypes in high-risk minority groups. Thus, this study aims to elucidate disparities in the CMRFs of African American and Hispanic adolescents with varying levels of adiposity in a real-world setting.

2. Materials and Methods

2.1. Study Design and Sampling

A cross-sectional retrospective analysis was conducted of 3-year clinical data (2018–2020 $\pm$ 6 weeks) from the patient records of AA and H adolescents aged 12–17 years under LDH. Patients were included regardless of insurance coverage (private, Medicaid, or none) or whether they were assigned to the practice by the payer or referred from outside primary care for co-management [6]. Patients who were pregnant, underweight (body mass index-for-age percentile [BMI%] < 5.0%), or had any known genetic cause of obesity were excluded. All procedures were performed in accordance with the Declaration of Helsinki. Prior to data collection, informed consent was obtained. No independent ethics committee or institutional review board was sought, as this study does not meet the definition of human subject research as defined in Federal Regulation 45 CFR 46.102 [7].

2.2. Physical and Biochemical Parameters

Weight was measured in light clothing, without shoes, using a calibrated scale (Digital Platform Scale Pro Plus 2101KL, Health-o-meter®, McCook, IL, USA). Height was measured using a digital stationary stadiometer (Seca® 264, Chino, CA, USA). Blood pressure (BP) was measured in the right arm, using appropriate cuff size, in a sitting position, with a aneroid sphygmomanometer (McKesson®, Irving, TX, USA) [8]. Blood glucose, glycated hemoglobin (HbA_{1c}), high-sensitivity C-reactive protein (hs-CRP), fibrinogen, triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine transaminase (ALT), and 25-OH-vitamin D were measured using random blood samples in a single certified laboratory (LabCorp, Burlington, NC, USA) [9].

2.3. Study Variables and Definitions

Participants self-reported as male or female and as African American and Hispanic. BMI% percentile was determined using age- and sex-based definitions of BMI, with individuals classified as having normal weight (<85th BMI%), overweight ($\geq$85th and <95th BMI%), moderate obesity ($\geq$95th and <99th BMI%), or severe obesity ($\geq$99th BMI%) [10,11]. This modified version of the World Health Organization criteria (BMI% $\geq$95th) was used to

define obesity due to limitations in using waist circumference (WC) to measure excess adiposity, including variability in cut-off points in age, race and ethnicity. Elevated blood pressure (BP) was defined for children aged 1–<13 years as ≥90th percentile or 120/80 mmHg (whichever was lower) and for children ≥13 years ≥120/<80 mmHg. Hypertension was defined for children aged <13 years as ≥95th percentile or ≥130/80 mmHg (whichever was lower) and for children ≥13 years as ≥130/80 mmHg or taking BP-lowering medication [12]. Dyslipidemia was defined as TC ≥ 200 mg/dL, LDL-C ≥ 130 mg/dL, TG ≥ 130 mg/dL, HDL-C < 40 mg/dL, or taking any lipid-lowering medications. Prediabetes was defined as HbA_{1c} ≥ 5.7% and <6.5%, and T2D as either HbA_{1c} ≥ 6.5% or a personal history of diabetes [13]. Fasting blood glucose was not used to define dysglycemia status due to practical difficulties in verifying the fasted state in a clinical setting. Metabolic syndrome (MetS) was defined as any ≥3 of the following criteria: overweight/obesity/severe obesity [14], elevated BP/hypertension, elevated TG, low HDL-C, and prediabetes/T2D [15], while elevated levels of liver enzymes, hs-CRP and fibrinogen, or vitamin D insufficiency were additional CMRFs [16,17]. High liver enzymes were defined as AST > 40 IU/L or ALT > 32 IU/L [9]. Vitamin D insufficiency was established if 25-OH-vitamin D was <30 ng/mL [18].

2.4. Statistical Analysis

Data were analyzed using R (version 3.6.2). Variables with normal distribution were presented as mean ± standard error, with differences between groups assessed by the student *t*-test. Frequencies were presented as percentages and 95% confidence intervals (CIs). Groups were compared using the χ^2 test. Multivariate logistic regression models were applied to explore the relationships between exposures (age, sex, ethnoracial group, and adiposity) and outcomes (impaired glucose metabolism [IGM], elevated BP, high TC, low HDL-C, high LDL-C, high TG, high AST/ALT, inflammation, vitamin D insufficiency), adjusted by age and sex. Odds ratios and 95% CIs were estimated across different groups. The significance threshold was 0.05 for all analyses.

3. Results

3.1. Population Characteristics

Overall, the analysis cohort consisted of 2284 adolescents meeting inclusion criteria. Of these, 25.8% had private health insurance, 67.6% had a Medicaid healthcare plan, and 6.6% were uninsured. Overall, 687 (30.1%) were African American and 1597 (69.9%) were Hispanic, 50.3% were female, and mean age, weight, and BMI was 13.9 years, 70.9 kg, and 26.9 kg/m^2, respectively. Sex distribution was similar between groups. Males had higher mean weight, height, BP, TG, blood glucose, AST, ALT, and vitamin D, while females had higher mean BMI, HDL-C, hs-CRP, and fibrinogen. African American adolescents had higher mean weight, height, BMI, BP, LDL-C, hs-CRP, and HbA_{1c}, and lower vitamin D, while Hispanic adolescents had higher TG, AST, and ALT. Hispanic males had lower HDL-C and higher blood glucose than African American males (Table 1).

3.2. CMRF Prevalence by Ethnoracial Group, Sex, and Adiposity

Of the total cohort, 40.7% were a normal weight, 20.2% were overweight, 25.0% had obesity, and 14.2% had severe obesity (Table 2). Prevalence was high for the following CMRFs: vitamin D insufficiency, inflammation, dyslipidemia, MetS, high TG, low HDL-C, IGM, prediabetes, hypertension, high liver enzymes, elevated BP, high TC, high LDL-C, and T2D (92.4%, 55.6%, 46.2%, 34.1%, 29.2%, 24.6%, 18.0%, 16.3%, 12.3%, 12.0%, 8.5%, 7.1%, 4.9%, and 1.7%, respectively; Table 2). Males had a higher frequency of severe obesity, elevated BP, hypertension, dyslipidemia, high TG, low HDL-C, MetS, and high liver enzymes, while females had significantly higher rates of inflammation and vitamin D insufficiency (Table 2).

Table 1. Cardiometabolic risk factors in adolescents by sex and ethnoracial group.

	Male (M)				Female (F)				Total	
	African American (AA)	Hispanic (H)	Total	*p* Value AA vs. H	African American (AA)	Hispanic (H)	Total	*p* Value AA vs. H	Total	*p* Value M vs. F
n (%)									2284 (100.0)	
Age, years			13.9 ± 0.05				13.9 ± 0.05		13.9 ± 0.04	
Weight [a], kg	82.2 ± 1.8	69.6 ± 0.7	73.3 ± 0.8	≤0.001	81.4 ± 1.5	62.2 ± 0.6	68.6 ± 0.7	≤0.001	70.9 ± 0.5	≤0.001
Height [a], cm	169.7 ± 0.6	163.3 ± 0.4	165.2 ± 0.3	≤0.001	161.8 ± 0.4	155.2 ± 0.2	157.4 ± 0.2	≤0.001	161.3 ± 0.2	≤0.001
BMI [a], kg/m^2	28.2 ± 0.6	25.8 ± 0.2	26.5 ± 0.2	≤0.001	30.8 ± 0.5	25.6 ± 0.2	27.4 ± 0.2	≤0.001	26.9 ± 0.2	0.01
SBP, mmHg [b]	112.5 ± 0.7	108.8 ± 0.4	109.8 ± 0.3	≤0.001	110.0 ± 0.6	104.3 ± 0.3	106.2 ± 0.3	≤0.001	108.0 ± 0.2	≤0.001
DBP, mmHg [b]	70.0 ± 0.5	67.9 ± 0.3	68.5 ± 0.3	≤0.001	70.3 ± 0.5	65.2 ± 0.3	66.9 ± 0.3	≤0.001	67.7 ± 0.2	≤0.001
Total cholesterol, mg/dL	153.3 ± 2.1	154.7 ± 1.4	154.8 ± 1.1	0.58	156.4 ± 1.9	154.9 ± 1.21	155.4 ± 1.0	0.51	155.1 ± 0.8	0.70
LDL-C, mg/dL	88.2 ± 1.9	82.9 ± 1.1	84.8 ± 0.1	0.02	90.5 ± 1.8	83.0 ± 1.1	85.4 ± 0.9	≤0.001	85.1 ± 0.7	0.64
HDL-C, mg/dL	47.9 ± 0.9	44.9 ± 0.5	45.7 ± 0.4	≤0.001	50.5 ± 0.8	49.2 ± 0.5	49.6 ± 0.4	0.14	47.7 ± 0.3	≤0.001
TG, mg/dL	87.6 ± 4.0	139.8 ± 3.7	125.8 ± 3.0	≤0.001	78.3 ± 2.5	117.2 ± 2.7	104.5 ± 2.1	≤0.001	114.7 ± 1.8	≤0.001
hs-CRP, mg/dL	3.42 ± 0.4	2.54 ± 0.2	2.82 ± 0.2	0.04	4.96 ± 0.4	2.94 ± 0.2	3.79 ± 0.2	≤0.001	3.3 ± 0.1	≤0.001
Fibrinogen, mg/dL	316.3 ± 6.5	318.3 ± 3.8	318.5 ± 3.3	0.77	368.3 ± 6.1	355.2 ± 3.8	360.6 ± 3.3	0.07	338.9 ± 2.4	≤0.001
Blood glucose, mg/dL	95.7 ± 1.2	99.7 ± 0.7	98.6 ± 0.6	≤0.001	95.4 ± 1.1	95.1 ± 0.6	95.2 ± 0.6	0.82	96.8 ± 0.4	≤0.001
HbA_{1c}, %	5.54 ± 0.03	5.38 ± 0.02	5.42 ± 0.01	≤0.001	5.56 ± 0.03	5.36 ± 0.02	5.43 ± 0.02	≤0.001	5.42 ± 0.01	0.55
AST, IU/L	23.4 ± 0.6	26.1 ± 0.5	25.4 ± 0.4	≤0.001	19.2 ± 0.4	20.9 ± 0.4	20.4 ± 0.3	≤0.001	22.8 ± 0.3	≤0.001
ALT, IU/L	20.3 ± 0.9	28.8 ± 1.0	26.6 ± 0.8	≤0.001	15.7 ± 0.8	18.8 ± 0.7	18.0 ± 0.5	≤0.001	22.2 ± 0.5	≤0.001
25-OH-Vitamin D, ng/mL	17.3 ± 0.6	19.4 ± 0.4	19.1 ± 0.3	≤0.001	15.0 ± 0.5	17.8 ± 0.4	16.9 ± 0.3	≤0.001	18.0 ± 0.2	≤0.001

Variables are expressed as mean ± standard error. Sex (male vs female for total sample) and ethno-racial group (African American vs Hispanic for each sex) groups were compared using *t*-test. The sample size for each variable differed between groups; therefore, the mean (standard deviation) was calculated based on observed numbers and not the total cohort. [a] These variables were not adjusted by adiposity. [b] SBP/DBP were adjusted by height (cm). Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglycerides.

Table 2. Prevalence of cardiometabolic risk factors, high liver enzymes, and vitamin D insufficiency in adolescents by sex and ethnoracial group.

	Male (M)				Female (F)				Total	
	African American (AA)	Hispanic (H)	Total	*p* Value AA vs. H	African American	Hispanic	Total	*p* Value AA vs. H	Total	*p* Value M vs. F
Overweight [a]	12.6 (9.3, 16.9)	20.7 (18.0, 23.7)	18.4 (16.3, 20.8)	≤0.001	13.8 (10.5, 17.8)	25.9 (22.9, 29.2)	21.8 (19.5, 24.3)	≤0.001	20.2 (18.6, 21.9)	0.05
Obesity [a]	17.7 (13.7, 22.4)	27.6 (24.6, 30.9)	24.8 (22.4, 27.4)	≤0.001	25.7 (21.4, 30.5)	24.6 (21.7, 27.8)	25.1 (22.6, 27.7)	0.75	25.0 (23.2, 26.8)	0.92
Severe obesity [a]	26.2 (21.5, 31.5)	11.4 (9.4, 13.9)	15.8 (13.8, 18.0)	≤0.001	27.3 (22.9, 32.2)	5.4 (4.0, 7.3)	12.5 (10.7, 14.6)	≤0.001	14.2 (12.8, 15.6)	0.03

Table 2. *Cont.*

		Male (M)				Female (F)				Total	
		African American (AA)	Hispanic (H)	Total	*p* Value AA vs. H	African American	Hispanic	Total	*p* Value AA vs. H	Total	*p* Value M vs. F
Abnormal BP	Elevated	13.2 (9.6, 18.0)	9.3 (7.3, 11.6)	10.4 (8.7, 12.5)	0.08	9.4 (6.5, 13.2)	5.2 (3.8, 7.2)	6.6 (5.2, 8.3)	0.02	8.5 (7.3, 9.8)	≤0.001
	HTN	15.8 (11.8, 20.8)	14.6 (12.2, 17.3)	14.9 (12.8, 17.3)	0.69	17.2 (13.3, 21.9)	6.2 (4.6, 8.3)	9.7 (8.0, 11.7)	≤0.001	12.3 (10.9, 13.8)	≤0.001
Abnormal HbA_{1c}	Pre-DM	33.1 (25.7, 41.4)	11.2 (8.3, 14.8)	17.1 (14.1, 20.5)	≤0.001	27.5 (21.4, 34.6)	8.9 (6.2, 12.5)	15.5 (12.6, 18.8)	≤0.001	16.3 (14.2, 18.6)	0.53
	T2D	2.7 (0.9, 7.2)	0.8 (0.2, 2.4)	1.3 (0.6, 2.7)	0.09	4.8 (2.3, 9.1)	0.9 (0.2, 2.7)	2.2 (1.2, 3.9)	0.01	1.7 (1.1, 2.7)	0.34
	IGM	35.8 (28.2, 44.1)	11.9 (9.0, 15.6)	18.3 (15.2, 21.8)	≤0.001	32.3 (25.8, 39.5)	9.8 (7.0, 13.5)	17.7 (14.6, 21.2)	≤0.001	18.0 (15.8, 20.4)	0.84
Dyslipidemia		41.8 (34.7, 49.2)	58.5 (54.1, 62.8)	54.3 (50.5, 57.9)	≤0.001	33.7 (28.0, 40.0)	41.8 (37.5, 46.2)	38.9 (35.5, 42.5)	0.04	46.2 (43.7, 48.8)	≤0.001
High total cholesterol		8.5 (5.1, 13.6)	7.2 (5.2, 9.9)	8.1 (6.2, 10.4)	0.7	6.8 (4.1, 10.9)	6.2 (4.3, 8.7)	6.2 (4.7, 8.2)	0.86	7.1 (5.9, 8.6)	0.19
High LDL-C		5.3 (2.7, 9.8)	4.9 (3.3, 7.3)	5.2 (3.7, 7.1)	0.98	8.0 (5.1, 12.3)	3.3 (2.0, 5.3)	4.7 (3.4, 6.5)	0.01	4.9 (3.9, 6.2)	0.76
High TG		13.2 (8.9, 19.1)	44.0 (39.7, 48.5)	35.8 (32.3, 39.5)	≤0.001	7.6 (4.8, 11.8)	30.6 (26.7, 34.8)	23.2 (20.3, 26.3)	≤0.001	29.2 (26.9, 31.6)	≤0.001
Low HDL-C		26.5 (20.4, 33.4)	32.9 (28.9, 37.2)	31.4 (28.0, 34.9)	0.12	18.9 (14.3, 24.4)	18.2 (15.0, 21.8)	18.3 (15.7, 21.2)	0.89	24.6 (22.4, 26.8)	≤0.001
Inflammation		55.3 (46.4, 63.9)	43.8 (38.2, 49.5)	47.6 (42.9, 52.3)	0.03	68.9 (61.1, 75.9)	60.6 (54.4, 66.6)	64.1 (59.4, 68.6)	0.1	55.6 (52.3, 58.9)	≤0.001
Metabolic syndrome		35.8 (28.2, 44.1)	42.6 (37.8, 47.7)	41.1 (37.0, 45.3)	0.18	30.1 (23.7, 37.3)	24.3 (20.0, 29.2)	27.0 (23.4, 30.9)	0.18	34.1 (31.3, 37.0)	≤0.001
High liver enzymes		9.0 (5.5, 14.3)	21.2 (17.8, 25.1)	18.1 (15.4, 21.1)	≤0.001	2.1 (0.8, 5.1)	7.8 (5.7, 10.6)	6.3 (4.7, 8.3)	≤0.001	12.0 (10.4, 13.7)	≤0.001
Vitamin D insufficiency		93.5 (88.4, 96.5)	90.6 (87.4, 93.0)	90.8 (88.2, 92.8)	0.32	96.9 (93.4, 98.6)	92.5 (89.6, 94.6)	93.9 (91.8, 95.5)	0.04	92.4 (90.8, 93.7)	0.04

Frequencies are expressed as percentages and 95% confidence intervals. Groups were compared using the χ^2 test. [a] These variables were not adjusted for adiposity. Abbreviations: BP, blood pressure; HbA_{1c}, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HTN, hypertension; IGM, impaired glucose metabolism; LDL-C, low-density lipoprotein cholesterol; pre-DM, prediabetes mellitus; T2D, type 2 diabetes; TG, triglycerides.

A higher proportion of Hispanic than African American males had overweight (20.7% vs. 12.6%) or obesity (27.6% vs. 17.7%), although severe obesity was more frequent in African American males (11.4% vs. 26.2%). IGM was also more frequent in African American males (primarily driven by prediabetes as T2D prevalence was similar between the groups). Hispanic males had higher prevalence of dyslipidemia, high TG, and high liver enzymes. Among females, overweight was more frequent in Hispanic than African American adolescents (25.9% vs. 13.8%), no difference in obesity was found, and severe obesity was more frequent in African American females (5.4% vs. 27.3%). Hispanic females also had a higher prevalence of dyslipidemia, high TG, and high liver enzymes, while African American females had a higher prevalence of prediabetes, T2D, elevated BP, hypertension, high LDL-C, and vitamin D insufficiency (Table 2).

In both groups, adiposity was the strongest determinant of cardiometabolic risk. CMRFs became more prevalent as severity of adiposity increased, except by high TG levels that were more prevalent in Hispanic adolescents regardless of level of adiposity (Table 3). As adiposity increased from overweight to severe obesity, Hispanic adolescents exhibited a significantly higher prevalence of dyslipidemia, lower HDL-C, and higher liver enzymes, with MetS prevalence also significantly higher in those with obesity and severe obesity. African American adolescents demonstrated a higher prevalence of elevated BP in the overweight category, prediabetes in the overweight to severe obesity categories, and T2D in the obesity category (Table 3).

3.3. Association between Demographics and BMI Categories with Cardiometabolic Abnormalities

Males were significantly more likely to have elevated BP, low HDL-C, high TG, and high liver enzymes, while inflammation and vitamin D insufficiency were more likely in females (Table 4). Older adolescents (aged 15–17 years) had 3.4-fold higher odds of hypertension and were more likely to have high TC, high LDL-C, and high liver enzymes than younger adolescents (12–14 years) (Table 4). Compared with Hispanic, African American ethnicity was associated with 3.0-fold greater odds of IGM, whereas Hispanic adolescents were more likely to have low HDL-C (1.7-fold higher odds), high liver enzymes (5.9-fold higher odds), and high TG (8.3-fold higher odds) than African American adolescents (Table 4).

All CMRFs tended to increase with adiposity, with a significantly increased risk of IGM, elevated BP, low HDL-C, high TG, and inflammation in overweight vs. normal weight individuals. The increased risk of high liver enzymes and vitamin D insufficiency was significant in adolescents with overweight vs. normal weight, while risk of high TC and high LDL-C became significant with severe obesity (Table 4).

Table 3. Prevalence of cardiometabolic risk factors, high liver enzymes, and vitamin D insufficiency by severity of adiposity (via BMI%) and ethnoracial group in the total sample.

%		Normal Weight				Overweight			
		African American	Hispanic	Total, % (Normal Weight)	*p* Value	African American	Hispanic	Total, % (Overweight)	*p* Value
Abnormal BP	Elevated	4.9 (2.6, 8.8)	3.3 (2.1, 5.1)	3.7 (2.5, 5.2)	0.38	15.4 (8.5, 25.7)	5.9 (3.7, 9.1)	7.5 (5.3, 10.6)	0.01
	Hypertension	6.2 (3.6, 10.4)	3.8 (2.5, 5.7)	4.4 (3.1, 6.0)	0.19	3.8 (1.0, 11.6)	6.2 (4.0, 9.5)	5.9 (3.9, 8.7)	0.59
Abnormal HbA_{1c}	Pre-DM	2.4 (0.1, 14.4)	2.3 (0.6, 7.0)	2.3 (0.7, 6.1)	1.00	15.2 (5.7, 32.7)	7.6 (4.5, 12.5)	8.6 (5.5, 13.1)	0.18
	DM	NA	1.5 (0.3, 5.9)	1.1 (0.2, 4.5)	1.00	NA	0.5 (0.0, 3.2)	0.4 (0.0, 2.7)	1.00
	IGM	2.4 (0.1, 14.4)	3.8 (1.4, 9.1)	3.4 (1.4, 7.6)	1.00	15.2 (5.7, 32.7)	8.1 (4.9, 13.1)	9.0 (5.8, 13.6)	0.2
Metabolic syndrome		2.6 (0.1, 15.1)	1.5 (0.3, 6.0)	1.7 (0.5, 5.4)	0.55	12.1 (4.0, 29.1)	23.3 (17.8, 29.8)	21.8 (16.9, 27.7)	0.23
Dyslipidemia		24.3 (17.0, 33.4)	30.1 (25.3, 35.4)	28.3 (24.3, 32.7)	0.29	20.0 (10.5, 34.1)	41.7 (35.7, 48.0)	38.1 (32.8, 43.7)	0.01
High total cholesterol		6.1 (2.7, 12.6)	4.8 (2.8, 7.8)	5.0 (3.3, 7.5)	0.76	12.0 (5.0, 25.0)	5.8 (3.4, 9.6)	7.0 (4.5, 10.5)	0.12
High LDL-C		5.2 (2.1, 11.5)	3.0 (1.5, 5.6)	3.5 (2.1, 5.7)	0.25	4.0 (0.7, 14.9)	4.3 (2.3, 7.7)	4.5 (2.6, 7.5)	1
High TG		4.3 (1.6, 10.3)	20.9 (16.7, 25.7)	16.3 (13.1, 20.1)	≤0.001	2.0 (0.1, 12.0)	32.4 (26.8, 38.6)	27.6 (22.8, 33.0)	≤0.001
Low HDL-C		10.4 (5.7, 17.9)	8.9 (6.2, 12.6)	9.3 (6.9, 12.5)	0.77	6.0 (1.6, 17.5)	20.5 (15.8, 26.0)	18.1 (14.1, 22.9)	**0.03**
Inflammation		NA	11.1 (2.9, 30.3)	7.0 (1.8, 20.1)	0.54	17.4 (5.7, 39.5)	36.8 (29.3, 45.1)	34.1 (27.2, 41.7)	0.11
High liver enzymes		1.9 (0.3, 7.5)	4.5 (2.6, 7.4)	4.0 (2.5, 6.4)	0.38	NA	8.9 (5.8, 13.3)	7.3 (4.7, 11.0)	**0.03**
Vitamin D insufficiency		91.2 (82.3, 96.1)	85.1 (80.2, 89.0)	86.2 (82.1, 89.6)	0.22	95.2 (82.6, 99.2)	89.7 (84.8, 93.1)	90.3 (86.1, 93.4)	0.39

Table 3. *Cont.*

%		Obesity				Severe Obesity				Sample	
		African American	Hispanic	Total, % (Obesity)	*p* Value	African American	Hispanic	Total, % (Severe Obesity)	*p* Value	%	*p* Value
Abnormal BP	Elevated	8.7 (4.6, 15.3)	11.9 (8.9, 15.6)	11.8 (9.2, 14.9)	0.4	19.8 (14.1, 26.9)	16.3 (10.6, 24.0)	17.8 (13.7, 22.7)	0.54	8.5 (7.3, 9.8)	≤0.001
	Hypertension	20.5 (14.0, 28.7)	17.3 (13.7, 21.5)	18.0 (14.9, 21.6)	0.5	34.0 (26.8, 41.9)	33.3 (25.4, 42.2)	33.9 (28.6, 39.6)	1.00	12.3 (10.9, 13.8)	≤0.001
Abnormal HbA_{1c}	Pre-DM	29.9 (21.6, 39.6)	11.4 (8.1, 15.6)	16.2 (12.9, 20.1)	≤0.001	40.4 (32.7, 48.5)	21.0 (13.9,30.2)	32.1 (26.7, 38.1)	≤0.001	16.3 (14.2, 18.6)	≤0.001
	DM	6.5 (2.9, 13.5)	0.3 (0.0, 2.1)	1.9 (0.9, 3.8)	≤0.001	3.8 (1.6, 8.6)	1.9 (0.3, 7.4)	3.0 (1.4, 6.0)	0.48	1.7 (1.1, 2.7)	0.17
	IGM	36.4 (27.5, 46.4)	11.7 (8.4, 15.9)	18.1 (14.6, 22.1)	≤0.001	44.2 (36.4, 52.4)	22.9 (15.5, 32.3)	35.1 (29.4, 41.1)	≤0.001	18.0 (15.8, 20.4)	≤0.001
Metabolic syndrome		29.8 (21.4, 39.7)	44.2 (38.6, 49.8)	41.1 (36.5, 45.9)	0.01	46.2 (38.3, 54.3)	64.2 (54.2, 73.1)	54.0 (47.9, 60.0)	0.01	34.1 (31.3, 37.0)	≤0.001
Dyslipidemia		38.4 (29.5, 48.1)	68.1 (62.7, 73.1)	60.4 (55.7, 65.0)	≤0.001	50.9 (43.0, 58.8)	77.8 (68.6, 85.0)	62.0 (56.0, 67.7)	≤0.001	46.2 (43.7, 48.8)	≤0.001
High total cholesterol		6.2 (2.8, 12.9)	7.1 (4.6, 10.5)	6.9 (4.8, 9.7)	0.94	8.1 (4.5, 13.7)	13.9 (8.2, 22.2)	11.1 (7.8, 15.5)	0.18	7.1 (5.9, 8.6)	0.02
High LDL-C		5.4 (2.2, 11.8)	3.1 (1.6, 5.8)	3.8 (2.3, 6.1)	0.26	10.0 (6.0, 16.0)	10.3 (5.5, 18.0)	9.7 (6.6, 14.0)	1.00	4.9 (3.9, 6.2)	≤0.001
High TG		8.9 (4.6, 16.2)	50.0 (44.6, 55.4)	39.1 (34.6, 43.8)	≤0.001	17.4 (12.1, 24.3)	61.1 (51.2, 70.2)	36.2 (30.6, 42.2)	≤0.001	29.2 (26.9, 31.6)	≤0.001
Low HDL-C		23.2 (16.0, 32.3)	39.1 (33.9, 44.7)	35.0 (30.7, 39.7)	≤0.001	34.8 (27.6, 42.7)	47.7 (38.1, 57.4)	40.0 (34.3, 46.0)	0.05	24.6 (22.4, 26.8)	≤0.001
Inflammation		55.0 (44.8, 64.9)	53.4 (47.5, 59.3)	54.1 (49.1, 59.1)	0.88	80.6 (73.4, 86.4)	77.7 (68.2, 85.0)	79.9 (74.4, 84.4)	0.67	55.6 (52.3, 58.9)	0.02
High liver enzymes		3.5 (1.1, 9.4)	20.9 (16.7, 25.8)	16.4 (13.2, 20.2)	≤0.001	9.9 (6.0, 15.9)	39.1 (30.1, 48.9)	22.4 (17.8, 27.8)	≤0.001	12.0 (10.4, 13.7)	≤0.001
Vitamin D insufficiency		94.5 (88.0, 97.8)	95.9 (93.0, 97.7)	95.2 (92.7, 97.0)	0.59	98.1 (94.2, 99.5)	98.2 (92.9, 99.7)	97.9 (95.2, 99.1)	1.00	92.4 (90.8, 93.7)	≤0.001

Frequencies are expressed as percentages and 95% confidence intervals, and groups were compared using the χ^2 test. Abbreviations: BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; HDL-C, high-density lipoprotein cholesterol; HbA_{1c}: glycated hemoglobin; IGM, impaired glucose metabolism; LDL-C, low-density lipoprotein cholesterol; NA, not available; TG, triglycerides.

Table 4. Association between demographics and BMI categories with cardiometabolic abnormalities in the total sample (logistic regression analysis adjusted by age and sex).

		IGM	Elevated BP [a]	High TC	Low HDL-C	High LDL-C	High TG	High AST/ALT	Inflammation	Vitamin D Insufficiency
Sex	Male	1	1	1	1	1	1	1	1	1
	Female	0.88 (0.63, 1.23)	0.48 (0.39, 0.60)	0.80 (0.54, 1.20)	0.54 (0.42, 0.70)	0.97 (0.61, 1.56)	0.63 (0.50, 0.81)	0.36 (0.25, 0.52)	2.51 (1.85, 3.39)	1.75 (1.15, 2.65)
Age	12–14 years	1	1	1	1	1	1	1	1	1
	15–17 years	1.27 (0.89, 1.81)	3.38 (2.69, 4.24)	1.68 (1.11, 2.54)	1.00 (0.75, 1.32)	1.83 (1.14, 2.95)	1.05 (0.79, 1.38)	1.65 (1.13, 2.40)	1.17 (0.83, 1.65)	1.33 (0.80, 2.19)
Ethnoracial group	Hispanic	1	1	1	1	1	1	1	1	1
	African American	3.01 (2.09, 4.32)	1.13 (0.83, 1.53)	0.87 (0.54, 1.40)	0.58 (0.43, 0.79)	1.16 (0.68, 1.98)	0.12 (0.08, 0.18)	0.17 (0.19, 0.27)	0.80 (0.56, 1.15)	1.30 (0.75, 2.26)
BMI percentile	Normal weight	1	1	1	1	1	1	1	1	1
	Overweight	3.43 (1.34, 8.79)	2.17 (1.56, 3.03)	1.50 (0.82, 2.76)	2.12 (1.39, 3.23)	1.43 (0.68, 2.99)	1.82 (1.27, 2.61)	1.88 (0.98, 3.61)	6.33 (1.85, 21.61)	1.6 (0.97, 2.65)
	Obesity	6.58 (2.79, 15.52)	5.23 (3.92, 6.98)	1.49 (0.85, 2.59)	5.11 (3.54, 7.37)	1.28 (0.65, 2.53)	3.62 (2.61, 5.01)	4.90 (2.84, 8.46)	16.6 (4.98, 55.19)	3.45 (2.02, 5.90)
	Severe obesity	11.30 (4.79, 26.82)	11.50 (8.35, 5.96)	2.45 (1.38, 4.38)	7.17 (4.77, 10.76)	2.84 (1.47, 5.49)	5.91 (3.94, 8.86)	10.50 (5.84, 18.81)	62.9 (18.39, 215.22)	7.15 (2.97, 17.23)

Data are odds ratios and 95% confidence intervals. Logistic regression analysis adjusted by sex and age was applied. Reference groups in the regression model were selected based on the lowest levels of perfect separation. [a] Elevated BP includes stage 1 and stage 2 hypertension. Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; HDL-C, high-density lipoprotein cholesterol; IGM, impaired glucose metabolism; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides.

4. Discussion

This study illustrates ethnoracial differences in the association of the progressive increase in adiposity with the CMRF profile of African American and Hispanic adolescents receiving care at LDH. The main finding of this study was that, independently of ethnoracial group, age, and sex, adiposity was the strongest determinant of cardiometabolic risk. Another relevant finding was that BMI% thresholds used in clinical practice were not sufficient at highlighting health implications in adolescents with adiposity in an appropriate amount of time. By the time individuals were submitted for evaluation (≥85th BMI%, classified as overweight) [14], the prevalence of IGM, elevated BP, low HDL-C, and high TG were already elevated and continued to progress with increasing adiposity. Established clinical guidelines recommend the evaluation and treatment of individuals only once they are overweight [14]. Thus, early identification of high-risk individuals in these minority groups is lacking and ultimately precludes the potential for earlier and more proactive interventions [19,20].

The rate of increase in obesity has been greater in children than in adults in many countries [21], The frequency of obesity in our study (39.2%) was higher than the 20.6% reported for US adolescents (12–19 years) in NHANES (2015–2016) [22]. In NHANES, no overall difference was found in the prevalence of obesity between Hispanic adolescents (25.8%) and African American [22] adolescents (22.0%), although obesity was more frequent in Hispanic than African American males (28% vs. 19%). We found that African American adolescents had a higher prevalence of severe obesity in both sexes, although overweight was more prevalent in Hispanic adolescents of both sexes. Our study is not a population-based sampling analysis like NHANES, but a representation of a multi-specialty clinical setting where most patients were assigned by insurance companies, which may explain these differences. Several factors may also account for the higher prevalence of obesity in our study. First, the sample was drawn from a predominantly minority population where the estimated prevalence of obesity exceeds the national average [1]. Moreover, subjects from low-income families that were uninsured or Medicaid-covered represented 74.2% of our sample and it is known that prevalence of obesity is higher among these groups [14,21,23]. Furthermore, LDH has implemented a diabetes risk stratification program, thus patients may have been referred from outside primary care for co-management [8]. Finally, the lifestyle impact of the COVID-19 pandemic could have exacerbated obesity in those evaluated in 2020 [14,24].

The overall obesity prevalence in our study (39.2%) was similar to that reported for adults in NHANES (2015–2016; 39.8%) [22]. A meta-analysis involving 200,777 subjects reported that approximately 80% of adolescents with obesity remain with obesity into adulthood [25]. Data from the Bogalusa Heart Study which followed 2392 children (aged 5–14 years) for 17.1 years showed that African American children with obesity were more likely to remain with obesity as adults (83%) than their non-Hispanic White counterparts (68%) [26]. The cumulative effect of multiple CMRFs associated with increased adiposity in childhood and adolescence may lead to adult cardiovascular disease (CVD). Earlier, more intensive interventions may be needed to ameliorate the impact of childhood obesity on adult cardiovascular outcomes [27].

Although only 1 ethnoracial disparity in CMRFs (high TG) was found in normal weight adolescents, more marked phenotypic differences were expressed as the degree of adiposity increased. Similar results have been reported in other studies using representative national samples of adolescents [28,29]. MetS is a cluster of risk factors for cardiometabolic diseases, including heart disease, stroke, and diabetes [30]. In NHANES (1999–2008), MetS in adolescents (n = 3385) was reported as 35.4% and 24.6% in boys and girls with Obesity, respectively, per CDC classification standards, compared to 0.8% and 1.7% in their normal weight counterparts [31]. Like NHANES, we found increases in the prevalence of MetS as severity of adiposity increased. We previously evaluated the prevalence of CMRFs in 759 adolescents and adults with overweight/obesity undergoing LDH's Diabetes Risk Stratification Program. Findings showed that once obesity is present, the proportion of

subjects with ≥3 CMRFs was similar regardless of age, suggesting that obesity similarly impacts the CMRF profile of adolescents and adults [32].

Dyslipidemia prevalence has been reported to be between 19–25% in US adolescents when defined as any abnormal lipid or apolipoprotein B [33]. We found a higher prevalence of dyslipidemia (46.2%), which was greater in Hispanic than African American adolescents. Compared with non-Hispanic White adolescents, both African American and Hispanic adolescents exhibit a higher degree of insulin resistance in response to increased adiposity [34,35], which has been linked to high TG and low HDL-C, referred to as metabolic dyslipidemia [36,37]. However, the NHANES (2001–2012) study showed that in African American adolescents the impact of obesity-induced insulin resistance is expressed distinctively and with fewer adverse lipid effects than in Mexican American adolescents [36,37]. In our study, African American subjects exhibited a lower baseline TG level and lower high TG prevalence compared with Hispanic subjects across all adiposity categories.

Metabolic-associated fatty liver disease (MAFLD) is associated with both metabolic dyslipidemia and progression to prediabetes and T2D [38]. A meta-analysis reported that MAFLD prevalence is higher in children with obesity than normal weight (34.2% vs. 7.6%), higher in males with obesity than females, and increases with severity of adiposity [39]. In our study, the prevalence of high liver enzymes was 12%, with a three-fold higher prevalence in males and a >two-fold higher prevalence in Hispanic subjects, regardless of sex. Although the NHANES (1999–2004) survey reported a lower prevalence of elevated ALT >40 U/L (3.6%) in adolescents aged 12–19 years and significantly higher prevalence in Mexican American adolescents (6.1%) than African American adolescents (2.3%) [40], another NHANES survey (1999–2010) in normal weight 12–18-year-old adolescents detected no ethnoracial differences in ALT levels after adjusting for sex, WC, and weight [41]. Similarly, our study found no ethnoracial differences in the prevalence of high liver enzymes in normal weight adolescents, although prevalence increased in Hispanic adolescents with increasing adiposity. The true prevalence of high liver enzymes could be even higher than reported here, since lower pediatric cut-offs for ALT (males, 25.8 U/L; females, 22.1 U/L) have been proposed [42].

Inflammation is another common pathophysiological risk factor for CVD [43]. In our study prevalence was higher among African American adolescents regardless of sex. We have previously reported that comparable levels of adiposity and insulin resistance result in a higher level of subclinical inflammation in African American adolescents than in non-Hispanic White adolescents [44].

The most prevalent CMRF was vitamin D insufficiency (92.4%), which was most frequent in African American females. Vitamin D deficiency has previously been reported as more prevalent in female adolescents than males [45], and NHANES data have consistently shown a higher prevalence of vitamin D deficiency in African American and Hispanic adolescents compared with non-Hispanic White adolescents [46]. Disparities between African American and Hispanic subjects may be attributed to several factors, including darker skin pigmentation, amount of sun exposure required to produce vitamin D [45], and higher adiposity.

There are some limitations to this study. This retrospective analysis is liable to residual confounders and causal inferences cannot be established. Since these data were drawn from a specific clinical setting, these results cannot be extrapolated to the general population. IGM was defined only using HbA_{1c}, which can cause bias IGM estimates in African American subjects; however, it is currently used by the American Diabetes Association to define prediabetes [13]. Moreover, not all blood samples were collected in a fasted state, which introduces variations in the lipid profile. However, strengths of this study include its large sample size and conduct in a translational clinical practice designed to approach patients with cardiometabolic conditions under a standardized protocol for screening, management, and data collection. The study also used the same laboratory testing protocol and analytic methodology throughout the 3-year observation period regardless of patient payment ca-

pacity. Importantly, most adolescents were from low-income ethnoracial minority groups, who are usually under-represented in clinical trials.

5. Conclusions

The prevalence of overweight/obesity in the LDH system is very high among African American and Hispanic adolescents. Increased adiposity was the most impactful determinant of the CMRF profile in this population, even at lower levels of excess adiposity. The current BMI-based approach is not sensitive enough to allow the early identification of adolescents at higher risk, and differences between CMRF profiles in African American and Hispanic adolescents in response to increased adiposity are not addressed in current management guidelines, delaying the implementation of appropriate primary preventive measures. Research within routine clinical settings, particularly in minority groups, is required to characterize the nature of these ethnoracial discrepancies. This will better guide healthcare providers in more precise interventions to ameliorate any adverse health impact or underlying burden to the health system as they transition to adulthood.

Author Contributions: P.A.V.-M. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. He directed the study and contributed to the analysis plan, discussion, drafting, and revision of the manuscript. R.N.-M. contributed to design of the data analysis, contributed on the writing of the first draft, discussion, and review. A.E.V. contributed to the concept and design, interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. X.M. designed the statistical models and performed the statistical analyses. S.Y.-M. was formally working with Novo Nordisk at the time the research was conducted and contributed on the interpretation of data, drafting of the manuscript, discussion, and review. J.I.M. contributed to concept and design, interpretation of data, drafting of the manuscript, and critical revision of the manuscript for important intellectual content. C.C.G. contributed to concept and design, interpretation of data, and critical revision of the manuscript for important intellectual content. C.P.N. contributed to implementing the clinical protocol, collecting the data, training and data recording, and reviewing of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Based on federal regulation 45 CFR 46, no IEC/IRB review was sought as this does not meet the definition of human subject research as defined in 45 CFR 46.102. Specifically, this study was designed as a retrospective review using historical datasets of proprietary, de-identified data that have been previously collected.

Informed Consent Statement: Written informed consent was obtained from all youth or parents/legal guardians as applicable, in the four original studies. This study, which used de-identified data from the previously conducted studies, was considered Non-Human Subject Research.

Data Availability Statement: Data are not publicly available due to legal restrictions on sharing/distribution of study data requiring Sponsor approval on a case-by-case basis. The data that support the findings of this study can be provided by the corresponding author (Velásquez) upon reasonable request.

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A Pilot Randomized Control Trial Testing a Smartphone-Delivered Food Attention Retraining Program in Adolescent Girls with Overweight or Obesity

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Abstract: Background/Objectives: Attention bias (AB) toward food is associated with obesity, but it is unclear if programs designed to reduce AB can impact adolescents' eating behavior. We investigated whether a two-week, smartphone-delivered attention retraining (AR) program (vs a control program) altered food AB in adolescent girls with overweight. Methods: Participants completed three food-cue visual-probe trainings/day. The AR and control programs directed attention away from food stimuli during 100% and 50% of trainings, respectively. Before and after completion of the programs, girls completed a food-cue visual-probe task while undergoing magnetoencephalography (MEG), and then a laboratory test meal. Results: Sixty-eight adolescents were randomized; 58 completed post-program visits. There was minimal effect of condition on AB scores (β [95%CI] = −1.9 [−20.8, 16.9]; d = −0.06). There was a small effect of condition on energy intake ($EMM_{control}$ = 1017 kcal, EMM_{AR} = 1088 kcal, d = 0.29). Within the AR group, there was slightly blunted initial engagement in brain areas associated with reward response and subsequent increased goal-directed attention and action control. Conclusions: We found preliminary support for efficacy of an intensive smartphone-delivered AR program to alter neural correlates of attention processing in adolescent girls with overweight or obesity. Studies with larger sample sizes are needed to elucidate if AR trainings disrupt the link between food AB and eating behavior.

Keywords: attention bias; obesity; adolescence; attention retraining; smartphone program

1. Introduction

Food attention bias may be a useful target for interventions aiming to reduce aberrant eating and slow unhealthy weight gain during adolescence. Attentional bias (AB) is a tendency to attend selectively to environmental stimuli, such as food, that have acquired salience or meaning [1,2]. Although food AB is not inherently problematic, several reviews support an association between increased food AB and aberrant eating behavior [3–5] and obesity [6–8]. While there is some evidence that children with and without overweight do not differ in their attention towards food [9,10], other reports indicate a positive cross-sectional association between food AB and body mass index (BMI, kg/m^2) in children and adolescents [11,12]. Moreover, food AB has been shown to prospectively predict weight gain in adolescents [11]. Food AB might promote weight gain due to its association with aberrant eating behavior, such as increased energy intake [4,13] and loss-of-control eating [3] (LOC-eating; the subjective experience of a lack of control over what or how much is eaten [14]). One study found that the link between food AB and weight was significant only among adolescents with recent experiences of LOC-eating [15]. Similar positive associations between AB and snack food intake [16], LOC-eating [3,17,18], and overweight and obesity (compared to average weight) [16,19,20] have been reported among adults. Therefore, adolescence might be an opportune period for disrupting the development of associations between food AB and obesity.

Assessments of neural activity have been a key part of attempts to understand food AB [6,11,21]. Evidence that weight is associated with food AB is stronger when AB processes are assessed by neuroimaging (e.g., electroencephalogram, functional magnetic resonance imaging), and weaker or mixed when assessed by behavioral tasks [4,8,22]. The neural processes involved in attention are deployed over short time scales (milliseconds), and are therefore optimally captured by electroencephalogram and magnetoencephalography, temporally sensitive neuroimaging methods [23]. Specifically, rapidly deployed "bottom-up" striatal circuitry is involved in unconscious attention deployment, as well as food cue processing and reward value encoding [24–30]. Unconscious attention deployment during food AB tasks has also been linked to hyperactivation in the insula, ventral anterior cingulate cortex (ACC), and orbitofrontal cortex (OFC) in adolescent girls [11] and adults with LOC-eating [21,31,32]. Conscious attention deployment, involving "top-down" brain regions that support task-related goal attainment including the dorsal ACC and the ventrolateral and dorsolateral prefrontal cortex (vlPFC, dlPFC, respectively; [2]), occurs after unconscious attention deployment. Thus, there are two interacting pathways through which interventions aiming to reduce AB might affect change [33]; by affecting unconscious attention deployment, e.g., by reducing the reward responsivity (Valence-Specific Models), or altering conscious attention deployment, e.g., by increasing attentional control (Attentional Control Models).

Attention retraining (AR) programs, also commonly referred to as attention bias modification programs, are designed to alter attention processing. Extant AR programs have demonstrated preliminary efficacy for reducing food AB and energy intake among adults [34–36]. A recent review of ten AR programs delivered in a laboratory setting found that the trainings acutely reduced food AB [37]. There is also preliminary evidence that AR programs evince changes in reward and attention related neural underpinnings of food AB, suggesting these interventions might exert their effect by reducing the reward valuation of food [38]. However, there are limited data demonstrating that food-based AR programs affect changes in eating behavior and weight [37,39], potentially due to a lack of understanding as to how each phase of attention (capture and deployment) relates to behavioral outcomes [4].

Additionally, the dose of extant food-based AR programs, which range from a single training to 30 trainings delivered over the course of 5 weeks [37], may be too low to impact eating behavior. Higher intensity AR programs that require the completion of several AR trainings delivered multiple times a day for multiple weeks may increase the effectiveness of AR programs [40–43]. The use of smartphones for intervention delivery makes more

intensive AR programs feasible, especially for adolescents. Smartphones have become an integral part of society, with rates of smartphone ownership among youth dramatically increasing in the last decade [44,45]. Compared to laboratory/clinic-based interventions, programs delivered on a smartphone allow for youth to complete more intensive AR programs in their natural environments.

We therefore conducted a double-blind randomized controlled pilot trial to test the impact of a two-week long AR program (compared to a control program) on food AB (assessed via a visual-probe task), energy intake (during a validated laboratory paradigm designed to simulate LOC-eating [46]) and brain activity (measured by magnetoencephalography) among adolescent girls with overweight or obesity. We hypothesized that following the smartphone program, girls who completed the AR program (vs a control program) would demonstrate a greater reduction in food AB scores, total energy intake, and intake of carbohydrates and fats, and increased intake of protein. We also hypothesized that following completion of the smartphone program, girls who completed the AR program would show decreased reward valuation of food cues via (1) decreased reward responsivity to food cues via increased oscillatory power in the striatum, ventral ACC and OFC during unconscious attention capture, and (2) increased attention control via decreased oscillatory power in the dorsal ACC, vlPFC, and dlPFC during attention deployment. We expected no change in the neural activity in any region of interest (ROI) among girls who completed the control program. Lastly, we explored recent LOC-eating as a moderator of the smartphone program's effects on food AB, energy intake, and brain activity.

2. Materials and Methods

2.1. Study Design

Study procedures for this double-blind randomized control pilot trial for adolescent girls with overweight or obesity (Clinical Trials Identifier: NCT02977403) were approved by the National Institutes of Health (NIH) Institutional Review Board. All study visits occurred at the NIH Clinical Center. Participants were recruited by mail to local area parents, flyers posted at local public facilities, and Facebook advertisements. Interested participants were screened for eligibility over the phone and during an initial in-person visit. During the screening visit, informed consent and assent were obtained from parents and/or guardians and the participant, respectively. Participants completed a dual-energy X-ray absorptiometry (DXA) scan for body composition, a physical exam by a medical provider, self-report questionnaires, and an interview to determine the presence of recent LOC-eating. If deemed eligible at the screening visit, girls were randomized to complete either the AR or control program and were scheduled for a pre-intervention visit.

Participants were instructed to fast starting at 10 PM the night prior to the study visit. Adolescents arrived at the pre-intervention visit in the morning and completed a fasting blood draw, consumed a breakfast shake (17% protein, 16% fat, 67% carbohydrate) calibrated to their age, height, weight, and activity level, and underwent an MEG scan. Immediately following the scan, girls participated in a laboratory test meal. Lastly, participants were trained on how to use a provided smartphone. Starting the day following their pre-intervention visit, participants completed the (AR or control) smartphone program for two-weeks in their natural environment. After two weeks, girls returned to the NIH Clinical Center to complete a post-intervention assessment. The procedures and temporal order of study activities (i.e., breakfast shake, MEG scan, and test meal) during the post-intervention visit were the same as the pre-intervention visit. In addition, participants completed a single structural MRI scan (3T) during either their screening, pre-intervention, or post-intervention visit.

2.2. Participants

English speaking, right-handed, adolescent females with overweight or obesity (BMI, $kg/m^2 \geq$ 85th percentile) aged 12–17 years were eligible to participate [47]. Exclusionary criteria were the presence of major medical illnesses (i.e., Cushing syndrome, untreated

hypothyroidism) or a health condition that required medical treatment (i.e., hypertension or fasting hyperglycemia consistent with diabetes); use of medications known to affect body weight or eating behavior; current or past pregnancy; presence of any significant and full-threshold psychiatric disorder, except for binge-eating disorder; current and regular substance use; history of significant or recent brain injury; current involvement in treatment for weight loss or eating behavior; and presence of conditions where MEG is contraindicated (e.g., braces, metal implants).

2.3. Smartphone Program

Food-cue visual probe task: The AR and control programs were designed by one of the authors (AJW) and delivered using a smartphone application ("Colors"). The Colors smartphone application can administer several reaction time tasks and has been used successfully to administer an AR program for smoking reduction [48]. The food-cue visual probe task used in this study requires participants to complete several trials during which two colored photographs are presented side-by-side on a screen for 200 ms. Then, both images disappear and a probe (left or right pointing arrow) appears in a location previously occupied by one of the pictures. Participants are instructed to press a left button if the probe is pointing to the left, and a right button if the probe is pointing to the right, regardless of the side of the screen the probe appears on.

The smartphone program used high-palatability food (HF) stimuli and non-food (NF) stimuli, so that during all trials, participants viewed high-palatability food and non-food stimuli pairs (HF-NF). The only difference between AR and control programs was in the placement of the probe. For the experimental condition, the visual probe always replaced the non-food (neutral) stimulus. Thus, there was a perfect correlation between stimulus type and probe location. Individuals assigned to the AR program should, therefore, learn to attend away from the high-palatability food stimuli and identify the probe direction more quickly. For the control condition, the probe was equally likely to replace the food stimulus and the non-food stimulus. Therefore, there was no correlation between stimulus type and probe location, and no training of attention towards either food or non-food cues should occur. Consistent with previous attention retraining studies [48,49], the same program for AR and control conditions was used so that the duration of AR and control trials did not differ. This approach also ensured participants randomized to AR and control programs were exposed to the same food and neutral stimuli and received equal practice on the motoric aspects of the visual probe task.

Training frequency and timing: Daily, for two weeks, participants were prompted to complete three smartphone trainings, each consisting of 80 trials of the visual probe task. In addition to the trainings, participants completed a once-daily food AB assessment. The assessment required completion of 40 trials of the food-cue probe task. However, daily AB assessments were not part of the intervention program; thus, the probe replaced food and non-food images equally for all participants, regardless of treatment condition. Additionally, participants reported on their eating patterns and mood during each of the prompted trainings and AB assessments.

On school days, girls were prompted to complete AR (or control) trainings before school and immediately after school. They were also randomly prompted two more times between 3:30 pm and their bedtime, once for training and once to complete the daily AB assessment, which were completed in random order. Bedtime was set individually for each person at a time between 7:30 pm and 10:30 pm, based on the participant's preference. On weekend days, the same schedule was used as on school days; however, participants were able to delay the first training by up to two hours to accommodate later waking times.

2.4. Outcome Measures

2.4.1. Laboratory Test Meal

All participants received a multi-item, buffet-style meal (~11,000 kcal, 12% protein, 33% fat, 55% carbohydrate) comprising foods typically consumed by youth (e.g., chicken

nuggets, white bread, turkey and ham, cheese slices, orange slices, carrots, tortilla chips, sandwich cookies, jellybeans). Prior to beginning the meal, girls were played tape-recorded instructions to "let yourself go and eat as much as you want", and were left alone to eat. This is a well-validated paradigm which has been used in both adolescents [46] and adult [50] samples. The amount consumed at each meal was calculated by weighing each item before and after the test meal. Energy content and macronutrient composition consumed by participants was determined for each item according to data from the U.S.D.A. Nutrient Database for Standard Reference [51] or Food and Nutrient Database for Dietary Studies.

2.4.2. Magnetoencephalography Scan

Brain magnetic fields were measured by a CTF 275 MEG system (CTF Systems, Inc., Coquitlam, BC, Canada) composed of a whole-head array of 275 SQUID sensors, in a magnetically shielded room (Vacuumschmelze, Hanau, Germany). Participants were in a seated position with the helmet placed around their heads. Head position within the magnetometer was determined by digitizing the position of the three indicator coils attached to the right and left preauricular and nasion fiducial points. Consistent with previous MEG research in pediatric samples [52,53], MEG data were sampled at 600 Hz (bandwidth 0–150 Hz).

Rest scan: At the beginning of the MEG session, participants completed a five-minute rest scan. Girls were instructed to keep their eyes open and focus on a black cross that was presented in the center of a white screen.

Food-cue visual probe task: Following the rest scan, participants completed 180 trials of the food-cue AB task. During the MEG scan, the images presented three types of stimuli: high-palatability food (HF), low-palatability food (LF), and non-food object (control stimuli, NF), with two differing stimuli simultaneously presented during each trial (on the right and on the left sides of the screen). Thus, there were three cue pairs (Figure S1; see Supplemental Materials): (1) HF-NF; (2) HF-LF; and (3) LF-NF. Each stimulus pair was presented 60 times, with the location of stimuli and location of the probe fully crossed. To minimize automaticity, the inter-trial interval was randomly jittered across three durations of 100 ms, 150 ms, and 500 ms. Additionally, to ensure participants understood the instructions, they engaged in a short practice session before completing the task. The practice trials resembled the main task, but no food stimuli were shown.

2.5. Other Measures

Anthropometric measures: Fasting weight was measured using a calibrated scale to the nearest 0.1 kg and height was measured in triplicate on a calibrated stadiometer to 0.1 cm. Weight and the average of three heights were used to calculate BMI, that was converted to BMI scores standardized for sex and age (BMIz) [54]. Total body fat mass (kg) was determined by a dual energy X-ray absorptiometry scan (GE Lunar iDXA, GE Healthcare, Madison WI; software GE encore 15), which is a validated body composition measure in youth [55].

Recent loss-of-control eating: The Eating Disorder Examination interview [56] is a semi-structured clinical interview of eating disorder psychopathology. Recent LOC-eating was determined as reporting at least one episode of LOC-eating (regardless of the amount of food consumed) in the prior 28 days. This interview has been found to be reliable and valid in adolescent samples [57–59]. All interviews were audio recorded. Twenty percent of the interviews conducted during the screening visit (n = 16) were reviewed and rated by a second, independent interviewer. There was 100% inter-rater agreement for presence of LOC-eating episodes in the prior month.

2.6. Sample Size Estimation

Sample size estimation was based on the power analysis for the first hypothesis (changes in AB scores following completion of the smartphone program). Meta-analytic studies of AR programs in adults found that AR programs produce large effects (d = 0.80–1.41)

on reduction in AB and medium effects (d = 0.51–0.61) on target behaviors, such as smoking and eating [41,42]. Based on recommended equations for estimating sample size [60] when using general linear models, and assuming 35% attrition due to incomplete visual probe or MEG data [53,61,62], 80 girls, with a planned recruitment of 40 with LOC-eating and 40 without LOC-eating, were estimated to provide >80% power to detect medium to large effects.

2.7. Randomization and Blinding

In the current double-blind randomized control trial, girls were randomized to complete the AR or control smartphone program in blocks of eight with stratification for recent LOC-eating presence (LOC-eating or no LOC-eating), age (12–14 year or >14 year), and race (White or Other Race). Participants were each assigned a unique color and number combination to maintain blinding throughout the study. A study member who was not involved in data collection or analysis completed randomization and blinding procedures. Smartphone condition was not disclosed to participants at any point in the study. The unique codes (color, number) assigned to each participant were used to set up the smartphones, and maintain blinding of research coordinators involved in smartphone setup and data collection. Program allocation was revealed for data analysis only after all participants completed their pre- and post-intervention visits.

2.8. Analytic Plan

2.8.1. Data Pre-Processing

AB reaction time scores: AB reaction time scores were derived from the dot probe task completed during the MEG scans. Reaction times scores were obtained for each of the stimulus pairings (3 total; HF-NF, LF-NF, HF-LF). Trials where the probe appeared behind the more salient food cue (e.g., a high-palatability food image, or low-palatability food image when the other image was a non-food image) were considered congruent trials. Trials where the probe appeared behind the less salient cue (e.g., non-food image, or low-palatability food image when the other image was a high-palatability food image) were considered incongruent trials. The participant's average reaction time during incongruent trials was subtracted from the participant's average reaction time during congruent trials. Thus, positive scores represent a quicker reaction time for (and bias towards) the more palatable stimulus, and negative scores represent a slower reaction time for (and bias away from) the more palatable stimulus. A difference score of 0 represents no bias towards or away from the more palatable stimulus. Consistent with prior studies, only trials where the participant responded correctly to the direction of the probe were included in computations.

Neural Oscillatory Power: Data processing was completed within MNE Python (v5.5.1) [63]. Data were structured into BIDS format using MNE-BIDS [64,65]. Standard MEG and MRI pre-processing steps were performed using Freesurfer (v7.4.1) [66] and the MNE-BIDS-Pipeline (v1.9) [67], including the following: cortical extraction and tessellation, source space generation (5 mm volumetric and 4096 nodes per hemisphere surface space), boundary element modeling, forward modeling, notch filtering (60/120/180 Hz), and epoching of the data. A structural MRI scan was co-registered to the MEG coordinate system. Three fiducial points (right and left preauricular and nasion) were marked via vitamin E capsules during the MRI scan to facilitate co-registration with MEG data. AFNI software (v24.04) [68] was used for co-registration with MEG data.

Additional processing was performed to generate the beamformer source localization using code developed for the study. Environmental noise was removed by applying reference channel third-order gradient compensation. Data were filtered from 13 to 35 Hz with a bandpass zero-phase FIR filter to extract the canonical beta band frequencies. The pre-stimulus baseline of 100 ms was used to generate the noise covariance matrix. The covariance matrix was generated using all data (0–500 ms following stimulus appearance). Linearly Constrained Minimum Variance (LCMV) beamformer weights were created using the data covariance. Trials were then segmented by (1) stimuli pairing (e.g., high-palatability

food vs. non-food); (2) attention phase (capture = 0–250 ms following stimulus appearance, deployment = 250–500 ms following stimulus appearance); and (3) probe placement (i.e., congruent or incongruent trial). The segmented data were then used to create stimuli by timing by probe placement-specific covariance matrices and projected through the common covariance beamformer weights to generate surface and volumetric source reconstructions. Next, within each stimuli-pairing and attention phase, oscillatory power (pseudo-Z) during the incongruent trials was divided by oscillatory power during the congruent trials. The resulting difference value was then log transformed. The oscillatory power log scores were then averaged across all voxels within each a priori specified ROI. Volumetric model Freesurfer labels corresponding to regions in the striatum were obtained from the aseg atlas [69]. Surface model Freesurfer labels corresponding to regions in the ACC, OFC, dlPFC, and vlPFC were obtained from the aparc atlas [70]. This resulted in six oscillatory power measures (3 pairing types, 2 attention phases) in each ROI, for each participant at both pre-intervention and post-intervention.

2.8.2. Hypothesis Testing

All analyses were conducted in python and are accessible on GitHub at https://github.com/Yanovski-Lab/AttentionRetraining_2weekOutcomes.git; (published on 19 July 2024). To examine changes in AB and oscillatory power from pre- to post-intervention, change scores were computed (Δ = post-intervention − pre-intervention) for AB reaction time scores and oscillatory power measures. Positive Δ represents an increase in AB or oscillatory power from pre- to post-intervention. Negative Δ represents a decrease in AB or oscillatory power from pre- to post-intervention. In general, there is an inverse association between the BOLD response and oscillatory power in the beta band; therefore, positive $\Delta_{\text{oscillatory power}}$ corresponds with decreased BOLD, and vice versa [71].

To compare the two conditions regarding change in food AB ($\Delta_{\text{AB scores}}$) and neural activity ($\Delta_{\text{oscillatory power}}$), linear mixed models were run with change scores as the dependent variable. Between-subject factors were condition, recent LOC-eating, and a term for the LOC-eating by condition interaction. AB and neural activity change scores were nested within subject. Models included a random intercept. Independent linear mixed models were run for $\Delta_{\text{oscillatory power}}$ in each a priori specified ROI, and for each attention phase (attention capture and attention deployment). All mixed models were adjusted for stimuli pairing (HF-NF, LF-NF, HF-LF), age, fat mass (kg) and height (cm) at pre-intervention, and race and ethnicity.

To test the effects of smartphone condition on energy intake, general linear models were used. Dependent variables were total caloric intake, and percentage intake from carbohydrates, fat, and protein. Models included condition as an independent variable, as well as a term for condition by LOC-eating interaction. Models were adjusted for age, fat mass (%), lean mass (kg), height (cm), race/ethnicity, and LOC-eating, as well as the respective intake variable (i.e., total calories, carbs, fat, protein) at the pre-intervention visit.

Participants who did not provide complete covariate data were excluded from analyses. The number of participants included in each outcome analysis varied, as participants were included in analyses only if they provided complete data on the outcome measure at both baseline and follow-up visits. Given the preliminary nature of the study, effect sizes and 95% confidence intervals, rather than statistical significance, were used to interpret changes in outcome measures. Cohen's *d* values were computed to compare outcomes between the AR and control groups and were interpreted as minimal to no effect (<0.02), small effect (0.2–0.49), medium effect (0.5–0.79), and large effect ($\geq$0.8) [60]. Cohen's *d* values for the effect of condition were computed as:

$$\text{Cohen's } d = \frac{EMM_{active} - EMM_{control}}{SD_{pooled}}.$$

3. Results

3.1. Recruitment and Retention

Participants completed the study visits from February 2017 to September 2023. As shown in the consort diagram (Figure 1), 82 girls completed the screening visit. Of the 68 girls randomized to a smartphone program (n_{AR} = 32, $n_{control}$ = 36), 7 did not complete it and an additional 3 girls did not complete the post-intervention visit. The demographic characteristics of girls randomized (n = 68) to the control and active conditions are reported in Table 1. Comparisons of participants who were and were not included in analyses are reported in the Supplemental Material. The NIH paused data collection and participant recruitment in March 2020, due to COVID-19 safety protocols. Study recruitment was concluded before the 80 were randomized due to a low recruitment rate following the onset of the COVID-19 pandemic.

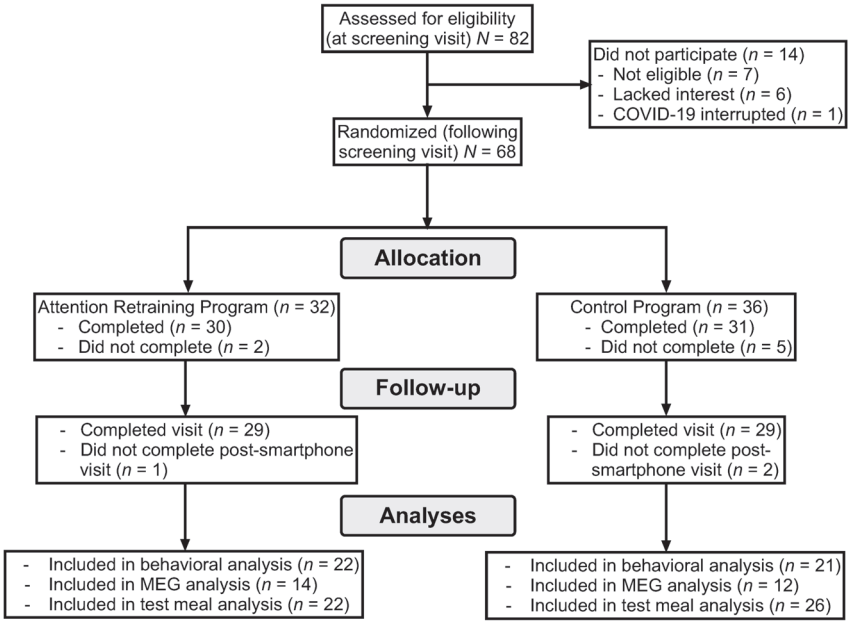

Figure 1. Consort diagram of participant retention.

Table 1. Characteristics of participants randomized to control and attention retraining programs.

Characteristic	Total Sample (N = 68)	Control Program (n = 36)	AR Program (n = 32)	Comparisons
				t, p
Age (year) [1]	14.93 ± 1.64	14.88 ± 1.70	15.00 ± 1.61	−0.31, 0.76
BMIz [1]	1.84 ± 0.60	1.88 ± 0.64	1.80 ± 0.55	0.54, 0.59
Fat mass (kg) [1]	33.71 ± 10.79	33.64 ± 11.31	33.79 ± 10.36	−0.06, 0.95
Height (cm) [1]	161.89 ± 6.46	161.29 ± 6.58	162.58 ± 6.35	−0.82, 0.42
				χ^2, p
Recent LOC-eating [2]	20 (29.41)	11 (30.56)	9 (28.13)	0.05, 0.83
Race [2]				3.18, 0.37
Asian	1 (1.5)	1 (2.8)	0 (0)	
Black	37 (54.4)	19 (52.8)	18 (56.3)	
Multiracial	7 (10.3)	2 (5.5)	5 (15.6)	
White	23 (33.8)	14 (38.9)	9 (28.1)	

Table 1. *Cont.*

Characteristic	Total Sample (*N* = 68)	Control Program (*n* = 36)	AR Program (*n* = 32)	Comparisons
Ethnicity [2]				0.97, 0.62
Hispanic	9 (13.2)	6 (16.7)	3 (9.4)	
Non-Hispanic	54 (79.4)	27 (75.0)	27 (84.4)	
Unreported	5 (7.4)	3 (8.3)	2 (6.2)	

Note. BMIz: body mass index adjusted for age and sex; recent LOC-eating: presence of 1 or more reported loss-of-control eating episodes as identified by the Eating Disorder Interview. One subject randomized to the active condition had missing baseline height and therefore BMIz data. [1] Mean ± Standard Deviation. [2] *n* (%).

3.2. Δ*AB Scores Outcomes*

There was minimal to no effect of condition on changes in the visual probe AB task scores derived from the food-cue visual probe reaction times (β [95%CI] = −1.948 [−20.790, 16.894]; Cohen's *d* = −0.057; Figure 2A). There was minimal to no interactive effect of LOC-eating by condition on $\Delta_{AB\ score}$ (β [95%CI] = −0.952 [−35.280, 33.377]; Cohen's *d* = −0.098–0.165; Figure 2B).

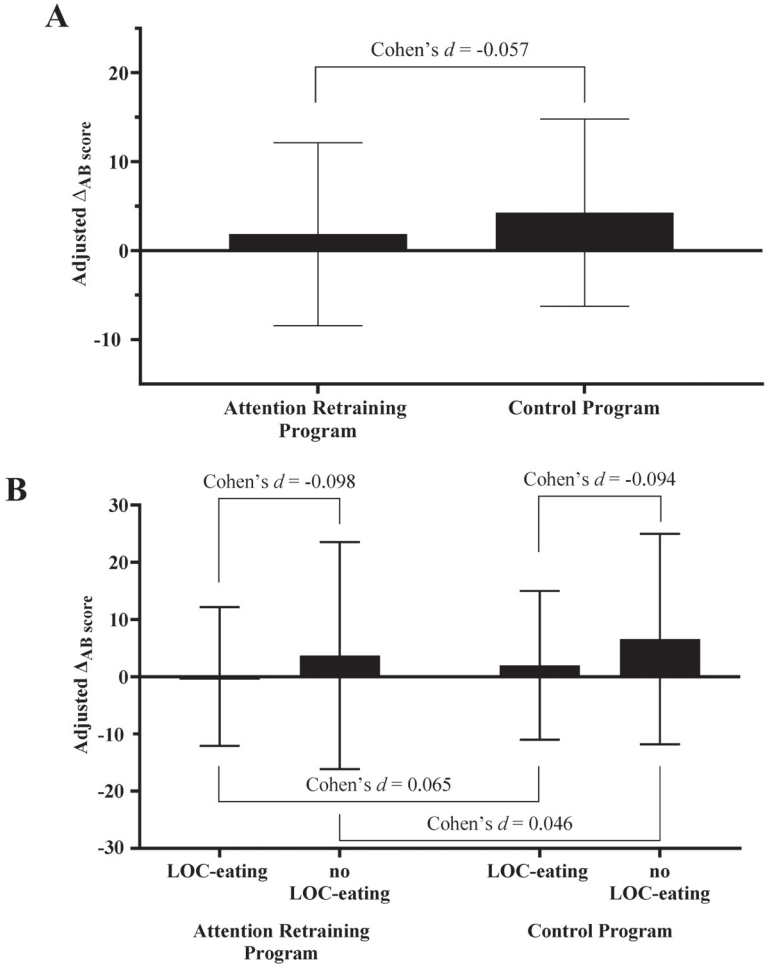

Figure 2. Effects of (**A**) condition and (**B**) condition by LOC-eating on change in $\Delta_{AB\ score}$. Note: LOC-eating; loss-of-control eating. Estimated marginal means (EMM) and 95% confidence intervals

presented for the total sample (EMM_{AR} 1.85 [−8.44, 12.13]; $EMM_{control}$ 4.27 [−6.25, 14.80]), AR group (EMM $_{LOC\text{-}eating}$ 0.02 [−12.13, 12.18]; EMM $_{no\ LOC\text{-}eating}$ 3.67 [−16.18, 23.52]), and Control group (EMM $_{LOC\text{-}eating}$ 1.97 [−11.02, 14.97], EMM $_{no\ LOC\text{-}eating}$ 6.57 [−11.81, 24.95]). The presented estimated marginal means for the effect of condition are from the models adjusted for LOC-eating and the LOC-eating × condition interaction term.

3.3. Energy Intake Outcomes

The effects of condition by LOC-eating on energy intake are reported in Tables 2 and 3, respectively. Unadjusted energy intake prior to and following completion of the smartphone program are reported in Supplemental Table S1. There was a small effect of condition on total energy intake, such that following the intervention the AR group consumed slightly more energy than the control group (d = 0.291). There was a medium effect of condition on carbohydrates (d = −0.544), with the AR group consuming a smaller percentage of energy from carbohydrates than the control group. There was also a medium effect of condition on fat (d = 0.615) and small effect of condition on protein (d = 0.216) intake with the AR group consuming a greater percentage of energy from these macronutrients than the control group (see Table 2).

Table 2. Effect of condition on energy intake.

	Effect of Condition	**Control (n = 26)**	**AR (n = 22)**	**Effect Size**
	β [95%CI]	EMM [95%CI]	EMM [95%CI]	Cohen's d
Total Calories (kcal)	66.322 [−133.345–265.988]	1017.024 [923.141–1110.906]	1088.188 [986.126–1190.249]	0.291 *
Carbohydrate (%)	−0.021 [−0.064–0.023]	78.3 [76.3–80.4]	75.4 [73.2–77.6]	−0.544 **
Fat (%)	0.024 [−0.008–0.057]	65.0 [63.5–66.5]	67.4 [65.8–69.1]	0.615 **
Protein (%)	−0.006 [−0.032–0.020]	37.3 [36.1–38.6]	38.0 [36.7–39.4]	0.216 *

Note. * small effect Cohen's d = 0.20; ** medium effect Cohen's d = 0.50. β: beta; EMM: estimated marginal mean; CI: confidence interval. β derived from general linear models adjusted for age, fat mass (%), lean mass (kg), height (cm), race and ethnicity (0 = non-Hispanic White, 1 = other race or ethnicity), and LOC-eating (0 = absent, 1 = present), as well as the respective intake variable (i.e., total energy intake, carbs, fat, protein) at baseline. The presented estimated marginal means for the effect of condition are from the models adjusted for LOC-eating and the LOC-eating × condition interaction term. Percentages were arcsin(sqrt)-transformed before analysis, so percentages do not add up to 100%. See Supplemental Materials for untransformed results.

Exploratory smartphone program × LOC-eating interaction effects: Within the control group, there was minimal to no effect of LOC-eating on energy intake (d = 0.101). However, there was a small effect of LOC-eating on carbohydrate intake (d = −0.288), minimal to no effect on fat intake (d = −0.023), and a medium effect on protein intake (d = 0.705), such that girls with LOC-eating consumed a somewhat greater percentage from carbohydrates and lower percentage from protein compared to girls without LOC-eating.

Within the AR group, there was minimal to no effect of LOC-eating on total energy intake (d = 0.067), carbohydrate intake (d = 0.046), fat intake (d = −0.005), nor protein intake (d = −0.095) following the intervention.

Table 3. Interactive effect of condition and LOC-eating on energy intake.

	Effect of Condition × LOC	Control No LOC (n = 16)	Control LOC (n = 10)	Control No LOC–LOC	AR No LOC (n = 15)	AR LOC (n = 7)	AR No LOC–LOC
	β [95%CI]	EMM [95%CI]	EMM [95%CI]	*d*	EMM [95%CI]	EMM [95%CI]	Cohen's *d*
Total Calories (kcal)	9.684 [−335.492–354.860]	1029.919 [907.552–1152.286]	1004.128 [849.345–1158.912]	0.101	1096.241 [969.861–1222.621]	1080.134 [895.132–1265.136]	0.067
Carbohydrate (%)	−0.017 [−0.092–0.058]	77.6 [74.9–80.3]	79.0 [75.6–82.4]	−0.288 *	75.5 [72.8–78.3]	75.3 [71.2–79.3]	0.046
Fat (%)	−0.001 [−0.056–0.055]	65.0 [66.9–63]	65.1 [67.5–62.6]	−0.023	67.4 [69.5–65.4]	67.4 [70.4–64.5]	−0.005
Protein (%)	0.026 [−0.019–0.071]	38.5 [36.9–40.1]	36.2 [34.1–38.2]	0.705 **	37.9 [36.2–39.5]	38.2 [35.7–40.6]	−0.095

Note. * small effect Cohen's d = 0.20; ** medium effect Cohen's d = 0.50. β: beta; EMM: estimated marginal mean; CI: confidence interval. β derived from general linear models adjusted for age, fat mass (%), lean mass (kg), height (cm), race and ethnicity (0 = non-Hispanic White, 1 = other race or ethnicity), and LOC-eating (0 = absent, 1 = present), as well as the respective intake variable (i.e., total energy intake, carbs, fat, protein) at baseline. The presented estimated marginal means for the effect of condition are from the models adjusted for LOC-eating and the LOC-eating × condition interaction term. Percentages were arcsin(sqrt)-transformed before analysis, so percentages do not add up to 100%. See Supplemental Materials for untransformed results.

3.4. $\Delta_{oscillatory\ power}$ during Unconscious Attention Capture (0–250 ms)

The effect of the smartphone program on change in oscillatory power during attention capture are reported in Table 4 and Figure 3. In "bottom-up" regions, there were small to medium effects of condition on the left pallidum (d = 0.307), left putamen (d = 0.447), caudal ACC ($d_{\text{left hemisphere}}$ = −0.569, $d_{\text{right hemisphere}}$ = −0.397), right rostral ACC (d = −0.235), lateral OFC ($d_{\text{left hemisphere}}$ = 0.291, $d_{\text{right hemisphere}}$ = 0.207), and left medial OFC (d = 0.237). In general, the AR group had an increase (or smaller decrease) in oscillatory power in regions of the striatum and OFC while the control group had a decrease in oscillatory power among these regions. Additionally, the AR group had a decrease (or smaller increase) in oscillatory power in the rostral ACC while the control group had an increase in oscillatory power in this region.

Outcome of within-group changes in neural activity and the interactive effects of LOC-eating x condition on changes in neural activity during attention capture are reported in the Supplemental Material and in Supplemental Table S1.

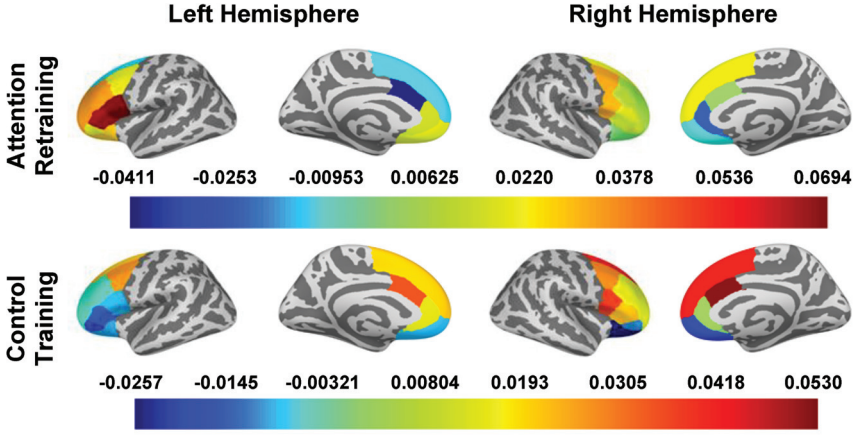

Figure 3. $\Delta_{\text{oscillatory power}}$ in Surface ROIs During Attention Capture (0–250 ms). Note. Estimated marginal means of change (post intervention − pre intervention) in beta band oscillatory power are presented for all a priori identified ROIs. To obtain oscillatory power estimates, we log transformed ratios (pseudo-Z oscillatory power in congruent trials/pseudo-Z oscillatory power in incongruent trials); therefore, estimated marginal means are unitless. The presented estimated marginal means for the effect of condition are from the models adjusted for LOC-eating and the LOC-eating × condition interaction term.

Table 4. Effects of treatment condition on Δoscillatory power during attention capture (0–250 ms following stimulus).

	Effect of Condition	**Control (n = 12)**	**AR (n = 14)**	**Effect Size**
ROI Sub-Regions	**β [95%CI]**	**EMM [95%CI] [a,b]**	**EMM [95%CI] [a,b]**	**Cohen's d**
Striatum				
Caudate-lh	0.047 [−0.025–0.119]	0.010 [−0.027–0.048]	0.021 [−0.014–0.056]	0.093
Caudate-rh	−0.002 [−0.085–0.082]	0.032 [−0.011–0.075]	0.023 [−0.017–0.063]	−0.066
Pallidum-lh	0.08 [−0.022–0.182]	−0.007 [−0.055–0.041]	0.038 [−0.006–0.083]	0.307 *
Pallidum-rh	0.013 [−0.086–0.113]	0.028 [−0.022–0.078]	0.009 [−0.037–0.056]	−0.121
Putamen-lh	0.083 [−0.001–0.167]	−0.013 [−0.056–0.03]	**0.046 [0.006–0.086]**	0.447 *
Putamen-rh	0.031 [−0.059–0.121]	0.017 [−0.029–0.063]	0.02 [−0.022–0.063]	0.020

Table 4. *Cont.*

	Effect of Condition	Control (n = 12)	AR (n = 14)	Effect Size
ROI Sub-Regions	**β [95%CI]**	**EMM [95%CI] [a,b]**	**EMM [95%CI] [a,b]**	**Cohen's *d***
ACC				
Caudal-lh	−0.042 [−0.149–0.065]	0.035 [−0.009–0.078]	**−0.041 [−0.081–−0.001]**	−0.569 **
Caudal-rh	−0.034 [−0.122–0.053]	**0.053 [0.011–0.095]**	0.002 [−0.037–0.041]	−0.397 *
Rostral-lh	0.042 [−0.041–0.126]	0.012 [−0.03–0.055]	0.012 [−0.027–0.051]	−0.003
Rostral-rh	0.021 [−0.06–0.102]	0.002 [−0.034–0.039]	−0.024 [−0.058–0.01]	−0.235 *
OFC				
Lateral-lh	**0.107 [0.03–0.185]**	−0.010 [−0.050–0.030]	0.026 [−0.011–0.063]	0.291 *
Lateral-rh	**0.092 [0.01–0.175]**	−0.026 [−0.065–0.014]	−0.001 [−0.037–0.036]	0.207 *
Medial-lh	0.046 [−0.032–0.123]	−0.010 [−0.048–0.029]	0.018 [−0.017–0.054]	0.237 *
Medial-rh	0.041 [−0.03–0.112]	−0.018 [−0.053–0.018]	−0.011 [−0.044–0.022]	0.062
dlPFC				
Caudal-lh	−0.037 [−0.125–0.052]	0.026 [−0.006–0.058]	0.02 [−0.01–0.049]	−0.068
Caudal-rh	0.001 [−0.091–0.093]	0.027 [−0.010–0.065]	0.031 [−0.003–0.066]	0.037
Rostral-lh	0.065 [−0.001–0.132]	−0.006 [−0.040–0.029]	**0.033 [0.001–0.066]**	0.365 *
Rostral-rh	0.023 [−0.055–0.101]	0.014 [−0.018–0.046]	0.008 [−0.021–0.038]	−0.061
Superior-lh	−0.032 [−0.116–0.051]	0.021 [−0.004–0.046]	−0.013 [−0.036–0.01]	−0.438 *
Superior-rh	−0.012 [−0.078–0.053]	**0.041 [0.017–0.064]**	0.019 [−0.002–0.041]	−0.294 *
vlPFC				
Pars opercularis-lh	**0.089 [0.004–0.174]**	−0.010 [−0.048–0.029]	**0.069 [0.034–0.105]**	0.672 **
Pars opercularis-rh	−0.016 [−0.113–0.081]	0.037 [−0.007–0.08]	0.031 [−0.009–0.071]	−0.045
Pars orbitalis-lh	**0.129 [0.049–0.209]**	−0.019 [−0.060–0.023]	0.019 [−0.019–0.058]	0.299 *
Pars orbitalis-rh	**0.126 [0.045–0.207]**	−0.011 [−0.053–0.030]	0.016 [−0.023–0.055]	0.214 *
Pars triangularis-lh	**0.115 [0.027–0.203]**	−0.015 [−0.059–0.029]	**0.064 [0.024–0.105]**	0.589 **
Pars triangularis-rh	0.037 [−0.053–0.127]	0.022 [−0.020–0.063]	0.016 [−0.023–0.054]	−0.047

Note. * small effect Cohen's d = 0.20; ** medium effect Cohen's d = 0.50.. ROI: region of interest; β: beta; EMM: estimated marginal mean; AR: attention retraining; -lh: left hemisphere; -rh: right hemisphere; ACC: anterior cingulate cortex; OFC: orbitofrontal cortex; dlPFC: dorsolateral prefrontal cortex; vlPFC: ventrolateral prefrontal cortex. Estimated marginal mean represents the group-level mean change score (post intervention − pre intervention) of beta band power. [a] A decrease in power corresponds to an increase in activity. Thus, a negative estimated marginal mean reflects increased activity in that brain region post-intervention. Thus, a negative estimated marginal mean reflects increased activity in that brain region post-intervention. [b] A positive estimated marginal mean reflects decreased activity in that brain region post-intervention. Estimated marginal means with a 95%CI that does not contain 0 are in bolded font. Linear mixed models were adjusted for stimuli pairing (HF-NF, LF-NF, HF-LF), age, fat mass (kg) and height (cm) at pre-intervention, race and ethnicity (0 = non-Hispanic White, 1 = other race or ethnicity), and LOC-eating (0 = absent, 1 = present), and included a LOC-eating × condition interaction term.

3.5. $\Delta_{oscillatory\ power}$ during Attention Deployment (250–500 ms)

The main effects of the smartphone program on the change in oscillatory power during attention deployment are reported in Table 5 and Figure 4. In "top-down" regions, there were small to large effects of condition on the caudal ACC (d left hemisphere = −0.817, d right hemisphere = −0.594), left dlPFC (d caudal = −0.228, d rostral = −0.472, d superior = −0.286), and left vlPFC (d pars opercularis = −0.802, d pars orbitalis = −0.545, d pars triangularis = −0.805). The AR group had a decrease in oscillatory power among these regions, except for the right pars orbitalis, where increased oscillatory power was observed. The control group had an increase in oscillatory power in these regions, except for in the left pallidum and right pars orbitalis, where decreased oscillatory power was observed.

Table 5. Effects of treatment condition on Δoscillatory power during attention deployment (250–500 ms following stimulus).

	Effect of Condition	**Control (*n* = 12)**	**AR (*n* = 14)**	**Effect Size**
ROI Sub-Regions	**β [95%CI]**	**EMM [95%CI] [a,b]**	**EMM [95%CI] [a,b]**	**Cohen's *d***
Striatum				
Caudate-lh	−0.021 [−0.092–0.05]	0.012 [−0.025–0.049]	**−0.052 [−0.086–−0.017]**	−0.561 **
Caudate-rh	−0.013 [−0.095–0.069]	0.030 [−0.013–0.073]	−0.031 [−0.07- 0.009]	−0.464 *
Pallidum-lh	−0.005 [−0.103–0.092]	−0.011 [−0.058–0.036]	**−0.051 [−0.095–−0.008]**	−0.281 *
Pallidum-rh	0.02 [−0.069–0.109]	0.037 [−0.009–0.084]	−0.026 [−0.069–0.017]	−0.445 *
Putamen-lh	−0.01 [−0.099–0.08]	<0.001 [−0.040–0.041]	**−0.062 [−0.10–−0.025]**	−0.505 **
Putamen-rh	0.013 [−0.069–0.095]	0.018 [−0.024–0.061]	−0.031 [−0.07–0.009]	−0.375 *
ACC				
Caudal-lh	−0.041 [−0.123–0.041]	0.026 [−0.012–0.064]	**−0.069 [−0.104–−0.033]**	−0.817 ***
Caudal-rh	−0.038 [−0.142–0.066]	**0.058 [0.011–0.105]**	−0.028 [−0.072–0.016]	−0.594 **
Rostral-lh	−0.023 [−0.105–0.059]	−0.005 [−0.048–0.038]	−0.03 [−0.07–0.01]	−0.189
Rostral-rh	−0.057 [−0.14–0.027]	0.032 [−0.010–0.074]	−0.012 [−0.051–0.027]	−0.341 *
OFC				
Lateral-lh	0 [−0.091–0.091]	0.021 [−0.022–0.063]	**−0.062 [−0.102–−0.023]**	−0.641 **
Lateral-rh	−0.01 [−0.092–0.071]	0.012 [−0.029–0.052]	0.002 [−0.035–0.04]	−0.075
Medial-lh	−0.013 [−0.101–0.076]	0.018 [−0.023–0.058]	**−0.038 [−0.076–−0.001]**	−0.454 *
Medial-rh	−0.033 [−0.11–0.043]	**0.041 [0.001–0.080]**	−0.009 [−0.046–0.028]	−0.404 *
dlPFC				
Caudal-lh	0.011 [−0.069–0.091]	0.016 [−0.024–0.056]	−0.012 [−0.049–0.025]	−0.228 *
Caudal-rh	0.054 [−0.037–0.146]	0.005 [−0.041–0.050]	0.006 [−0.036–0.048]	0.008
Rostral-lh	0.002 [−0.061–0.065]	0.015 [−0.017–0.047]	**−0.031 [−0.061–−0.002]**	−0.472 *
Rostral-rh	0.032 [−0.05–0.115]	0.010 [−0.024–0.044]	−0.006 [−0.037–0.026]	−0.149
Superior-lh	−0.016 [−0.075–0.042]	0.004 [−0.025–0.033]	−0.022 [−0.049–0.005]	−0.286 *
Superior-rh	0.03 [−0.039–0.099]	0.023 [−0.009–0.055]	0.01 [−0.02–0.039]	−0.138
vlPFC				
Pars opercularis-lh	−0.016 [−0.097–0.066]	**0.043 [0.003–0.082]**	**−0.054 [−0.09–−0.017]**	−0.802 ***
Pars opercularis-rh	0.053 [−0.051–0.156]	0.001 [−0.037–0.039]	−0.018 [−0.053–0.017]	−0.163
Pars orbitalis-lh	0.015 [−0.061–0.091]	0.013 [−0.026–0.053]	**−0.052 [−0.089–−0.016]**	−0.545 **
Pars orbitalis-rh	0.036 [−0.047–0.119]	−0.027 [−0.067–0.012]	0.023 [−0.014–0.06]	0.412 *
Pars triangularis-lh	−0.017 [−0.092–0.057]	0.023 [−0.015–0.062]	**−0.071 [−0.107–−0.036]**	−0.805 ***
Pars triangularis-rh	0.07 [−0.019–0.159]	−0.009 [−0.055–0.037]	0.013 [−0.03–0.056]	0.156

Note. * small effect Cohen's d = 0.20; ** medium effect Cohen's d = 0.50; *** large effect Cohen's d = 0.80. ROI: region of interest; β: beta; EMM: estimated marginal mean; AR: attention retraining; -lh: left hemisphere; -rh: right hemisphere; ACC: anterior cingulate cortex; OFC: orbitofrontal cortex; dlPFC: dorsolateral prefrontal cortex; vlPFC: ventrolateral prefrontal cortex. Estimated marginal mean represents the group-level mean change score (post intervention − pre intervention) of beta band power. [a] A decrease in power corresponds to an increase in activity. Thus, a negative estimated marginal mean reflects increased activity in that brain region post-intervention. [b] A positive estimated marginal mean reflects decreased activity in that brain region post-intervention. Estimated marginal means with a 95%CI that does not contain 0 are in bolded font. Linear mixed models were adjusted for stimuli pairing (HF-NF, LF-NF, HF-LF), age, fat mass (kg) and height (cm) at pre-intervention, race and ethnicity (0 = non-Hispanic White, 1 = other race or ethnicity), and LOC-eating (0 = absent, 1 = present), and included a LOC-eating × condition interaction term.

The outcome of within-group changes in neural activity and the interactive effects of LOC-eating × condition on changes in neural activity during attention deployment are reported in the Supplemental Material and in Supplemental Table S2.

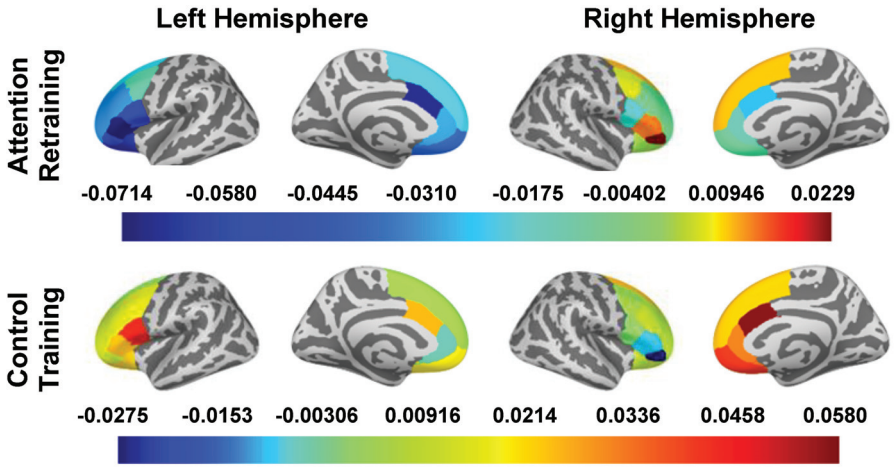

Figure 4. $\Delta_{\text{oscillatory power}}$ in Surface ROIs During Attention Deployment (250–500). Note: Estimated marginal means of change (post intervention − pre intervention) in beta band oscillatory power are presented for all a priori identified ROIs. To obtain oscillatory power estimates, we log transformed ratios (pseudo-Z oscillatory power in congruent trials/pseudo-Z oscillatory power in incongruent trials); therefore, estimated marginal means are unitless. The presented estimated marginal means for the effect of condition are from the models adjusted for LOC-eating and the LOC-eating × condition interaction term.

3.6. Adverse Events

All participants completed the smartphone program (AR or control) they were randomized to complete. No adverse events were reported during completion of the smartphone program. However, adverse events occurred during the MEG scans: nausea or vomiting (three participants in the AR group), headache (one participant in the control group and one participant in the AR group), and sore neck (one participant in the control group).

4. Discussion

This pilot, double-blind, randomized control trial investigated whether a two-week long, smartphone-delivered AR program (versus a control program) altered food AB in adolescent girls with overweight or obesity. Our findings provide preliminary support for the potential effectiveness of an intensive, smartphone-delivered food AR program to alter neural activity associated with attention processing and changes in eating behavior. Specifically, our AR program did not promote significant changes in AB as measured by a food-cue visual probe task, but produced detectable changes in neural correlates of attention capture and deployment. The observed changes in neural activity broadly indicate a slightly blunted initial reward response, reduced stimulus-driven attention, and increased goal-directed attention and action control. The AR program also promoted less carbohydrate intake in the laboratory. However, greater fat and protein intake as a percentage of energy offset this reduction and resulted in slightly greater total energy intake. However, no changes in energy intake were clinically significant.

We observed no statistically significant change in (reaction time-based) food AB scores among girls who completed the AR or control programs. These results are contrary to our hypothesis and reported outcomes from prior food-based AR interventions [37]. Although reaction time scores are the most common outcome measure of AR programs [37,72], they have several limitations, including reliance on discrete events (when a stimulus is shown) rather than continuous assessment of shifts in attention [73]; potential influence by non-attention specific processes, such as response execution [74]; and poor psychometric reliability and stability [75,76], which likely introduced noise into our reaction time

assessments. To improve outcome measurement of food AR programs, future studies could employ multiple approaches to assess attention processes, such as using a behavioral task and eye tracking, as well as computational modeling [74,77].

Following completion of the smartphone program there was a modest difference (~71 kcal) in energy intake and fat intake (2.4%) between the AR and control groups. The difference in energy intake was opposite to what we hypothesized. Our total energy intake results are consistent with results from other food AB programs, which have failed to consistently produce improvements in appetite and eating behavior [37]. However, consistent with hypotheses, we observed a lesser consumption of percentage intake from carbohydrates and a greater percentage of energy intake from protein among the AR group compared to the control group. Exploratory analyses showed that girls in the control condition with LOC-eating consumed more carbohydrates and lesser protein than their counterparts without LOC-eating, which is consistent with the phenotypic eating behavior of adolescents with LOC-eating [46,78]. However, energy and macronutrient intake did not differ between girls with and without LOC-eating who completed the AR program. The observed pattern of macronutrient intake among the AR group, regardless of LOC-eating status, suggest our AR program might produce increased control of energy intake by promoting foods higher in protein and lower in carbohydrates, rather than reducing self-served portion sizes. Studies with longer follow-up periods and are needed to determine whether these changes are generalizable to eating episodes that occur in naturalistic environments.

We observed changes in brain regions among girls that completed the AR program that are suggestive of reduced biases in attentional processing. Among the AR group during attention capture, there were no changes in activity in the caudate, pallidum, ventral ACC or OFC, but there was decreased engagement of the left putamen. The putamen is a region of the striatum associated with stimulus-reward associations [79]. Consistent with our hypotheses, we observed more robust changes among the AR group during attention deployment. Specifically, among the AR group (compared to the control group) we observed (1) relatively lower engagement in regions of the vlPFC and OFC and (2) increased engagement of the pallidum and OFC. These changes are suggestive of greater direction of attention towards goal-related stimuli [80–82], stimulus–outcome action learning [83,84], and goal-directed decision making [85,86]. These findings are particularly promising because the opposite pattern of activation in these areas has been associated with having a higher BMI and/or future increases in BMI [11]. Interestingly, although attention processes are thought to be lateralized to the right hemisphere of the brain [87,88], most of the changes among our AR group occurred in the left hemisphere of the brain. Greater engagement in left hemisphere might support attentional control by promoting greater goal-driven and object-based orienting of attention [89,90]. Therefore, the AR program appears to have largely bolstered the engagement of attention support systems.

The effects of the AR program were somewhat different among girls with and without LOC-eating. During attention capture, girls in the AR condition with LOC-eating experienced minimal to no change in oscillatory power in any ROI. Thus, the observed changes in neural activity among girls who completed the AR program appeared to be driven by girls without LOC-eating. However, there was no clear pattern for the effect of LOC-eating on neural outcomes during attention deployment for the AR group. Changes in brain regions associated with reward-related motivation [91,92], stimulus–outcome action learning [83,84], and stimulus-directed reorienting [85,93] seem to be driven by girls with LOC-eating. Alternatively, changes in goal-directed attention [86] might be driven by girls without LOC-eating. Changes in impulsivity and attentional switching [80,81] were observed in both girls with and without LOC-eating. The pattern of results might suggest that girls with LOC-eating have greater difficulty suppressing reward response and stimulus-driven attention compared to girls without LOC-eating. These differences in changes in neural activity did not correspond to differences in AB reaction time scores or energy intake. Thus, there may be different neural pathways for reducing the impact of

food AB on energy intake among people with and without LOC-eating. Studies with larger samples of girls with LOC-eating are needed to bolster support for this interpretation.

Consistent with our hypotheses, the control smartphone program produced minimal to no changes in neural activity. Following completion of the smartphone program, the control group had no changes in neural activity associated with reward valuation or responsivity during attention capture. During attention deployment, the control group had decreased engagement in some brain regions associated with direction of attention towards goal-related stimuli [82], and an updating of stimulus–reward associations [83,84] and goal-driven attention and action selection [23,94,95]. Additionally, exploratory interaction analyses revealed that girls in the control condition with LOC-eating had lower engagement in most ROIs during both attention capture and deployment. The observed decreases in inhibition and goal-oriented attention were likely driven by girls with recent LOC-eating during attention deployment. Thus, in the absence of any AR intervention, girls with LOC-eating are likely to continue to exhibit a greater vulnerability to food AB.

In general, findings from this study provide tentative support for Attentional Control models of AB modification [33]. Attentional Control Models assert that observed reductions in inhibitory control and increased goal-oriented action, regardless of reward responsivity, promote decreased AB [33], and subsequently would improve energy intake. Alternatively, Valence-Specific Models suggest blunting of the reward response drives reductions in AB and associated behaviors [33]. Response patterns among the AR group seem to provide more support for Action Control models. Specifically, during attention capture we observed reduced engagement only in the left putamen, and during attention deployment we saw increased engagement in regions of the striatum, ventral ACC, and OFC. Additionally, the response pattern observed among girls who completed the control program provides additional support for Attentional Control Models. The control group experienced a decreased engagement in some brain regions associated with reward responsivity and inhibitory control. Girls in the control group also consumed a greater percentage of energy from carbohydrates and less from protein during a laboratory meal than the AR group following completion of the smartphone program. This pattern is in contrast with Valence-Specific Models, but consistent with Attention Control models. Some recent research among adults also supports the notion that increasing attentional control (e.g., through cognitive reappraisal) reduces food AB [96]. However, additional research is needed to map theory and neural mechanisms of change onto behavioral outcome from food AR programs.

This study has several strengths. We recruited a sample of racially and ethnically diverse girls, potentially increasing the generalizability of findings to diverse populations. LOC-eating was assessed by a validated interview and energy consumption with an in-laboratory feeding paradigm. We used DXA to assess body composition, which is a more appropriate measure of adiposity than BMI [97]. We also used MEG, an ideal neuroimaging methodology for measuring the minute temporal changes that underly attention processing [62,98]. The high temporal acuity of MEG scans allowed us to gain a more in-depth understanding of food AB processes by disaggregating magnetic field changes during attention capture and deployment phases. A limitation of this study is its small sample size, such as that of the participants who responded positively regarding occurrences of LOC-eating. Additionally, participants who provided complete data for MEG analyses were, on average, older than participants who were not included in MEG analyses. A large percentage of MEG data were missing due to head motion, adverse events (e.g., feeling sick), and technical issues during data collection; common challenges experienced when collecting MEG scans of young people [99,100].

5. Conclusions

Findings from this pilot double-blind randomized clinical trial provide preliminary evidence that a 2-week smartphone AR program promoted changes in food choices leading to a decreased percentage of carbohydrate intake during a laboratory meal designed to induce LOC-eating. This change may be due to alterations in neural activity consistent

with blunted reward responsivity during unconscious attention capture and increased attentional control and goal-directed action during attention deployment towards food cues. This preliminary study increases our understanding of the mechanisms involved in the association between food AB and aberrant eating behavior. However, additional research with larger samples is needed to demonstrate the validity of our findings and determine the optimal frequency and duration of food AR programs to maximize positive outcomes and minimize burdens.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nu16203456/s1, Figure S1: Schematic of the food-cue visual probe task; Table S1: Unadjusted energy intake prior to and following completion of the smartphone program; Table S2: Interactive effects of condition and LOC-eating on $\Delta_{\text{oscillatory power}}$ during attention capture (0–250 ms following stimulus); Table S3: Interactive effects of condition and LOC-eating on $\Delta_{\text{oscillatory power}}$ during attention deployment (250–500 ms following stimulus).

Author Contributions: M.T.-K., J.A.Y., A.J.W. and M.M.S. designed the study. S.M.B., B.F.B., S.A.T., S.B.Y. and M.E.B. collected data. M.N.P. managed the dataset, preprocessed the neuroimaging data, and conducted statistical analyses. A.C.N. oversaw the collection of the MEG data. J.D.S. supervised the analysis of neuroimaging data. K.Y.C. supervised collection of dual-energy X-ray absorptiometry data. N.A.S. performed randomization procedures. M.N.P. and B.F.B. prepared the first draft of the manuscript, tables, and figures. M.T.-K. and J.A.Y. completed substantial editing of the manuscript and supervised the project. M.T.-K. and J.A.Y. had primary responsibility for final content. All authors edited the manuscript and approved the final draft submitted. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of The National Institutes of Health protocol 17-CH-0014, approved 17 November 2016, and is registered at clinicaltrials.gov as NCT02977403.

Informed Consent Statement: Prior to data collection, informed assent and consent was obtained from all subjects and their parent/legal guardian, respectively.

Data Availability Statement: Data are available at https://github.com/Yanovski-Lab/AttentionRetraining_2weekOutcomes.git (published on 19 July 2024).

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Continuous Glucose Monitor Metrics That Predict Neonatal Adiposity in Early and Later Pregnancy Are Higher in Obesity Despite Macronutrient-Controlled Eucaloric Diets

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Abstract: Background: Fasting glucose is higher in pregnancies with obesity (OB); less is known about postprandial (PP) and nocturnal patterns when the diet is eucaloric and fixed or about the continuous-glucose-monitor (CGM) metrics that predict neonatal adiposity (NB%fat). We hypothesized that continuous glucose monitors (CGMs) would reveal higher glycemia in OB vs. normal weight (NW) during *Early* (14–16 weeks) and *Later* (26–28 weeks) gestation despite macronutrient-controlled eucaloric diets and elucidate unique predictors of NB%fat. Methods: In a prospective, parallel-group comparative study, a eucaloric diet (NW: 25 kcal/kg; OB: 30 kcal/kg) was provided (50% carbohydrate [20% simple/30% complex; of total calories], 35% fat, 15% protein) to Early and Later gestation groups wearing a blinded CGM for three days. CGM metrics (mean fasting; 1 h and 2 h PP; daytime and nocturnal glucose; percent time-in-range (%TIR: 63–140 mg/dL); PP excursions; and area-under-the-curve [AUC]) were interrogated between groups and as predictors of NB%fat by dual X-ray absorptiometry(DXA). Results: Fifty-four women with NW (BMI: 23 kg/m^2; n = 27) and OB (BMI: 32; n = 27) provided their informed consent to participate. Early, the daytime glucose was higher in OB vs. NW (mean ± SEM) (91 ± 2 vs. 85 ± 2 mg/dL, p = 0.017), driven by 2 h PP glucose (95 ± 2 vs. 88 ± 2, p = 0.004). Later, those with OB exhibited higher nocturnal (89 ± 2 vs. 81 ± 2), daytime (95 ± 2 vs. 87 ± 2), 1 h (109 ± 3 vs. 98 ± 2), and 2 h PP (101 ± 3 vs. 92 ± 2) glucose (all $p < 0.05$) but no difference in %TIR (95–99%). Postprandial peak excursions for all meals were markedly blunted in both the Early (9–19 mg/dL) and Later (15–26 mg/dL). In OB, the Later group's 24 h AUC was correlated with NB%fat (r = 0.534, p = 0.02). Despite similar weight gain, infants of OB had higher birthweight (3528 ± 107 vs. 3258 ± 74 g, p = 0.037); differences in NB%fat did not reach statistical significance (11.0 vs. 8.9%; $p > 0.05$). Conclusions: Despite macronutrient-controlled eucaloric diets, pregnancies with OB had higher glycemia Early and Later in gestation; the Later 24 h glucose AUC correlated with NB%fat. However, glycemic patterns were strikingly lower than current management targets.

Keywords: pregnancy; obesity; diet; eucaloric; CGM; glucose metrics; infant adiposity

1. Introduction

The high prevalence of obesity in young women continues to challenge the landscape of pregnancy and obstetric care. In 2015, the American College of Obstetricians and Gynecologists recognized maternal obesity as the most common threat to health in women of reproductive age [1]; its impact on short-term and particularly long-term offspring health is acknowledged and requires further elucidation. Although typically associated with maternal diabetes, large-for-gestational-age (LGA) infants are most common in pregnancies affected by obesity outside of diabetes [2,3]. Even more predictive than LGA or birthweight (BW) of child obesity risk is infant body composition, particularly neonatal adiposity (%NB fat) [4]. Elevated pre-pregnancy BMI, excessive gestational weight gain, and exacerbated maternal insulin resistance associated with heightened exposure to excess glucose and lipids have been implicated in obesity-associated fetal macrosomia [5].

We previously reported normative data on patterns of glycemia in normal pregnancy by systematic review [6], and subsequently demonstrated using continuous glucose monitoring (CGM) that pregnant women with obesity (OB) had higher 24 h patterns of glycemia compared to normal-weight (NW) controls both ad libitum and when their diet was controlled, although analysis on each diet was limited to 24 h [7]. It was surprising that despite the higher patterns of glycemia in OB, the strongest predictor of NB%fat was fasting triglycerides (TG) at 14 weeks gestation, and fasting free fatty acids (FFA) at 28 weeks. In this new cohort, when the dietary macronutrient content was controlled and calories fixed according to maternal BMI during all metabolic testing, we previously reported that independent of glucose, 1 h and 2 h postprandial (PP) TGs at 14–16 weeks gestation were, in fact, the strongest predictors of NB%fat [8].

This study addresses the long-believed posit that women with OB have higher 24 h glycemia, especially apparent with CGM technology, and this may explain higher BW, LGA, and adiposity. However, whether differences in CGM metrics are due to the OB vs. NW metabolic phenotype or differences in diet composition or caloric intake is a subject of continued debate and has not been clearly delineated. In a highly controlled prospective cohort study, we set out to characterize phenotypic differences in maternal glucose and lipid metabolism between NW and OB pregnancies using tightly controlled gestational windows, 72 h of controlled eucaloric diets, and CGM metrics that have been associated with fetal overgrowth in diabetes and obesity [9]. Because NB%fat is viewed as a sensitive marker of intrauterine nutrient exposure [4] and better-predicts childhood obesity than BW [10], dual X-ray absorptiometry (DXA) was employed to measure NB%fat in infants and in women post-delivery. We tested the hypothesis that CGM metrics would reveal higher fasting and PP glucose (primary outcome) in women with OB vs. NW in *Early* (14–16 weeks) and *Later* (26–28 weeks) gestation despite controlled diets, and unique glucose metrics by CGM would add to prediction models of NB%fat that included maternal TG.

2. Materials and Methods

This was an NIH-funded prospective trial (R56 DK078645; R01 DK078645). Results from the primary analysis characterizing TG patterns during liquid-breakfast test meals between OB and NW groups and their role in predicting fetal fat accretion are reported elsewhere [8]. The analyses herein were planned a priori with the goal of ascertaining differences in CGM metrics on 3 days of a provided, macronutrient- and calorie-controlled diet and their prediction of NB%fat. The study was approved by the Colorado Multiple Institutional Review Board (COMIRB, #07-0535); all women gave their written informed consent. Persons who self-identified as women and were pregnant (18–35 years') and their infants were studied at University of Colorado Hospital from 2009 to 2017. Fifty-four healthy English-speaking women with singleton pregnancies were enrolled (n = 27 NW; pre-pregnancy BMI 20–26 kg/m^2 and n = 27 OB; pre-pregnancy BMI 30–38 kg/m^2). To minimize the risk of growth restriction or preterm birth that would confound measures of infant body composition, all chronic medical conditions (i.e., hypertension, HIV, cardiac dysfunction) were exclusions. The women with OB were screened for glucose intolerance

before enrollment [11] and were not included if they failed an early 100 g glucose tolerance test (OGTT). The term "women" is used in this manuscript because all of the participants identified themselves as such. The use of the term "women or maternal" in this manuscript is intended to be used inclusively to respect previously published reports.

Women were studied both *Early* (14–16 weeks) and *Later* (26–28 weeks) during pregnancy. They wore a CGM for 72 h while consuming a eucaloric diet matched for macronutrient composition. Three women with OB could not be studied *Later* due to gall bladder disease, pregnancy loss, or relocation. At 28 weeks, both groups underwent a 100 g OGTT to diagnose gestational diabetes (GDM) [11]; glucose and insulin were measured at baseline/fasting, 1, 2, and 3 h for insulin sensitivity estimates using assays previously reported [7,8]. Those who met the diagnostic criteria for GDM were excluded from this analysis. Only term ($\geq$37 weeks), healthy NB were included in the final NB%fat analysis, given that fetal fat accretion was rapid near term. One NB from an NW mother was excluded due to pre-term delivery. Exclusions and birth complications were previously reported [8]; 26 NW and 19 OB offspring were included for term NB%fat analysis.

Macronutrient-controlled eucaloric diets: Ad libitum dietary fat and carbohydrate markedly affect glucose and lipids [12] and would be expected to confound the comparison of CGM metrics between groups. Thus, women were provided with standardized diets prepared by the Colorado Clinical Translational Science Institute (CCTSI) Bionutrition kitchen for 3 days while wearing a CGM. The 3-day diets for women with OB and NW were matched for calories and macronutrients: 50% carbohydrate (30% complex/20% simple carbohydrates; of total calories); 35% fat (12% saturated/12% monounsaturated/11% polyunsaturated); 15% protein. Energy requirements were based on the Institute of Medicine guidelines (OB: 25 kcal/kg; NW: 30 kcal/kg). During standard time periods, women consumed 25% of calories at breakfast (between 0600 and 1000), 30% at lunch (1100–1400), 35% at dinner (1700–2000), and 10% as a bedtime snack (after 2000). CGM metric analyses were completed within tight gestational windows of 14–16 and 26–28 weeks due to the progressive insulin resistance of pregnancy.

Other biochemical measures: The collection of fasting TG and PPTG data was previously reported [8] and were used in the regression analysis to determine if CGM metrics added predictive value to TGs in the relationship with NB%fat. Maternal insulin resistance (IR) from the OGTT was estimated using the product of glucose and insulin area-under-the-curve (AUC) [8] using the 28 week, 3 h, 100 g OGTT.

Blinded Continuous Glucose Monitoring: Interstitial glucose was initially measured using CGMS GOLD (Medtronic MiniMed, Symlar, CA, USA), followed by wireless iPro®1 and then iPro®2 (Medtronic MiniMed) when CGMS GOLD was no longer supported. Data procedures were applied with extraction of pregnancy-relevant glucose variables as previously described [13]. 24 h %time-in-range (%TIR) was defined as 63–140 mg/dL [14]. All CGM measures represent an average over 48–72 h during controlled diet. Despite precautions to avoid lost data, 3 NW participants did not have CGM data at 14 weeks, and 2 different participants did not have data at 28 weeks (n = 24 and n = 25 evaluable cases, respectively). In the OB cohort, there were n = 24 evaluable cases at 14 weeks (gallstones exclusion, 2 cases sensor malfunction) and n = 23 cases at 28 weeks (gallstones, intrauterine fetal demise, relocation, sensor malfunction).

Physical Activity: Physical activity was assessed at 14 and 28 weeks using the validated 36-item Pregnancy Physical Activity Questionnaire (PPAQ; One-week test–retest reliability demonstrated by intraclass correlations of 0.78–0.93) [15]. The women were asked not to vigorously exercise while CGMS was worn.

Delivery Outcomes and Maternal and Newborn Adiposity: Delivery outcomes including delivery type, complications, birth length, and BW were extracted from the medical record. Ponderal Index was calculated (weight [kg]/height^3). Forty-five term NB underwent dual energy X-ray absorptiometry (DXA) at Children's Hospital Colorado at ~2 weeks (mean 15.6 days, range = 12–20 days; QDR Discovery fan beam densitometer, Hologic Delphi-W, Hologic Inc., Waltham, MA, USA; Apex version 3.2 software), as described

previously [8,16]. The DXA was performed at 2 weeks because of the expected newborn diuresis affecting total body water in the first week of life and the return of fat mass by 7 to 14 days [16,17]. In 2 NB, the DXA revealed a calibration error; for these measures, we applied a regression equation based on our previous data [16] to predict the NB%fat as previously described [8]. On the same day as the neonatal DXA, a maternal DXA was also performed (Hologic Delphi-W, Hologic Inc., Waltham, MA, USA).

Power Analysis: Power was calculated a priori (PASS 2005 software, Kaysville, UT, USA) to test the hypothesis that pregnancies affected by OB have higher fasting and postprandial glucose in *Early* and *Later* gestation. Based on our NIH-funded pilot study (R56 DK078645), 15 women/group would detect a between-group fasting-glucose difference of 6 ± 4.6 mg/dL (SD) *Early* and 14 ± 9.7 mg/dL *Later* for 84–91% power ($\alpha = 0.01$) using a 2-sided/2-sample *t*-test. To detect a clinically meaningful 15 mg/dL difference in postprandial glucose in the NW vs. OB participants ($\alpha = 0.05$, $1 - \beta = 0.8$), 12 women per group were required.

Statistical Analyses: Area-under-the-curve (AUC) was calculated to represent total potential fetoplacental nutrient exposure [13] for CGM measures to characterize patterns of glycemia. Data are presented as mean ±SEM; between-group [OB minus NW] and within-group differences [*Later* minus *Early*] with 95% confidence intervals (CI) are provided for CGM and OGTT variables. All variables approximated a normal distribution with the exception of plasma insulin, which was log-transformed for analysis. Between-group differences were assessed using *t*-tests for independent groups and within-group differences by paired *t*-tests for primary and secondary outcomes. Correlations were assessed using Pearson's *r.* For this analysis, multivariate regression models were constructed to include TG, maternal characteristics (maternal BMI at delivery, and %BF after delivery), insulin sensitivity derived from OGTT measures, and CGM metrics that demonstrated a between-group difference. Multiple and univariate linear regression were used to test for predictive associations (IBM SPSS Statistics v24, Armonk, NY, USA). For these analyses, $p < 0.05$ was considered statistically significant.

3. Results

Maternal and Newborn Characteristics: Fifty-four participants (27 per group) were studied during gestational week 15.9 ± 0.2 (*Early*) and 27.8 ± 0.1 (*Later*). Participants with NW and OB were similar in age, gravida, and were mostly Caucasian (Table 1). By design, participants with OB had a significantly higher BMI; however, gestational weight gain was similar between maternal BMI groups. There were no between-group differences in physical activity either *Early* or *Later* [8], as previously reported. The cohort with OB had a higher percentage of cesarean deliveries. All included infants were healthy and born at term (39.7 weeks, both groups). Infants born to mothers with OB had a significantly higher BW compared to those born to NW mothers (3528 g vs. 3258 g, $p = 0.037$, respectively). There was a higher percentage of females born to NW vs. OB (50% vs. 30%, $p > 0.05$). Although there was a trend for NB%fat at 2 weeks to be higher in neonates of those with OB vs. NW, the difference did not meet statistical significance (11.0 vs. 8.9%, $p > 0.05$). At 2 weeks postpartum, women with OB had significantly higher %fat compared to NW (41% vs. 33%, respectively, $p < 0.0001$).

Group Differences in Patterns of Glycemia by CGM: Table 2 and Figure 1 show differences in patterns of glycemia between maternal groups *Early* and *Later* in gestation. On the eucaloric diets, there were no between-group differences in fasting glucose by CGM *Early* or *Later* in gestation. Our hypothesis was largely supported by a pattern of higher PP glucose responses in women with OB both *Early* and *Later*. This was true across individual meals and as an average across 1 h and 2 h PP glucose. In Later gestation, women with OB averaged a 10 mg/dL statistically higher 1 h and 2 h PP glucose across meals (Table 2) compared to NW.

Table 1. Maternal biochemical characteristics during *Early* (14–16 weeks) and *Later* (26–28 weeks) gestation, delivery, and postpartum. *p*-values are for between-group comparisons at the same timepoint (NS = $p > 0.05$). Data are mean ± SEM. For OGTT variables, between-group [OB minus NW] and within-group differences [*Later* minus *Early*] with 95% confidence intervals (CI) are provided.

	NW (*n* = 27)		**Obese (*n* = 27)**		**NW vs. OB, Same Time Point *p*-Value Difference [95% CI]**
Baseline Maternal Characteristics					
Age (years)	31 ± 0.6		30 ± 0.8		NS
Pre-pregnancy BMI (kg/m^2)	22.3 ± 0.3		32.0 ± 0.6		<0.0001
Primigravida (%total)	52		41		NS
Caucasian (%total)	93		93		NS
Early and *Later* Maternal Measures	*Early*	*Later*	*Early*	*Later*	
Fasting TG, mg/dL *	89.2 ± 3.98	135.1 ± 7.8	126.2 ± 8.7 †	174.9 ± 12.2 ‡	-
1 h PP TG, mg/dL *	95.3 ± 4.6	153.2 ± 8.0	143.4 ± 10.8 †	201.2 ± 13.3 ‡	-
2 h PP TG, mg/dL *	86.6 ± 5.2	137.9 ± 8.1	135.3 ± 10.7 †	189.1 ± 13.1 †	-
100 g OGTT, 28 weeks					
Fasting glucose, mg/dL		77.3 ± 1.2		83.0 ± 1.4	0.004 5.54 [1.89, 9.20]
1 h glucose		121.0 ± 5.7		144.0 ± 3.9	0.002 22.85 [8.74, 36.96]
2 h glucose		104.0 ± 3.5		120.1 ± 5.0	0.01 16.24 [4.14, 28.35]
3 h glucose		90.0 ± 3.9		98.0 ± 5.1	NS 8.09 [−4.83, 21.00]
3 h glucose AUC, mg × dL/h		18,616 ± 536		21,243 ± 540	0.001 2626 [1095, 4157]
Fasting insulin, uIU/L		11.1 ± 1.0		19.8 ± 2.0	0.01 8.60 [4.33, 12.87]
1 h insulin		98.0 ± 18.0		136.4 ± 13.4	NS 38.75 [−6.58, 84.08]
2 h insulin		80.0 ± 7.5		118.4 ± 18.1	NS 38.80 [0.40, 77.19]
3 h insulin		46.0 ± 6.6		76.4 ± 11.2	NS 30.42 [4.71, 56.12]
3 h insulin AUC, uIU × h/L		12,479 ± 1471		18,173 ± 1891	NS 5694 [918, 10,470]
GlucoseAUC × InsulinAUC		248,118,444 ± 33,167,536		396,303,263 ± 47,676,620	0.013 148,184,819 [32,145,562, 264,224,075]
Delivery and Postpartum					
Gestational weight gain, kg	13.7 ± 0.8		14.2 ± 1.6		NS
Gestational Age at Delivery, weeks	39.7 ± 0.2		39.7 ± 0.2		NS
Cesarean (%total)	23		40		NS
Birthweight, g	3258 ± 74 (*n* = 26)		3528 ± 107 (*n* = 20)		0.037
Female (% total)	50		30		NS
Ponderal Index	2.6 ± 0.04		2.7 ± 0.05		0.03

Table 1. *Cont.*

	NW (n = 27)	Obese (n = 27)	NW vs. OB, Same Time Point p-Value Difference [95% CI]
2 weeks NB %fat	8.9 ± 0.7	11.0 ± 1.2 (n = 19)	NS
2 weeks Total mass, g	3864.8 ± 95.4	4123 ± 137	NS
2 weeks Maternal BMI, kg/m^2	24 ± 0.4	33 ± 0.5	<0.0001
2 weeks Maternal %fat	33 ± 1	41 ± 1	<0.0001

95% CI = 95% confidence interval; NS = $p > 0.05$. * After 3 days of control diet while wearing a blinded CGM at 14–16 and 26–28 weeks, women reported to the CCTSI clinic after fasting × 10 h. Baseline labs were collected, followed by a liquid breakfast shake (30% total calories) and frequent blood sampling over 4 h for plasma TG using assays previously reported [7,8] in the CCTSI Laboratory (paired samples run on same batch). ‡ $p \leq 0.001$, NW vs. OB same timepoint. ‡ $p < 0.01$, NW vs. OB same timepoint.

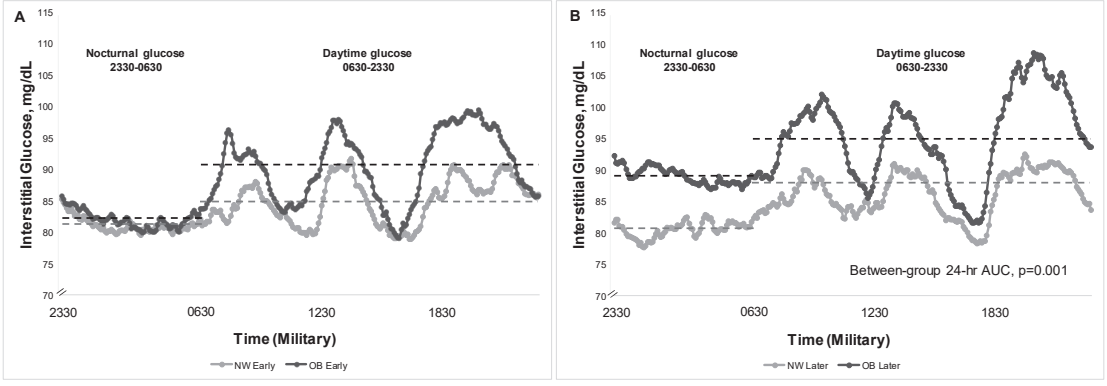

Figure 1. Patterns of 24 h glycemia measured by CGM in participants with NW and OB, both *Early* (14–16 weeks, Panel (**A**)) and *Later* (26–28 weeks, Panel (**B**)) in pregnancy. Gray and black dashed lines show mean nocturnal and daytime glucose between the groups.

In *Early* gestation, women with OB had a higher daytime mean glucose (91.0 vs. 84.8 mg/dL [95% CI for difference: 1.10, 10.53]) vs. NW and a correspondingly higher daytime glucose AUC (Table 2, p = 0.01 for both). This was explained by a pattern of higher 1 h PP (100.2 vs. 93.4 mg/dL [95% CI: −0.06, 13.66]) and 2 h PP glucose (95.3 vs. 87.3 mg/dL [95% CI: 2.65, 13.42,) in OB vs. NW, respectively, because nocturnal AUC was similar (Figure 1). The % TIR (63–140 mg/dL) was similar in women with OB (97.7 ± 1.1%) compared to those with NW (98.5 ± 1.1%)(95% CI: −3.21, 1.60).

In *Later* gestation, women with OB averaged a 10 mg/dL statistically higher 1 h PP glucose across meals (109.0 mg/dL vs. 98.3 mg/dL [95% CI for difference:, 4.0, 16.94]) as well as 2 h PP glucose (101.2 vs. 92.0 mg/dL [95% CI: 3.48, 15.40] compared to NW, but much lower than current gestational diabetes treatment targets [14,18]. By *Later* gestation, women with OB had higher glycemia across the 24 h period with a higher 24 h mean glucose (93.0 vs. 85.4 mg/dL, respectively [95% CI: 3.52, 13.14]), and a correspondingly higher 24 h glucose AUC by 9% (p = 0.001 for both, Table 2, Figure 1). The OB group vs. NW *Later* also had higher mean nocturnal glucose (88.5 vs. 81.3 [95% CI: 1.43, 12.84]; p = 0.015). Importantly, the %TIR remained similar between groups (OB: 98.8 ± 0.5%; NW: 95.1 ± 1.7% [95% CI: −0.03, 7.32]).

Table 2. Maternal CGM metrics from *Early* (14–16 weeks) and *Later* (26–28 weeks) gestation. Data are mean ± SEM. Between-group [OB minus NW] and within-group differences [*Later* minus *Early*] with 95% confidence intervals (CI) are provided.

	NW *Early*	OB *Early*	NW vs. OB *Early* *p*-Value Difference [95% CI]	NW Later	OB Later	NW vs. OB *Later* *p*-Value Difference [95% CI]	NW *Early* vs. NW *Later* * *p*-Value Difference [95% CI]	OB *Early* vs. OB *Later* * *p*-Value Difference [95% CI]
n	24	24		25	23			
Fasting glucose †	81.3 ± 1.9	82.7 ± 2.1	1.47 [−4.20, 7.14]	81.0 ± 1.9	86.0 ± 2.3	4.8 [−1.27, 10.80]	−0.41 [−4.55, 3.74]	2.45 [−0.89, 3.27]
Meals								
Preprandial Lunch	78.3 ± 1.7	85.0 ± 3.7	6.78 [−1.23, 14.78]	80.1 ± 1.9	84.0 ± 1.8	3.7 [−1.67, 9.10]	2.1 [−2.76, 6.91]	−2.06 [−10.72, 6.61]
Preprandial Dinner	78.0 ± 1.7	82.9 ± 3.0	4.93 [−2.04, 11.91]	77.2 ± 1.2	82.4 ± 1.7	0.016 5.21 [1.01, 9.40]	−1.66 [−4.98, 1.65]	−1.55 [−9.41, 6.30]
1 h PP breakfast	90.3 ± 2.8	99.0 ± 2.9	0.036 8.59 [0.60, 16.58]	96.0 ± 2.1	112.2 ± 4.1	0.001 16.2 [7.21, 25.25]	0.033 5.37 [0.46, 10.28]	0.010 12.6 [3.31, 21.90]
1 h PP lunch	96.0 ± 2.9	100.5 ± 3.3	4.59 [−4.24, 13.41]	100.0 ± 2.2	106.1 ± 2.4	0.049 6.57 [0.04, 13.10]	3.55 [−3.21, 10.3]	5.20 [−3.54, 13.94]
1 h PP dinner	94.0 ± 2.9	101.6 ± 2.3	0.042 7.8 [0.31, 15.30]	99.6 ± 2.2	108.1 ± 3.4	0.038 8.53 [0.49, 16.58]	4.04 [−3.45, 11.52]	5.89 [−1.98, 13.76]
2 h PP breakfast	85.2 ± 2.3	90.4 ± 2.3	5.24 [−1.23, 11.72]	89.2 ± 2.1	100.2 ± 3.1	0.005 11.0 [3.51, 18.47]	3.99 [−1.75, 9.73]	0.02 7.21 [1.29, 13.14]
2 h PP lunch	89.1 ± 2.2	94.6 ± 2.3	5.47 [−0.94, 11.89]	92.6 ± 2.0	98.1 ± 2.4	5.55 [−0.69, 11.78]	2.99 [−2.22, 8.21]	3.74 [−3.10, 10.58]
2 h PP dinner	87.6 ± 2.1	101.0 ± 2.8	<0.0001 13.24 [6.25, 20.23]	93.5 ± 2.1	105.2 ± 3.2	0.003 11.78 [4.23, 19.33]	0.036 5.39 [0.40–10.39]	4.67 [−3.42, 12.77]
1 h PP across 3 meals	93.4 ± 2.6	100.2 ± 2.2	0.052 6.80 [−0.06, 13.66]	98.3 ± 1.8	109.0 ± 2.7	0.002 10.44 [4.0, 16.94]	4.32 [−1.19, 9.82]	0.027 8.02 [1.02, 15.01]
2 h PP across 3 meals	87.3 ± 1.8	95.3 ± 2.0	0.004 8.04 [2.65, 13.42]	92.0 ± 1.6	101.2 ± 2.5	0.003 9.44 [3.48, 15.40]	0.039 4.13 [0.22, 8.03]	5.08 [−0.74, 10.90]
PP excursion breakfast	8.8 ± 2.7	11.1 ± 3.0	2.35 [−5.81, 10.52]	15.0 ± 2.2	23.5 ± 4.3	9.01 [−0.42, 18.46]	0.047 4.83 [0.07, 9.59]	0.024 12.88 [1.84, 23.92]
PP excursion lunch	17.8 ± 3.1	15.5 ± 3.9	−2.28 [−12.27, 7.71]	19.4 ± 1.5	22.3 ± 1.9	2.86 [−1.94, 7.66]	1.38 [−6.21, 9.0]	7.26 [−2.80, 17.32]
PP excursion dinner	15.9 ± 2.6	18.8 ± 2.9	2.87 [−4.97, 10.72]	22.3 ± 1.9	26.0 ± 2.5	3.34 [−2.88, 9.57]	5.70 [−1.60, 12.99]	7.46 [−1.95, 19.87]

Table 2. *Cont.*

	NW *Early*	OB *Early*	NW vs. OB *Early* *p*-Value Difference [95% CI]	NW Later	OB Later	NW vs. OB *Later* *p*-Value Difference [95% CI]	NW *Early* vs. NW *Later* * *p*-Value Difference [95% CI]	OB *Early* vs. OB *Later* * *p*-Value Difference [95% CI]
Diurnal								
Daytime Mean glucose	84.8 ± 1.6	91.0 ± 1.8	0.017 5.82 [1.10, 10.53]	86.9 ± 1.5	94.5 ± 1.7	0.002 7.60 [2.96, 12.24]	1.24 [−1.84, 4.34]	3.09 [−1.02, 7.19]
Nocturnal glucose	81.6 ± 1.5	83.1 ± 1.8	1.53 [−3.20, 6.26]	81.3 ± 2.2	88.5 ± 1.7	0.015 7.13 [1.43, 12.84]	0.07 [−3.36, 3.50]	0.020 4.63 [0.80, 8.47]
Mean 24 h glucose	83.8 ± 1.4	88.0 ± 1.7	4.21 [−0.25, 8.67]	84.5 ± 1.7	93.0 ± 1.7	0.001 8.33 [3.52, 13.14]	0.43 [−2.53, 3.41]	0.03 4.19 [0.44, 7.94]
Time in range, 63–140, % of 24 h	98.5 ± 1.1	97.7 ± 1.1	−0.81 [−3.21, 1.60]	95.1 ± 1.7	98.8 ± 0.5	0.052 3.65 [−0.03, 7.32]	0.023 −4.45 [−8.21, −0.68]	0.50 [−1.99, 2.99]
AUCs								
2 h AUC breakfast	10,737 ± 288	11,485 ± 269	748 [−44.62, 1541]	11,048 ± 213	12,572 ± 347	<0.0001 1523 [719, 2327]	278 [−220, 775]	0.017 944 [190, 1698]
2 h AUC lunch	10,955 ± 241	11,565 ± 307	610 [−173, 1392]	11,160 ± 239	11,859 ± 260	0.054 699 [−11.0, 1409]	177 [−383, 738]	342 [−501, 1186]
2 h AUC dinner	10,792 ± 284	11,763 ± 273	0.018 971 [178, 1763]	11,167 ± 190	12,118 ± 362	0.022 951 [146, 1756]	233 [−409, 874]	306 [−510, 1122]
24 h AUC	120,622 ± 1968	126,297 ± 2451	5675 [−611, 11,961]	120,993 ± 2386	133,320 ± 2364	0.001 12,328 [5558, 19,098]	275 [−3637, 4187]	0.029 6020 [700, 11,339]
Daytime AUC	86,341 ± 1564	92,109 ± 1783	0.019 5768 [1007, 10,530]	87,961 ± 1523	95,988 ± 1762	0.001 8027 [3359, 12,694]	1216 [−1797, 4228]	3238 [−969, 7445]
Nocturnal AUC	33,683 ± 758	34,543 ± 741	859 [−1274, 2993]	33,781 ± 912	36,739 ± 724	0.016 2958 [587, 5329]	269 [−1246, 1783]	0.021 1906 [318, 3495]

* Within-group (*Early* to *Later*) difference, paired *t*-tests, (19–22 cases). 95% CI = 95% confidence interval. † Fasting glucose was defined as the average six consecutive values starting at 06:00 h and/or after at least 7 h fasting. All CGM metric definitions have been previously published [13]. *p*-values ≤ 0.05 are reported.

Group Differences in Metabolic Measures: As previously reported, the fasting, 1 h, and 2 h PP TG were ~30% different between groups both *Early* and *Later* [8]. In response to the 3 h 100 g OGTT, women with OB had significantly higher fasting plasma glucose and (log)insulin compared to NW (83.0 vs. 77.3 mg/dL, respectively [95% CI for difference: 1.89, 9.20]), followed by ~20 mg/dL higher 1 and 2 h post-load glucose concentrations (p = 0.004–0.01, Table 1). Although the post-load insulin concentrations appeared higher in OB vs. NW, the log-transformed concentration differences were not statistically significantly different. Participants with OB were more insulin-resistant compared to those with NW, demonstrated by higher 3 h glucose AUC and a higher glucose × insulin AUC product (Table 1).

Within-group Changes in Glycemia from Early to Later Gestation: Table 2 shows within-group changes in patterns of glycemia for the NW and OB groups. Notably, fasting glucose did not change in NW from *Early* to *Later* (81.3 to 81.0 mg/dL), and the slight increase in fasting glucose in the OB group *Early* to *Later* (82.7 to 86.0 mg/dL) was not statistically significant. In NW, the 1 h PP glucose across all meals did not increase statistically from *Early* to *Later* (93.4 vs. 98.3 mg/dL) but did statistically increase in the OB group (100.1 to 109.0 mg/dL, p = 0.027). The peak PP breakfast excursion (highest PP glucose within 2 h [13]) increased statistically from *Early* to *Later* within the NW group (8.8 to 15.0 mg/dL, p = 0.047) without a change in fasting glucose. In the OB group, the peak PP breakfast excursion increased from *Early* to *Later* (11.1 to 24.0 mg/dL, p = 0.024), but the maximum peak PP excursion in either group, at any time point, or after any meal was only 26 mg/dL. The pregnancies affected by OB demonstrated a pattern of increased glycemia postprandially over time between *Early* and *Later* gestation, particularly after breakfast, in 1 h PP measures, mean nocturnal and 24 h glucose, and in nocturnal and 24 h AUC glucose (all $p < 0.05$; Table 2).

Correlates with Neonatal Birthweight and Adiposity

Total Cohort: Across 45 mother–infant pairs, none of the CGM or metabolic measures were correlated with infant BW. In addition, maternal characteristics, including pre-pregnancy BMI and gestational weight gain (GWG), were not correlated with infant BW or NB%fat. Across women in *Later* pregnancy, there was a pattern of moderate correlation between PP meal responses and NB%BF (1 h PP dinner [r = 0.331, p = 0.02], 2 h PP breakfast [r = 0.322, p = 0.03], 2 h PP dinner AUC [r = 0.331, p = 0.02]. Moreover, the 24 h glucose AUC (r = 0.310, p = 0.04) and mean 24 h glucose (r = 0.305, p = 0.04) were modestly correlated with NB%fat.

By Group: There were no correlations between any CGM measures and NB%fat in those with NW *Early*. In *Later* pregnancy, the PP breakfast excursion (~15 mg/dL; Table 2) was correlated with NB%fat (r = 0.519, p = 0.008) in NW. In the OB group, the *Early* 2 h PP glucose across meals was correlated with NB%fat (r = 0.469, p = 0.05 [borderline significant]). By *Later* gestation, significant correlations between the mean 24 h glucose and NB%fat (r = 0.538, p = 0.02), and 24 h glucose AUC and NB%fat (r = 0.532, p = 0.02) were demonstrated in the OB group.

Predictors of Neonatal Adiposity by Univariate and Multivariate Regression: There were no correlations between insulin sensitivity estimates based on the 100 g OGTT and NB%fat, nor were there correlations between GWG or maternal %BF after delivery or with NB%fat. As reported previously [8], across the 45 mother–infant pairs, a 1 h or 2 h PPTG both *Early* and *Later* in gestation predicted NB%fat and explained ~30% of the variance, respectively (R^2 = 0.32 and R^2 = 0.29, $p < 0.001$ for both) [8]. In the OB cohort, the *Early* 1 h or 2 h PPTG predicted 50% of the variance in NB%fat (R^2 = 0.50; $p < 0.01$) [8]. None of the CGM or glucose variables here added predictive value to the fasting and PPTG measures on NB%fat across the cohort or in mothers with OB specifically.

4. Discussion

We set out to evaluate the premise that pregnancies affected by OB have higher patterns of glycemia using CGM metrics associated with fetal overgrowth [9], even when diets are carefully controlled. In this parallel-group comparative study of OB and NW pregnant women without significant co-morbidities, the gestational week of measurement was fixed at 14–16 and 26–28 weeks to reduce variability from the expected weekly increase in insulin resistance. Notably, a controlled diet that was both eucaloric and equivalent in macronutrient composition was provided for 3 days to further reduce variation in patterns of glycemia from ad libitum diet consumption that would be expected to influence CGM metrics. Both *Early* and *Later* in gestation, pregnancies with OB manifested higher glycemia despite highly controlled diets compared to their NW counterparts, supporting our a priori hypothesis. *Early* in pregnancy, fasting and nocturnal glucose was similar between groups but participants with OB had higher PP meal responses. Between *Early* and *Later* pregnancy, both groups of women demonstrated higher nocturnal glucose and increased PP glucose responses. Those with OB had larger increases, such that by 26–28 weeks gestation, CGM metrics demonstrated glucoses that were consistently statistically higher after meals, over the 24 h period, and throughout the night. Remarkably, the 1 h PP (109.0 vs. 98.3 mg/dL) and the 2 h PP response (101.2 vs. 92.0 mg/dL) averaged across all meals *Later* in both the OB and NW group, respectively, were much lower than current 1 h PP and 2 h PP therapeutic targets for diabetes in pregnancy (<140 mg/dL, <120 mg/dL, respectively [18]), and were similar to our previously reported normative data [6]. CGM predictors of NB%fat measured by DXA were identified: In women with NW, only the *Later* gestation PP breakfast response (~15 mg/dL) was positively correlated with NB%fat. The strongest correlation in OB *Later* was the 24 h mean glucose and the 24 h glucose AUC.

Because the majority of large-for-gestational age (LGA) deliveries are accounted for by pregnancies with OB rather than GDM [3], we sought to examine differences in CGM metrics with a macronutrient- and calorie-controlled diet. This otherwise healthy cohort with OB displayed normal glucose tolerance [18] and demonstrated lower patterns of glycemia than expected, likely due to consuming the provided eucaloric diet. Fasting glucose by CGM was slightly higher in the OB group *Later*, but did not reach statistical significance. However, fasting glucose on the OGTT at 28 weeks was higher in the OB group as was the mean nocturnal glucose *Later* in pregnancy. Moreover, on the controlled diet, the PP meal excursions in both groups *Early* in gestation were surprisingly low (9–16 mg/dL for NW, 11–19 mg/dL for OB). Similarly, the 1 and 2 h PP glucoses across meals in the NW group throughout pregnancy was ~30–40 mg/dL below current targets, and in the OB group, they were ~20–30 mg/dL below targets. In our previously published systematic review [6], the pattern of glycemia in late normal pregnancy (~34 weeks, BMI range 22–28 kg/m^2) was lower than had been formerly appreciated: fasting glucose was 71 $\pm$ 8, 1 h PP glucose was 109 $\pm$ 13, 2 h PP glucose was 99 $\pm$ 10, and mean 24 h glucose was 88 $\pm$ 10 mg/dL (mean $\pm$ SD). In this current study, while on a eucaloric diet, both groups of women fell within these ranges. In another of our previous studies, women with NW (n = 22) and OB (n = 16) wore a CGM while consuming a controlled diet and in addition, while consuming their typical ad libitum diet. Analysis of the CGM data was limited to only 1 day of each diet. In that observational study, those with OB (vs. NW) had higher fasting glucose both *Early* and *Later*, and higher 24 h patterns of glycemia by ~9% on ad libitum and ~8% when diet was controlled [7]. In this current study, 72 h of CGM data on controlled study diets produced patterns of 24 h glycemia that were 13–14% lower compared to the previous study [7]. Taken together, these data suggest that lower glucose concentrations may be achieved by consuming a healthy eucaloric diet pattern for a longer period (3 days) across pregnancy in both NW and OB individuals, effectively blunting peak PP excursions in the range of 9–26 mg/dL.

Others have employed CGM technology in pregnancy to characterize 24 h glucose patterns, but this is the only study to have provided controlled diets to participants with NW and OB, both *Early* and *Later* in pregnancy, within tightly controlled gestational

windows. Chandler-Laney and colleagues [19] studied 40 pregnant Black women (BMI 21.3–43.9 kg/m^2, 32.0–34.6 weeks) who wore a CGM while consuming an ad libitum diet; the fasting glucose was similar to women with OB in this study (86.5 ± 12.7 mg/dL, mean ± SD), but glycemia in NW vs. OB was not reported. In a randomized cross-over study, Kizirian and colleagues [20] studied 17 women in Australia with risk factors for GDM (BMI 23.8 ± 4.7 kg/m^2, 29.3 ± 1.3 weeks) who were provided a low glycemic- and a higher-glycemic-load diet while wearing a CGM for 24 h each. The %TIR (70–140 mg/dL) was 95.1 ± 1.7% on the low-glycemic-load diet day (vs. 87.7 ± 3.2% on higher-glycemic-load day, $p = 0.031$), and the low-glycemic-load diet %TIR was similar to both groups of women, both *Early* and *Later*, in this study. This high %TIR also coincides with a recent report in uncomplicated pregnancies [21]. While our controlled diets were not designed based on glycemic load, the diets contained 50% of total energy from carbohydrates, the majority being complex carbohydrates (30% of total calories) with mostly low–medium-glycemic index foods.

Maitland and colleagues [22], in the UK, conducted a 3-arm randomized trial ($n = 16$; BMI 37 ± 4.7 kg/m^2, 24–28 weeks) in women with OB, in which one arm involved consuming an ad libitum diet (2 days) while wearing a CGM. Women in that study demonstrated lower glucose than women with OB in this study (fasting glucose 76, 24 h mean glucose 85, daytime mean 87, and nocturnal mean 78 mg/dL). In Brazil, Rahmi and colleagues [23] studied 10 women with NW (pre-gestational BMI 22.1 [range 21.7–23.8] kg/m^2) and 10 women with OB (39.9 [35.8–41.9]) who wore a CGM for 3 days while on an ad libitum diet at ~25 weeks gestation (range 24–28 weeks). Although diet was not controlled, their findings mirror those in this study, wherein women with OB showed higher glycemia over 24 h compared to those with NW. While the women with NW [23] demonstrated similar glucose patterns to the NW women in our study, the women with OB demonstrated lower PP glucose (by ~9 mg/dL) across 24 h compared to OB in our study. Finally, in the recently reported large observational prospective cohort by Durnwald and colleagues [24], pregnant individuals wore GCM throughout gestation, and glycemic metrics were compared in the individuals who developed GDM versus those who did not. Although NW vs. OB pregnancies were not compared, the %TIR in the individuals who did not develop GDM was 93–94% in the first and third trimester of pregnancy, slightly less than our range in both NW and OB groups (95–99%). However, mean glucose was higher in the first and third trimesters in the individuals who did not develop GDM (101 and 99 mg/dL, respectively) compared to women in this study with NW and OB at 14–16 weeks (83 and 88 mg/dL, respectively) and at 26–28 weeks (85 and 93 mg/dL, respectively) [24]. This difference might also be related to the eucaloric diet consumed by our participants as opposed to the ad libitum diet. None of the above studies measured NB%fat.

Newborn adiposity is a marker sensitive to intrauterine nutritional exposures and more strongly predicts risk for childhood obesity than BW [4,10]. Unique to this study was our measurement of NB%fat by DXA, which some experts still consider the gold standard in precision for infant body composition [16], to evaluate associations between CGM glucose metrics and fetal growth. While BW was higher in the offspring of women with OB (vs. NW), the difference in NB%fat did not reach statistical significance (11.0% vs. 8.9%). Nonetheless, when the NB%fat was used as a continuous dependent variable, some CGM predictors were revealed in this NW and OB population with relatively low glycemic patterns. Across the total cohort, PP glucose and 24 h glycemia at 26–28 weeks gestation were correlated with NB%fat. Unique to women with NW, the PP breakfast excursion during 26–28 weeks was associated with NB%fat. In women with OB, the average 2 h PP glucose response across meals (95 mg/dL) was already associated with NB%fat at 14–16 weeks gestation. In those with OB, at 26–28 weeks, the average 24 h glucose and 24 h glucose AUC explained ~28% of the variance in NB%fat ($r = 0.538$, $r = 0.532$, respectively). However, linear regression models constructed using the fasting TG and PPTG data from our previously reported breakfast test meal studies [8] demonstrated that the 1 h and 2 h PP TG at 14–16 weeks gestation were the strongest predictors of NB%fat. Adding

insulin sensitivity estimates from the 100 g OGTT and CGM metrics from this study did not improve the predictive model. Given that both glucose and TG are sensitive to maternal diet patterns, and both increase postprandially from the ingestion of simple sugars, these data add evidence to suggest that diet intervention across pregnant women, and particularly in women with OB, might be more intentionally targeted throughout pregnancy to mitigate fetal overgrowth patterns.

This study has strengths and limitations. Incorporating measures of fasting TG and PPTG and estimates of insulin resistance to the CGM metrics to predict fetal overgrowth are strengths, as well as utilizing DXA to measure NB%fat. Although BW was higher in the offspring of women with OB, we did not find a between-group difference in %NBfat. This may be in part due to a higher percentage of female offspring being born to NW mothers (50%) compared to OB mothers (30%) and females tend to have higher NB%fat compared to males. We did not have adequate sample size to evaluate sex differences. Furthermore, the measurement of NB%fat was at ~2 weeks of life, which might have been influenced by early feeding patterns and partially account for the lack of difference in adiposity. Providing a highly controlled maternal diet while wearing the CGM allowed us to minimize the confounding variable calories and macronutrients which substantially influence CGM metrics between the NW and OB groups but could also limit generalizability to ad libitum diet conditions. CGM technology has evolved since this study was conducted with more precise sensors, but focusing on between-group comparisons rather than attempting to define precise individual time point measures would seem to attenuate this limitation.

5. Conclusions

On eucaloric diets matched for macronutrient composition, pregnancies affected by OB demonstrated higher patterns of 24 h glycemia both *Early* and *Later* in pregnancy. While PP glucose responses increased within NW women from *Early* to *Later*, the patterns across 24 h increased with greater magnitude within OB, supporting our hypothesis that the OB metabolic phenotype contributes to higher 24 h glycemia in pregnancy, independent of dietary macronutrient composition and calories. However, despite these differences, mean glucose and PP excursions in both groups were saliently lower than current therapeutic targets for diabetes in pregnancy when macronutrients and calories were controlled. *Later* in pregnancy, the mean and 24 h glucose AUC correlated with NB%fat in OB. However, the addition of CGM metrics in this study did not contribute to the prediction of NB% fat beyond fasting and PPTG measures in NW and OB pregnancies. This observation supports the premise that lipid metabolism may be at least as, if not more, important than glucose metabolism in predicting fetal overgrowth in OB pregnant populations without diabetes. Given both groups exhibited high %TIR (95–99%) both *Early* and *Later* in pregnancy when defined as 63–140 mg/dL, a lower %TIR range may be necessary to differentiate glycemic patterns associated with fetal overgrowth in NW vs. OB individuals without pre-existing diabetes for GDM. Because early nutritional interventions that extend through delivery which limit simple carbohydrate and saturated fats are likely to have favorable effects on both TG and glucose patterns, nutritional interventions may be important not only in GDM, but also in pregnancies affected by OB, at high risk for fetal overgrowth and offspring metabolic disease.

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Data Availability Statement: Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request. The data are not publicly available due to ongoing pre-planned analyses that are not yet complete.

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Weight Loss After Sleeve Gastrectomy According to Metabolic Dysfunction-Associated Steatotic Liver Disease Stage in Patients with Obesity: A Liver Biopsy-Based Prospective Study

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Abstract: Background: The role of metabolic dysfunction-associated steatotic liver disease (MASLD) in sleeve gastrectomy (SG)-related outcomes remains uncertain. In this study, we aimed to assess the influence of preoperative biopsy-proven MASLD and its stages on weight loss after SG. Methods: One hundred sixty-three patients with obesity undergoing SG with concomitant intraoperative liver biopsy were followed up for 1 year. Fifty-eight participants were categorized as no MASLD, thirty-eight as metabolic dysfunction-associated steatotic liver (MASL), and sixty-seven as metabolic dysfunction-associated steatohepatitis (MASH). Percentage total weight loss (%TWL) and percentage excess weight loss (%EWL) 1 year after SG were calculated for the different groups. We also evaluated the association between preoperative MASLD (and its stages) and weight loss, after adjusting for potential confounders. Results: Significant differences among groups were detected in %EWL ($p = 0.004$, ANOVA test), but not in %TWL ($p = 0.079$). However, significant differences in %TWL were found when MASH and no MASH (i.e., participants with MASL and participants without MASLD) groups were compared (27.3 ± 9.9 vs. 30.7 ± 9, respectively, $p = 0.025$). In the linear regression model for predicting %EWL 1 year after SG, the presence of MASH was independently associated with a lower %EWL, after adjusting for age, sex, baseline body mass index (BMI), and baseline glycated hemoglobin (HbA1c) (Beta −7.1; 95% CI −13.6, −0.5; $p = 0.035$). The presence of MASLD, liver fibrosis, or advanced liver fibrosis (≥F2) was also associated with lower %EWL after SG in crude models, although they did not remain significant after adjusting for these confounders. The presence of MASH was inversely related to %TWL, although the association did not remain significant after adjustment (Beta −2.7; 95% CI −5.7, 0.2; $p = 0.069$). Conclusions: MASH may be independently associated with lower %EWL 1 year after SG in patients with obesity.

Keywords: metabolic dysfunction-associated steatotic liver disease (MASLD); metabolic dysfunction-associated steatohepatitis (MASH); liver biopsy; obesity; sleeve gastrectomy; weight loss

1. Introduction

Obesity is a chronic disease associated with major health, social, and economic burdens [1]. In the last decades, the prevalence of obesity has reached pandemic proportions, with over 800 million adults suffering from this disease worldwide [2], and it is expected that this rising trend will continue in the coming years [2,3].

Lifestyle interventions, including diet and physical activity, are the mainstay of treatment of obesity [4]. Additionally, some medications, such as glucagon-like peptide-1 (GLP-1) receptor agonists, can help to achieve and maintain weight loss [4]. However, bariatric surgery (BS), recommended for patients with a body mass index (BMI) $\geq$ 35 kg/m^2 or $\geq$30 kg/m^2 with metabolic disease [5], is currently the most effective treatment for the management of obesity and related comorbidities [4,6,7].

Despite the remarkable effects of BS on the treatment of obesity, it should be noted that weight loss-related outcomes after this procedure may be influenced by several preoperative factors, including genetic and neurohormonal factors, which have been postulated to affect postoperative weight loss [8,9]. Recently, we showed that the gut microbiome may have a role in the success of BS in terms of percentage excess weight loss (%EWL) [10]. On the other hand, different studies have evaluated the roles of clinical factors, such as baseline weight, age, or sex, in the success of postoperative weight loss [11,12]. Interestingly, some studies have reported that different preoperative comorbidities associated with obesity, including type 2 diabetes (T2D), may lead to lower weight loss following BS [13,14].

Metabolic dysfunction-associated steatotic liver disease (MASLD) often coexists with obesity and other metabolic comorbidities [15,16]. It is associated with an increased risk for cardiovascular disease [17] and has become the first cause of liver transplantation in Western countries [18]. It has been demonstrated that BS is effective for the treatment of MASLD [19–21], although only a few studies have evaluated the role of MASLD as a potential predictor of weight loss after BS. In this regard, a previous study conducted in 143 participants with obesity (all of them undergoing gastric bypass) showed that the presence of MASLD before surgery was associated with lower weight loss in the short term following the intervention [22]. However, studies assessing the influence of biopsy-proven MASLD on weight loss after sleeve gastrectomy (SG), the most commonly performed bariatric procedure worldwide, are lacking.

Therefore, in this study, we evaluate the role of MASLD and the different stages of the disease, including metabolic dysfunction-associated steatohepatitis (MASH), in predicting weight loss after SG in patients with obesity.

2. Materials and Methods

2.1. Study Design and Participants

This was a prospective observational study that included 163 consecutive participants with obesity undergoing SG at Virgen de la Victoria University Hospital from April 2018 to December 2022, with an available intraoperative liver biopsy. All participants underwent laparoscopic sleeve gastrectomy according to international indications [23] and followed a standardized Enhanced Recovery After Surgery (ERAS) protocol for postoperative care [24].

Eligibility criteria to participate in this study included an age of 18–65 years, a BMI $\geq$ 35 kg/m^2 or $\geq$30 kg/m^2 with relevant comorbidities associated with obesity, laparoscopic SG as the BS technique, and written informed consent to obtain intraoperative liver biopsy. Exclusion criteria were alcohol consumption (>30 g/day in men and >20 g/day in women), use of drugs that could cause liver steatosis, and liver disease different from MASLD.

Participants were categorized as no MASLD, metabolic dysfunction-associated steatotic liver (MASL), or MASH according to the histological evaluation of intraoperative liver biopsies, and were followed up for 1 year after SG.

2.2. Histological Evaluation

The histological evaluation of wedge liver biopsies was done by expert liver pathologists. This evaluation was based on the Brunt semi-quantitative classification, including the assessment of liver steatosis, necroinflammatory activity, and fibrosis [25]. Therefore, participants were categorized as no MASLD (no steatosis, no necroinflammatory activity, and no fibrosis), MASL (at least grade 1 steatosis with no necroinflammatory activity nor fibrosis), and MASH (at least grade 1 steatosis with necroinflammatory activity ≥ 1, with or without fibrosis). Further details regarding the histological evaluation can be found elsewhere [26].

2.3. Clinical, Anthropometric, and Biochemical Evaluation

Baseline sociodemographic and clinical variables were obtained at a clinical interview. Baseline and 1-year anthropometric data were collected, and included weight, height, and BMI (calculated as weight in kilograms divided by the square of height in meters). Percentage total weight loss (%TWL) at 1 year after SG was calculated by the formula (preoperative weight—weight at 1 year)/preoperative weight $\times$ 100. %EWL at 1 year after SG was calculated by the formula (preoperative weight—weight at 1 year)/(preoperative weight—ideal weight) $\times$ 100. Ideal weight was calculated for a BMI of 25 kg/m^2.

Baseline blood samples were collected after a 12 h fast. Serum biochemical parameters were measured by standardized methods (Advia Chemistry XPT autoanalyzer, Siemens Healthcare Diagnostics). Low-density lipoprotein cholesterol (LDL-c) was estimated by Friedewald's formula [27]. Serum insulin was measured by immunoassay (ADVIA Centaur autoanalyzer, Siemens Healthcare Diagnostics). The formula fasting insulin ($\mu IU/mL$) $\times$ fasting glucose ($mmol/L$)/22.5, was used to calculate the homeostasis model assessment of insulin resistance (HOMA-IR) [28].

2.4. Statistical Analysis

IBM SPSS statistical software (Version 29.0, IBM Corporation, Chicago, IL, USA) was used for statistical analyses. The normal distribution of variables was assessed using the Kolmogorov–Smirnov test. Comparisons among groups were made using the ANOVA test (continuous variables with a normal distribution) or the Kruskal–Wallis test (continuous variables without a normal distribution), followed by a Bonferroni test. The Pearson's Chi-squared test was performed to compare proportions. The Student's *t* test was used to compare continuous variables with a normal distribution between 2 groups. Univariable general linear models were performed considering %TWL or %EWL as the dependent variable and selecting relevant histopathological variables and clinical/biochemical parameters as the fixed factor or covariate. Linear regression models considered %TWL or %EWL as the dependent variable, and different binary histopathological classifications as the independent variable, together with relevant clinical and biochemical parameters for adjustment. Data are given as mean $\pm$ standard deviation (SD), or mean (95% confidence interval), unless otherwise indicated. Statistical significance was set for a p value < 0.05.

3. Results

3.1. Basal Characteristics of the Study Population

Data from 163 participants with obesity (58 without MASLD, 38 with MASL, and 67 with MASH) were analyzed. The mean age was 45.7 $\pm$ 8.8 years, and 113 (69.3%) were women. The characteristics of the study population at baseline according to MASLD status are shown in Table 1.

Fasting glucose and glycated hemoglobin (HbA1c) levels were higher in patients with MASL or MASH, compared with patients without MASLD. On the other hand, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglyceride levels, and homeostatic model assessment of insulin resistance (HOMA-IR) values were higher in patients with MASH, compared with patients without MASLD. Data regarding the

histopathological evaluation of liver biopsies (i.e., steatosis, necroinflammatory activity, and fibrosis) according to these groups can also be found in Table 1.

Table 1. Basal characteristics of the study population according to metabolic dysfunction-associated steatotic liver disease (MASLD) stage.

	No MASLD (n = 58)	MASL (n = 38)	MASH (n = 67)	*p* Value
Sex (F, M)	41/17	27/11	45/22	0.882
Age (years)	43.6 ± 9.2 [a]	45.8 ± 7.7 [ab]	47.4 ± 8.7 [b]	0.048
Weight (kg)	128.8 ± 19.0	130.9 ± 19.3	137.3 ± 26.6	0.204
BMI (kg/m^2)	46.6 ± 5.9	46.9 ± 5.4	48.9 ± 7.1	0.163
Hypertension (n, %)	22 (37.9%)	12 (31.6%)	36 (53.7%)	0.060
SBP (mm Hg)	131.6 ± 21.0	127.1 ± 12.1	131.5 ± 17.0	0.364
DBP (mm Hg)	83.1 ± 12.4	80.6 ± 9.9	82.0 ± 11.2	0.574
Type 2 diabetes (n, %)	16 (27.6%)	13 (34.2%)	32 (47.8%)	0.060
Glucose (mg/dL)	98.8 ± 18.6 [a]	107.6 ± 20.0 [b]	109.1 ± 24.5 [b]	0.006
HbA1c (%)	5.6 ± 0.7 [a]	5.9 ± 0.7 [b]	6.2 ± 1.3 [b]	0.008
Insulin (μIU/mL)	16.5 ± 8.2 [a]	17.8 ± 10.0 [ab]	23.0 ± 13.5 [b]	0.010
HOMA-IR	4.0 ± 2.1 [a]	4.7 ± 2.6 [ab]	6.4 ± 4.6 [b]	0.003
Cholesterol (mg/dL)	182.0 ± 42.5	185.0 ± 37.1	188.4 ± 39.6	0.680
HDL-C (mg/dL)	44.5 ± 12.4	44.4 ± 13.9	42.7 ± 12.1	0.707
LDL-C (mg/dL)	113.1 ± 38.1	114.6 ± 27.0	115.4 ± 32.6	0.929
Triglycerides (mg/dL)	125.4 ± 63.8 [a]	137.1 ± 77.4 [ab]	156.9 ± 72.6 [b]	0.011
AST (U/L)	25.4 ± 16.1 [a]	28.1 ± 9.9 [ab]	31.1 ± 13.9 [b]	0.006
ALT (U/L)	28.3 ± 18.3 [a]	35.3 ± 17.5 [ab]	38.8 ± 18.1 [b]	<0.001
AST/ALT ratio	0.9 ± 0.3	0.9 ± 0.4	0.9 ± 0.3	0.167
Albumin (g/dL)	3.9 ± 0.4	3.8 ± 0.4	3.8 ± 0.4	0.499
Platelets (10^3/μL)	278.9 ± 91.0	253.3 ± 60.1	257.9 ± 75.5	0.626
Histopathological parameters				
Steatosis (grade 0/1/2/3)	58/0/0/0	0/29/2/7	0/39/17/11	<0.001
Necroinflammatory activity (grade 0/1/2/3)	58/0/0/0	38/0/0/0	0/47/19/1	<0.001
Fibrosis (grade 0/1/2/3/4)	58/0/0/0/0	38/0/0/0/0	16/30/12/8/1	<0.001

Data are given as mean ± standard deviation (SD) or n (proportion). Comparisons among groups were performed using an ANOVA test for continuous variables with a normal distribution (i.e., age, DBP, cholesterol, LDL-C, and albumin), or a Kruskal–Wallis test for continuous variables without a normal distribution (i.e., body weight, BMI, SBP, glucose, HbA1c, insulin, HOMA-IR, HDL-C, triglycerides, AST, ALT, AST/ALT ratio, and platelets), followed by a Bonferroni post hoc analysis. To compare proportions, a Pearson's Chi-squared test was used. Statistical significance was set for a p value < 0.05. Different superscript letters denote statistically significant differences within each row between the groups. MASLD, metabolic dysfunction-associated steatotic liver disease; MASL, metabolic dysfunction-associated steatotic liver; MASH, metabolic dysfunction-associated steatohepatitis; F, female; M, male; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycated hemoglobin; HOMA-IR, homeostatic model assessment of insulin resistance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; AST, aspartate aminotransferase; ALT, alanine aminotransferase.

3.2. Weight Loss After Sleeve Gastrectomy According to MASLD Stage

Weight loss 1 year after SG was evaluated in the different groups of the study population (Table 2). Notably, although no significant differences among the three groups were found for %TWL (p = 0.079), significant differences were detected for this outcome when MASH and no MASH (i.e., participants with MASL and participants without MASLD) groups were compared (27.3 ± 9.9 vs. 30.7 ± 9.0., respectively p = 0.025).

Table 2. Weight loss outcomes 1 year after sleeve gastrectomy according to metabolic dysfunction-associated steatotic liver disease (MASLD) stage.

	No MASLD (n = 58)	MASL (n = 38)	MASH (n = 67)	*p* Value
Weight (kg)	88.9 ± 17.2 [a]	91.2 ± 18.5 [ab]	98.7 ± 18.4 [b]	0.008
BMI (kg/m^2)	32.2 ± 5.9 [a]	32.7 ± 6.6 [ab]	35.3 ± 5.9 [b]	0.007
%EWL	69.4 ± 21.8 [a]	67.8 ± 23.1 [ab]	57.4 ± 20.1 [b]	0.004
%TWL	30.9 ± 8.8	30.3 ± 9.3	27.3 ± 9.9	0.079

Data are given as mean ± standard deviation (SD). Comparisons among groups were performed using an ANOVA test for continuous variables with a normal distribution (i.e., weight, %EWL, and %TWL), or a Kruskal–Wallis test for continuous parameters without a normal distribution (i.e., BMI), followed by a Bonferroni post hoc analysis. Statistical significance was set for a p value < 0.05. Different superscript letters denote significant differences within each row between the groups. BMI, body mass index; %EWL, percentage excess weight loss; %TWL, percentage total weight loss. %EWL at 1 year after SG was calculated by the formula (preoperative weight—weight at 1 year)/(preoperative weight—ideal weight) × 100. Ideal weight was calculated for a BMI of 25 kg/m^2. %TWL at 1 year after SG was calculated by the formula (preoperative weight—weight at 1 year)/preoperative weight × 100.

On the other hand, we found significant differences among groups regarding %EWL (p = 0.004). Therefore, a lower %EWL following SG was observed for patients with MASH, compared with patients without MASLD (57.4 ± 20.1 vs. 69.4 ± 21.8, p = 0.006). No differences were found between patients with MASL and patients without MASLD (67.8 ± 23.1 vs. 69.4 ± 21.8, p = 1.000). A non-significant difference was detected between participants with MASH and participants with MASL (57.4 ± 20.1 vs. 67.8 ± 23.1, p = 0.052). %EWL and %TWL stratified by sex are shown in Supplementary Table S1.

3.3. Histopathological Factors Associated with Weight Loss After Sleeve Gastrectomy

We performed univariable general linear models to explore baseline histopathological factors associated with weight loss following SG (Table 3). First, we found an inverse relationship between the presence of MASH and %TWL after SG [−3.4% (−6.3 to −0.4)] (Table 3A).

Table 3. (**A**) Univariable general linear model (unadjusted) for predicting percentage total weight loss (%TWL) 1 year after sleeve gastrectomy according to histopathological classifications and clinical/biochemical parameters. (**B**) Univariable general linear model (unadjusted) for predicting percentage excess weight loss (%EWL) 1 year after sleeve gastrectomy according to histopathological classifications and clinical/biochemical parameters.

(A)			
	Beta	**95% CI**	***p* Value**
MASLD (yes vs. no)	−2.5	(−5.6, 0.5)	0.105
MASH (yes vs. no)	−3.4	(−6.3, −0.4)	0.025
Liver fibrosis (yes vs. no)	−2.4	(−5.6, 0.7)	0.129
Liver fibrosis ≥ F2 (yes vs. no)	−4.3	(−8.7, 0.1)	0.053
Age (years)	−0.39	(−0.55, −0.23)	<0.001
Sex (female vs. male)	−0.57	(−3.77, 2.64)	0.728
Baseline weight (kg)	0.07	(0.01, 0.14)	0.029
Baseline BMI (kg/m^2)	0.16	(−0.06, 0.39)	0.152
Baseline diabetes (yes vs. no)	−4.5	(−7.5, −1.6)	0.003
Baseline HbA1c (%)	−1.5	(−2.9, −0.1)	0.039
Baseline hypertension (yes vs. no)	−3.0	(−6.0, −0.1)	0.046
Baseline triglycerides (mg/dL)	−0.02	(−0.01, 0.04)	0.073

Table 3. *Cont.*

Baseline AST (U/L)	−0.03	(−0.13, 0.08)	0.598
Baseline ALT (U/L)	0.03	(−0.05, 0.11)	0.460
	(B)		
	Beta	**95% CI**	***p* Value**
MASLD (yes vs. no)	−8.3	(−15.3, −1.3)	0.021
MASH (yes vs. no)	−11.4	(−18.1, −4.7)	0.001
Liver fibrosis (yes vs. no)	−10.4	(−17.6, −3.2)	0.005
Liver fibrosis ≥ F2 (yes vs. no)	−13.5	(−23.5, −3.5)	0.008
Age (years)	−0.64	(−1.00, −0.26)	0.001
Sex (female vs. male)	−0.99	(−8.40, 6.42)	0.793
Baseline weight (kg)	−0.17	(−0.31, −0.02)	0.030
Baseline BMI (kg/m^2)	−1.07	(−1.57, −0.58)	<0.001
Baseline diabetes (yes vs. no)	−8.9	(−15.9, −2.0)	0.012
Baseline HbA1c (%)	−4.4	(−7.7, −1.2)	0.008
Baseline hypertension (yes vs. no)	−4.0	(−10.9, 2.9)	0.253
Baseline triglycerides (mg/dL)	−0.03	(−0.08, 0.02)	0.206
Baseline AST (U/L)	−0.12	(−0.37, 0.13)	0.341
Baseline ALT (U/L)	−0.01	(−0.20, 0.18)	0.937

CI, confidence interval; MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; HbA1c, glycated hemoglobin; AST, aspartate aminotransferase; ALT, alanine aminotransferase. Beta values denote the coefficient of the general linear model.

Interestingly, the presence of MASLD and MASH were inversely related to %EWL after SG [−8.3% (−15.3 to −1.3), and −11.4% (−18.1 to −4.7), respectively] (Table 3B). Also, the presence of liver fibrosis, or advanced liver fibrosis (≥F2), was inversely associated with %EWL after SG [−10.4% (−17.6 to −3.2), and −13.5% (−23.5 to −3.5), respectively] (Table 3B).

The role of additional baseline clinical and biochemical variables of interest regarding this outcome are also shown in Table 3. We found that age, the presence of T2D or hypertension, and baseline HbA1c were inversely associated with %TWL, whereas a direct association was observed between baseline weight and %TWL (Table 3A). On the other hand, age, baseline weight, BMI, the presence of T2D, and baseline HbA1c were inversely associated with %EWL after SG (Table 3B).

3.4. MASH Is Independently Associated with 1-Year Excess Weight Loss but Not with Percentage Total Weight Loss After Sleeve Gastrectomy

Linear regression models considering %EWL after SG as the dependent variable, and the different histopathological parameters, together with other clinically relevant baseline parameters as independent variables, were performed. Notably, the model that better explained %EWL included age, sex, preoperative MASH, preoperative BMI, and HbA1c. Thus, we observed that the presence of MASH was independently associated with a lower %EWL, after adjusting for age, sex, baseline BMI, and baseline HbA1c [−7.1% (−13.6 to −0.5); p = 0.035] (Table 4A), with an adjusted R^2 of 0.21 for the model. However, the association between MASLD, liver fibrosis, or advanced liver fibrosis, and %EWL after SG did not remain significant after adjusting for these variables [−3.1% (−9.9 to 3.7), p = 0.364; −6.4% (−13.3 to 0.4), p = 0.066; and −8.9% (−18.3 to 0.5), p = 0.062, respectively] (Supplementary Table S2).

On the other hand, in the linear regression model considering %TWL as the dependent variable, the association between MASH and %TWL did not remain significant after adjusting for age, sex, baseline weight, and baseline HbA1c [−2.1% (−5.7 to 0.2); p = 0.069] (Table 4B).

Table 4. (**A**) Linear regression model for predicting percentage excess weight loss (%EWL) 1 year after sleeve gastrectomy (dependent variable) according to MASH status (adjusted for age, sex, baseline BMI, and baseline HbA1c). (**B**) Linear regression model for predicting percentage total weight loss (%TWL) 1 year after sleeve gastrectomy (dependent variable) according to MASH status (adjusted for age, sex, baseline weight, and baseline HbA1c).

(A)			
	Beta	**95% CI**	***p* Value**
MASH (yes vs. no)	−7.1	(−13.6, −0.5)	0.035
Age (years)	−0.7	(−1.1, −0.3)	<0.001
Sex (female vs. male)	−1.2	(−7.9, 5.5)	0.729
Baseline BMI (kg/m^2)	−1.2	(−1.7, −0.7)	<0.001
Baseline HbA1c (%)	−0.8	(−4.0, 2.4)	0.602
(B)			
	Beta	**95% CI**	***p* Value**
MASH (yes vs. no)	−2.7	(−5.7, 0.2)	0.069
Age (years)	−0.3	(−0.5, −0.2)	<0.001
Sex (female vs. male)	0.4	(−2.9, 3.8)	0.796
Baseline weight (kg)	0.06	(−0.01, 0.13)	0.115
Baseline HbA1c (%)	−0.6	(−2.0, 0.8)	0.386

MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; HbA1c, glycated hemoglobin. Beta values denote the coefficient of the linear regression model.

4. Discussion

The main findings of this study suggest that MASLD status may play a role in postoperative %EWL after SG in the short term (1 year). Specifically, we showed that baseline MASH was independently associated with a lower %EWL after SG in our study population. Conversely, the observed inverse association between MASH and %TWL did not remain significant after adjusting for potential confounders. Therefore, our results add relevant information regarding the role of MASLD and its histopathological stages in weight loss-related outcomes after SG, the most commonly performed bariatric procedure globally, which had remained poorly explored.

BS is the most effective treatment for the management of obesity and related comorbidities, including MASLD. As weight loss is the mainstay of treatment of MASLD, substantial weight loss achieved after BS leads to the improvement and even to the resolution of the disease [19,20,29,30]. Moreover, several studies have also reported favorable results in advanced stages of the disease, such as MASH [31] or liver fibrosis [19,32]. Therefore, patients with obesity and different stages of MASLD can benefit from BS [21].

However, only a few studies have assessed the impact of baseline MASLD on weight loss after BS, and were mainly performed in patients undergoing gastric bypass. In a prospective study that involved 143 patients with obesity undergoing laparoscopic gastric bypass with concomitant intraoperative liver biopsies, the non-alcoholic fatty liver disease (NAFLD) activity score was reported to be a predictor of %EWL at 6 months [22]. Nevertheless, some sample size disproportions among groups were found in this study, as only 13 participants without MASLD (9%) were included in the cohort. On the other hand, in a retrospective cohort of patients undergoing Roux-en-Y gastric bypass, Abbassi et al. showed that %EWL and change in BMI were similar in subjects with MASH and MASL [33]. Sabench et al. found that baseline MASH had a different influence on weight loss depending on the surgical technique, as worse outcomes were reported for patients with MASH that underwent SG, but not in the Roux-en-Y gastric bypass group [34]. However, only women aged 30–55 years, and not men, were included in this study, a fact that could limit external validity. In the recent study by Abu-Rumaileh et al., including participants who

underwent BS, preexisting MASLD was independently associated with a lower %TWL and %EWL after the intervention [35]. Notably, these data were retrospectively reviewed, and the definition of MASLD was mainly based on non-invasive criteria (i.e., ICD-9 and ICD-10 coding in electronic medical records, and evidence of hepatic steatosis on imaging studies), and only 38 participants (5% of the study population) had available liver biopsies [35]. Since the non-invasive assessment of MASLD has important limitations for the diagnosis of the disease (e.g., the low reliability of ultrasound to detect hepatic steatosis when <20%, or in individuals with a BMI > 40 kg/m^2) [36], some of the participants of this study might have been misclassified. Indeed, only 221 participants (31% of the study population) were identified with a diagnosis of MASLD at baseline, which contrasts with the reported higher estimated prevalence of the disease in people living with obesity [37]. Therefore, this prevalence might be explained by the absence of an ICD-9 /ICD-10 diagnosis or available/accurate imaging study in some patients, which may not be enough to rule out the presence of MASLD. Moreover, as MASH diagnosis can only be established by liver biopsy, this stage of the disease was not considered in the study.

Regarding non-surgical weight loss, steatohepatitis was a negative predictor of preoperative weight loss in a cohort of patients with obesity undergoing BS [38]. Additional findings also suggest that patients with overweight/obesity and MASLD might be less responsive to non-surgical approaches to the disease, including lifestyle interventions [39], although further research is needed regarding this point. Also, the mechanisms involved in the potential influence of MASLD on post-BS/non-surgical weight loss are yet to be elucidated. In this regard, it could be speculated that some differences in the gut–liver–brain axis between subjects with and without MASLD might play a role [40]. Another possible explanation of our results might be related to insulin resistance or different hormonal responses following BS, including GLP-1 secretion [41]. Indeed, less weight loss after BS has been observed in other metabolic comorbidities, such as T2D, and some of these mechanisms may play a role [13,42]. However, further research is needed.

Despite the fact that our results suggest that a lower %EWL following SG may be expected in patients with obesity and baseline MASH, it should be noted that, although statistically significant, differences between subjects with and without MASH regarding this outcome were relatively small after adjusting for potential confounders. In fact, participants with baseline MASH achieved a mean %EWL > 50% after SG. Also, clinical differences in %TWL between participants with and without MASH were moderate in this study, and the association between preoperative MASH and %TWL did not remain significant after adjusting for confounders. Therefore, our findings reinforce the fact that BS, including SG, is effective in patients with MASLD (including MASH stage) in terms of weight loss.

On the other hand, we also evaluated the role of histopathological liver fibrosis in %EWL after SG. Although liver fibrosis and advanced liver fibrosis were also associated with lower %EWL in crude models, they did not remain significant after adjusting for confounders. However, a trend towards significance was observed for these two histopathological parameters. In this regard, recent results from the Cologne cohort (including patients undergoing gastric bypass, but not SG) showed that baseline histological fibrosis did not predict %TWL [43]. Given that only a limited proportion of participants had liver fibrosis or advanced liver fibrosis in our study, these results should be cautiously interpreted, and larger studies are needed to evaluate the impact of liver fibrosis on weight loss after SG.

Some of the strengths of this study are its prospective design and the criteria for defining MASLD, which was based on liver biopsy, the gold standard technique for diagnosing the disease. However, despite these strengths, several limitations should be acknowledged. First, sex imbalance should be taken into account, as a predominance of women (69.3%) was observed in our cohort, although these proportions are similar to those observed in clinical practice in patients undergoing BS. Also, the evaluation of weight loss after SG was only considered in the short term. The sample size was relatively small after sub-grouping, which reduced statistical power, especially when analyzing liver fibrosis. Therefore, long-term, large-scale, biopsy-based prospective studies are needed to confirm

these results. Furthermore, future perspectives in this area may include evaluating the role of MASLD in weight loss maintenance or weight regain after BS in the long term. Finally, for ethical reasons, no postoperative biopsies were obtained. As previous reports have shown that the persistence of MASH after BS might be associated with less weight loss following the intervention, and changes in liver histology may affect weight loss [32], this could be an important point to consider, which was not evaluated in our study and could have impacted our results.

5. Conclusions

In a cohort of patients with obesity undergoing SG, baseline MASH was an independent predictor of lower %EWL, but not %TWL, after the intervention. Further research is needed to unravel the potential mechanisms involved in this association.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/nu16223857/s1; Table S1: Weight loss outcomes 1 year after sleeve gastrectomy according to metabolic dysfunction-associated steatotic liver disease (MASLD) stage (stratified by sex); Table S2: Beta, 95% confidence interval, and *p* value for predicting percentage excess weight loss 1 year after sleeve gastrectomy according to presence of MASLD/liver fibrosis/liver fibrosis $\geq$F2, after adjusting for age, sex, baseline BMI, and baseline HbA1c.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Research Committee of Malaga (reference number PI-S0103-22, 1 February 2018).

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data will be available on reasonable request to the corresponding authors due to ethical reasons.

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Positive Changes in Body Composition and Profiles of Individuals with Diabetes 3 Years Following Laparoscopic Sleeve Gastrectomy in Japanese Patients with Obesity

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Abstract: Background and Objectives: We analyzed the changes in obesity, glucose metabolism, and body composition over a 3-year period in Japanese patients with obesity following laparoscopic sleeve gastrectomy (LSG). Methods: Body weight, parameters related to diabetes such as glycated hemoglobin (HbA1c), and electrical impedance analysis were used to assess body composition in forty-eight Japanese patients with obesity before surgery and 6 months, 1 year, 2 years, and 3 years after LSG. Results: At 6 months, 1, 2, and 3 years post-LSG, there were significant reductions in body weight, body mass index, blood pressure, fasting plasma glucose, triglyceride, and HbA1c levels. Six months after LSG, fat mass (FM), muscle mass (MM), and %FM all showed a decrease compared to pre-treatment values (all $p < 0.05$). FM and %FM remained in a decreased state until 3 years had passed. In contrast, %MM increased at 6 months post-LSG and was maintained up to 3 years post-LSG (all $p < 0.05$). Furthermore, changes in FM and %FM were associated with changes in body weight and A1C. In contrast, change in %MM exhibited a negative correlation with body weight and A1C following LSG. Finally, multivariate regression analyses demonstrated that alterations in FM were independent factors affecting body weight in patients with obesity 3 years after LSG. Conclusions: We observed improvements in FM, fasting plasma glucose, and HbA1c levels over a 3-year period in Japanese patients after LSG. The reduction in FM and maintenance of %MM after LSG were suggested as possible links between the effects of LSG on obesity and diabetes over 3 years.

Keywords: body composition; laparoscopic sleeve gastrectomy; obesity; diabetes; fat mass

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1. Introduction

Obesity and type-2 diabetes mellitus (T2DM) lead to several complications, such as cardiovascular and renal diseases, and are important issues worldwide, including Japan [1–3]. Appropriate drug therapy and bariatric surgery are important in the treatment of obesity and T2DM [4]. Among the obese surgical treatments, laparoscopic sleeve gastrectomy (LSG) has been performed extensively in European countries and the United States for the treatment of severe obesity and T2DM [5,6].

However, there are relatively few cases of LSG in Japan compared to those in European countries and the United States; therefore, there are limited studies reporting the long-term

effects of LSG on body weight (BW), body composition, and profiles of individuals with diabetes in Japanese patients [7].

A method to assess the outcomes of bariatric surgery is by utilizing the body mass index (BMI) [8]. Nevertheless, weight loss and BMI by themselves are inadequate for evaluating the impact of LSG on obesity and T2DM. Increases in BMI and body composition, especially visceral fat mass (FM), are associated with an increased risk of T2DM [9]. Decrease of muscle mass (MM) and excessive fat accumulation are both associated with T2DM [10,11]. MM was analyzed in terms of total MM and skeletal MM (TMM and SMM), %MM, upper and lower extremity MM, and the ratio of lower limb MM to body weight (L/W) [12,13].

Bioelectrical impedance analysis (BIA) has proven to be an easy approach for evaluating body composition [14–16]. Recent studies have investigated the impact of LSG using BIA [17–19]. Nonetheless, the long-term impact of bariatric surgery on body composition in Japanese patients with obesity is still unclear.

Body composition varies greatly depending on race and the environment [20]. The proportion of body fat is different in Japanese people compared to European and American Caucasians [21–23]. Although some studies in Western countries have reported on the body composition of patients with obesity, long-term changes in glucose metabolism and body composition, such as MM and FM after LSG in Japanese patients, are unclear. Therefore, to investigate the long-term benefits of LSG in Japanese patients with obesity, we attempted to identify new findings based on changes in glucose homeostasis and body composition, including FM and MM, after LSG. This study examined long-term changes in glucose parameters and body composition assessed using BIA. We report the 3-year long-term changes in BW, body composition, and glucose metabolism after LSG.

2. Materials and Methods

2.1. Research Design and Participants

We enrolled 95 consecutive patients who performed LSG at Oita University Hospital between January 2014 and December 2021. Patients were treated according to the LSG surgical standards of the Japanese Ministry of Health, Labor, and Welfare. At 6 months and 1, 2, and 3 years after LSG, we examined the 3-year changes in glucose metabolism and body composition using BIA. Three years after LSG, changes in body composition and blood profiles were evaluated. There were 47 patients who lost follow-up due to the following reasons: transfer to another hospital (n = 23), COVID-19 infection (n = 5), and treatment interruption (n = 19) (Figure 1). Excluding patients who were unable to attend due to COVID-19, treatment interruption, or transfer to another hospital during this study, 48 patients (19 male and 29 females, mean body mass index [BMI] = 43.7 $\pm$ 8.7 kg/m^2, mean age = 42.2 $\pm$ 9.3 years) ultimately participated in this study. Among 48 patients, 26 patients have T2DM, and 32 patients have hypertension. The present research complied with the Declaration of Helsinki and received approval from the Ethics Committee of Oita University.

2.2. Surgery

The surgical techniques for LSG procedures have been previously reported [24,25]. In summary, following visual access to the intraperitoneal cavity using a 10 mm Visiport trocar (US Surgical, Norwalk, CT, USA) placed 18 cm below the xiphoid process, the abdomen was inflated to 15 mmHg. Subsequently, a 15 mm port, a 12 mm port, and two 5 mm ports were positioned in the upper abdomen, and a Nathanson liver retractor (Automated Medical Products Corp, Edison, NJ, USA) was introduced through a 5 mm skin incision in the subxiphoid area to hold back the left lateral segment of the liver. The vessel sealing system (LigaSure system, Valleylab, Boulder, CO, USA) was used to dissect the greater omentum from 5 cm proximal to the pyloric ring to the angle of His. Following the placement of a 10.5 mm (32-Fr) endoscope with or without a 15 mm (45-Fr) endoscopy tube directly along the lesser curvature of the stomach, endoscopic linear staplers (45 or 60 mm in length,

EndoGIA, US Surgical) were applied in a sequential manner, commencing 6 cm from the pylorus. Subsequently, the staple lines were reinforced to avoid bleeding and leakage. The specimen was obtained via the 15 mm port site. Ultimately, oral endoscopy was used to examine stenosis of the remnant stomach as well as hemorrhaging and air leakage from the staple line.

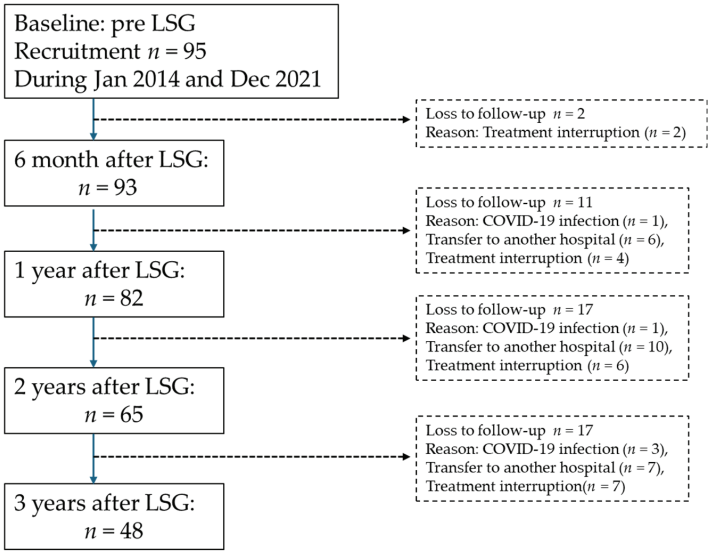

Figure 1. Flow chart of this study participants.

2.3. Collection of Measurement Parameters and Blood Data

Blood was collected between 8:00 and 11:00 h from the antecubital vein of subjects who had fasted overnight. Blood was collected from the participating patients and fasting plasma glucose (FPG), HbA1c, low-density lipoprotein (LDL), triglycerides, high-density lipoprotein (HDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma GTP, blood urea nitrogen (BUN), and creatinine (Cr) levels. Blood samples were collected prior to LSG at the first consultation, 6 months, 1 year, 2 years, and 3 years after LSG.

2.4. Analysis of Body Weight and Body Composition

According to previous report, ΔBMI was calculated as (Initial BMI) − (Post-LSG BMI). Percent of total weight loss (%TWL) was determined by [(Initial Weight) − (Post-LSG Weight)]/[(Initial Weight)] × 100. Percent excess weight loss (%EWL) was calculated as [(Initial Weight) − (Post-LSG Weight)]/[(Initial Weight) − (Ideal Weight)] × 100 [26]. Body composition, including FM, total muscle mass (TMM), skeletal muscle mass (SMM), body fluid, and bone mineral content (BMC), was measured periodically using a BIA device (InBody 770; InBody Japan., Ltd., Tokyo, Japan) according to previous reports. TMM is also defined as MM in the device. Body FM, MM, percentage of fat mass (%FM), and MM (%MM) were calculated using the following formula: %FM was determined by multiplying the percentage of fat by body weight (kg). %MM was calculated as MM (kg)/body weight (kg) × 100. %SMM was determined by SMM (kg)/body weight (kg) × 100. Body composition data were collected before bariatric surgery and at 6 months, 1 year, 2 years, and 3 years following LSG.

2.5. Statistical Analyses

Continuous variables are represented as mean ± standard deviation (SD). We have used a Shapiro–Wilk and Levene's test regarding the normality of the data distribution. Considering the normal distribution, analysis of variance (ANOVA) was utilized to evaluate the differences between baseline and postoperative data. The data at every time point were assessed using post-hoc multiple comparisons. The independent associations of these variables were evaluated using multiple regression analyses and simple correlation coefficients were examined. To evaluate the relationship between body composition and obesity, multiple regression analyses were performed, controlling potential confounders such as BMC. A *p*-value of less than 0.05 was considered statistically significant. All analyses were conducted using the JMP software (JMP14.1; SAS Institute, Cary, NC, USA).

3. Results

3.1. Clinical Characteristics of Patients and Changes in BW and BMI Following LSG

Table 1 displays the characteristics of the participants prior to surgery. Table 1 shows the changes in BW after LSG. None of the patients experienced serious adverse events or died during this study's period. BW and BMI both decreased at 6 months and 1, 2, and 3 years after LSG compared with preoperatively ($p < 0.01$). There were no notable differences in BW and BMI after 6 months and at the 3 years. There were no differences in BW and BMI change between men and women after LSG (BW (men vs. women: $p = 0.22$) and BMI (men vs. women: $p = 0.19$).

Table 1. Basal clinical characteristics and time-course changes in BW, blood pressure, plasma parameters, and antidiabetic, antihypertensive, and lipid-lowering medications use.

	Pre-LSG	6 Months	1 Year	2 Years	3 Years
Body weight (kg)	116.1 ± 24.4	82.4 ± 17.2 **	80.0 ± 18.5 **	81.6 ± 22.0 **	84.6 ± 21.6 **
%TBWL		28.5 ± 8.3	30.7 ± 10.5	29.6 ± 12.7	27.2 ± 11.9
%EBWL		60.6 ± 19.5	64.8 ± 23.5	63.0 ± 27.7	57.3 ± 24.7
BMI (kg/m^2)	43.7 ± 8.7	31.4 ± 6.7 **	30.2 ± 6.5 **	30.4 ± 7.4 **	31.7 ± 7.6 **
Systolic blood pressure (mmHg)	137.0 ± 17.5	119.9 ± 19.4 **	120.8 ± 16.0 **	125.1 ± 18.0 **	125.3 ± 16.6 **
Diastolic blood pressure (mmHg)	83.7 ± 12.4	73.0 ± 11.4 **	72.7 ± 11.1 **	75.6 ± 12.5 **	75.5 ± 12.5 **
Fasting plasma glucose (mg/dL)	116.5 ± 35.2	99.8 ± 26.7 **	96.8 ± 20.3 **	97.5 ± 26.9 **	92.9 ± 24.6 **
HbA1c (%)	6.8 ± 1.3	5.6 ± 0.8 **	5.7 ± 0.9 **	5.7 ± 0.9 **	5.7 ± 0.8 **
Triglycerides (mg/dL)	170.3 ± 84.8	103.4 ± 47.5 **	100.3 ± 77.8 **	97.4 ± 49.2 **	100.1 ± 55.2 **
HDL cholesterol (mg/dL)	48.1 ± 11.1	58.5 ± 16.2 **	63.7 ± 16.3 **	68.0 ± 19.0 **	70.6 ± 19.5 **
LDL cholestrol (mg/dL)	125.4 ± 32.3	121.5 ± 30.7	119.2 ± 32.1	112.6 ± 29.4	111.4 ± 29.0
BUN (mg/dL)	12.6 ± 3.8	13.2 ± 4.7	14.1 ± 4.3	13.6 ± 4.7	13.7 ± 4.1
Creatinine (mg/dL)	0.7 ± 0.2	0.7 ± 0.1 *	0.7 ± 0.2	0.7 ± 0.2 **	0.7 ± 0.2 **
AST (IU/L)	35.0 ± 26.1	17.1 ± 5.5 **	17.7 ± 4.5 **	17.7 ± 5.3 **	18.2 ± 4.5 **
ALT (IU/L)	49.6 ± 37.6	14.6 ± 5.8 **	16.5 ± 6.9 **	16.0 ± 7.5 **	17.5 ± 8.4 **
GTP (IU/L)	48.3 ± 30.5	17.9 ± 12.0 **	16.6 ± 7.4 **	16.3 ± 7.5 **	17.9 ± 8.2 **
Antidiabetic drugs use (%, n)	50.0(24/48)	6.2(3/48)	6.2(3/48)	12.5(6/48)	14.6(7/48)
Antihypertensive drugs use (%, n)	66.7(32/48)	14.6(7/48)	25.0(12/48)	27.1(13/48)	22.9(11/48)
Lipid-lowering drugs use (%, n)	29.2(14/48)	16.7(8/48)	16.7(8/48)	25.0(12/48)	27.1(13/48)

TBWL, total body weight loss; EBWL, excessive body weight loss; BMI, Body mass index; BW, body weight. * $p < 0.05$; ** $p < 0.01$ (indicate significant changes compared to pre-LSG assessed by ANOVA).

3.2. Changes in Plasma Metabolic Parameters and Antidiabetic, Antihypertensive, and Lipid-Lowering Medications Use Following LSG

The glucose metabolic parameters FPG and HbA1c both decreased at 6 months and 1, 2, and 3 years after LSG compared to before treatment ($p < 0.01$, respectively) (Table 1). AST, ALT, GTP, and triglyceride levels decreased at 6 months and 1, 2, and 3 years after LSG compared to preoperative levels ($p < 0.01$). Conversely, plasma HDL-C levels increased at 6 months and 1, 2, and 3 years after LSG compared to preoperative levels (Table 1). There were no notable differences between 1 year and 2 years, 2 years vs. 3 years, or 1 year and 3 years for FPG, HbA1c, AST, ALT, GTP, LDL-C, and triglyceride levels ($p > 0.1$).

There were no significant changes in BUN levels throughout this study's period ($p > 0.1$). In the present study, 50% (24/48) of patients were taking antidiabetic medications (DPP-4 inhibitors, 12 patients; glucagon-like peptide 1 receptor agonists, 5 patients; SGLT2 inhibitors, 6 patients; metformin, 13 patients; sulfonylureas, 5 patients; insulin, 3 patients; and others, 7 patients) as type-2 diabetes prior to bariatric surgery. In addition, 67% (32/48) of patients were taking antihypertensive medications (angiotensin II receptor blockers, 18 patients; mineralocorticoid receptor antagonists, 1 patient; calcium channel blockers, 16 patients; and others, 7 patients). Additionally, 29% (14/48) of patients were taking lipid-lowering medications (Statins, 11 patients; Fibrates, 3 patients; and others, 1 patient). As shown in Table 1, the proportion of patients who were taking any glucose-lowering, antihypertension, or lipid-lowering medications all decreased 1, 2, and 3 years after surgery compared to the levels at pre-surgery, respectively (Table 1). The percentage of patients with diabetes remission was 62% (16/26) at 3 years after surgery. In addition, the percentage of patients with normal blood pressure without antihypertensive drugs was 56% (18/32).

3.3. Time-Course Changes of FM and, % FM After LSG

Table 2 shows changes in body composition following LSG surgery. Both FM and %FM were significantly reduced and maintained at 6 months, 1, 2, and 3 years compared to pre-LSG ($p < 0.01$, respectively) (Table 2). There were no notable differences in FM and %FM between years 1 and 2 and between years 2 and 3 ($p > 0.1$, respectively). There was no difference in FM change between men and women after LSG ($p = 0.41$).

Table 2. Time-course changes in body composition.

	Pre-LSG	6 Months	1 Year	2 Years	3 Years
FM (kg)	53.2 ± 15.1	30.0 ± 13.1 *	27.8 ± 13.0 *	29.2 ± 15.5 *	32.1 ± 15.6 *
FM (%)	47.3 ± 6.5	35.7 ± 10.0 *	33.9 ± 9.4 *	34.6 ± 10.5 *	37.2 ± 10.0 *
Total MM (kg)	55.2 ± 10.2	48.9 ± 9.4 *	48.6 ± 9.3 *	48.4 ± 9.8 *	48.1 ± 9.6 *
Total MM/BW	0.50 ± 0.06	0.60 ± 0.09 *	0.62 ± 0.09 *	0.61 ± 0.10 *	0.59 ± 0.09 *
Skeletal MM (kg)	32.4 ± 6.4	28.3 ± 5.9 *	28.1 ± 5.9 *	28.0 ± 6.2 *	27.9 ± 6.1 *
Skeletal MM/BW	0.29 ± 0.04	0.35 ± 0.06 *	0.36 ± 0.05 *	0.36 ± 0.06 *	0.35 ± 0.07 *
Upper Skeletal MM/BW	0.06 ± 0.01	0.07 ± 0.01 *	0.07 ± 0.01 *	0.07 ± 0.01 *	0.07 ± 0.01 *
Lower Skeletal MM/BW	0.16 ± 0.03	0.20 ± 0.03 *	0.20 ± 0.03 *	0.20 ± 0.03 *	0.20 ± 0.04 *
Body fluid	42.0 ± 9.6	37.2 ± 8.5 *	37.0 ± 8.5 *	36.8 ± 8.8 *	36.6 ± 8.7 *
Bone mineral content	2.95 ± 0.72	2.98 ± 0.57	2.96 ± 0.54	2.91 ± 0.54 *	2.86 ± 0.53 *
The ratio of extra cellular fluid	0.387 ± 0.009	0.394 ± 0.009 *	0.394 ± 0.010 *	0.393 ± 0.009 *	0.391 ± 0.008 *

FM, fat mass; MM, muscle mass. * $p < 0.01$ (indicates significant changes compared to pre-LSG assessed by ANOVA).

3.4. Changes in Muscle Mass and the Ratio of Extra Cellular Fluid and Bone Mineral Content After LSG

TMM and SMM at 6 months, 1 year, 2 years, and 3 years decreased compared to the preoperative values ($p < 0.01$) (Table 2); however, TMM and SMM were maintained from 6 months to 3 years after LSG ($p > 0.1$). Compared with the pre-LSG values, both %TMM and %SMM increased at 6 months and 1, 2, and 3 years, respectively (Table 2). No significant differences were observed in MM or %MM between 1 and 2 years and between 2 and 3 years ($p > 0.1$). Body fluid was significantly reduced and maintained at 6 months and at 1, 2, and 3 years compared to pre-LSG ($p < 0.01$). Conversely, the ratio of extracellular fluid significantly increased at 6 months, 1 year, 2 years, and 3 years after LSG ($p < 0.01$). BMC was reduced at 2 and 3 years after LSG compared to preoperative levels. There were no differences in TMM and SMM changes between men and women after LSG (TMM (men vs. women: $p = 0.27$) and SMM (men vs. women: $p = 0.25$).

The upper and lower leg muscles both decreased at 6 months post-LSG ($p < 0.01$) and were maintained for up to 3 years post-LSG (Table 2). In contrast, the ratio of the lower leg muscles and body weight (L/W) and upper leg muscles and body weight (U/W) both increased at 6 months post-LSG ($p < 0.01$) (Table 2) and were maintained up to 3 years

post-LSG. The ratio of the upper Skeletal MM to the lower Skeletal MM (U/L) significantly decreased at 6 months, 1 year, 2 years, and 3 years after LSG. This result showed that the lower MM was maintained more than the upper MM after LSG.

3.5. Relationship Between Alterations in Body Compositions and Variations in BW and Glycemic Metabolic Parameters 3 Years Post-LSG

Changes in BW were associated with changes in FM (r = 0.91; $p < 0.01$), %FM (r = 0.71; $p < 0.01$), TMM (r = 0.68; $p < 0.01$), %TMM (r = −0.67; $p < 0.01$), % SMM (r = −0.54; <0.01), U/W (r = −0.46; $p < 0.01$), and L/W (r = −0.55; $p < 0.01$). Changes in FPG levels were associated with changes in FM (r = 0.30; $p = 0.05$). Changes in HbA1c levels were correlated with changes in FM (r = 0.47; $p < 0.01$), %FM (r = 0.35; $p = 0.02$), TMM (r = 0.36; $p = 0.02$), and % total MM (r = −0.33; $p = 0.03$). Figure 2 shows the associations among delta-BW-FM, delta-FPG-FM, and delta-HbA1c-%TMM.

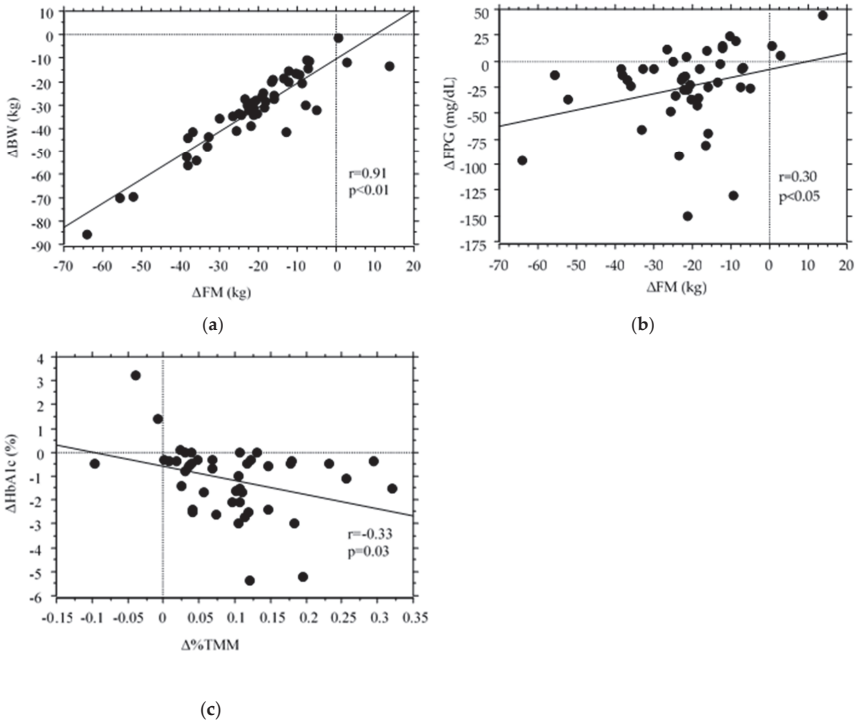

Figure 2. The relationship between variations in glycemic metabolic parameters and body composition. (**a**) Association between BW and FM, (**b**) association between FPG and FM, and (**c**) association between HbA1c and % TMM. Variables: Δ (0–3 years) variables, BW: body weight, MM: muscle mass, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c. Correlation coefficients were simply calculated. r = correlation coefficient.

3.6. 3 Years Post-LSG, Multiple Regression Analyses Regarding Changes in Body Weight, FPG, and HbA1c

Multiple regression analyses were conducted to evaluate the relationship between body composition and glycemic parameters while controlling for possible confounders, such as BMC. Changes in BW were used as the dependent variable in multiple regression analyses, with FM, %FM, MM, %MM, U/W, and L/W as the independent variables. Alterations in FM were the factor independently linked to variations in BW ($p < 0.01$) (Table 3). Conversely, no individual factor showed a correlation with alterations in FPG or HbA1c levels ($p > 0.05$).

Table 3. Multiple linear regression models with body weight as the dependent variable.

Variables	*t*-Value	*p*-Value
FM	5.44	<0.01 *
% FM	−0.40	0.69
Total MM	1.01	0.31
%Total MM	−0.29	0.77
Upper Skeletal MM/BW	0.91	0.37
Lower Skeletal MM/BW	−0.59	0.56

FM, fat mass; MM, muscle mass, * $p < 0.01$ significant correlation between factors.

4. Discussion

This is the first research to assess prolonged alterations in weight loss, glucose/lipid metabolism, and body composition over a 3-year period after LSG in Japanese patients with obesity. Body weight, FPG, HbA1c, FM, and %FM decreased over 3 years. In contrast, % TMM and % SMM increased over the long period of 3 years after LSG. A continuous reduction of around 50% of excess body weight is regarded as successful in weight loss [26–28]. In this study, compared to the preoperative weight, the %EBWL was almost 60% after three years, which was largely maintained.

Our previous analysis of body composition 12-month after LSG in Japanese patients with obesity showed a significant decrease in FM [17,29]. This is significant considering that the loss of FM is important for the improvement of glucose and lipid metabolic disorders 12-month after surgery. In the present study, FM decreased even 3 years after LSG compared to preoperative levels. In addition, FPG and HbA1c levels decreased at 6 months and 1, 2, and 3 years after LSG compared with preoperative levels.

The loss of MM could be associated with a higher risk of developing diabetes. During the ongoing 3-year study, TMM at 6 months, 1 year, 2 years, and 3 years showed a decrease in comparison to the preoperative values, but TMM was maintained from 6 months to 3 years after LSG. In addition, for pre-LSG, both %TMM and %SMM increased at 6 months and 1, 2, and 3 years, respectively. Similarly, MM in the upper and lower extremities was maintained compared to the preoperative values. This finding regarding the maintenance of TMM and increase in % TMM may be related to the improvement in glucose metabolism even 3 years after surgery. As mentioned above, FPG and HbA1c levels both improved and were maintained over a long period of 3 years after LSG. Strengthening skeletal muscle benefits the glycemic profile through mechanisms such as improved glucose utilization, and enhanced muscle maintenance benefits glucose metabolism [13,30]. The results indicated that FM, %FM, and %MM were important risk factors for obesity and diabetes after 3 years of LSG. In the present study, univariate analysis showed that ΔBW and ΔFM were related, indicating an association between weight loss and fat mass reduction with LSG surgery. Reducing fat mass correlates with a decrease in fasting blood glucose levels. In contrast, %TMM and ΔHbA1c were inversely correlated, indicating that HbA1c levels increased as total muscle mass decreased. The weight loss induced by LSG was related to a decrease in fat mass, indicating that the enhancement in glucose tolerance parameters might be due to both a reduction in fat mass and an increase in muscle percentage. Because the MM is an energy-metabolizing active organ, a sustained significant decrease in the MM may lead to weight rebound via a decrease in the basal metabolic rate. Taken together, the decrease in FM, maintenance of MM, and increase in %MM may have contributed to the improved glucose metabolism. Finally, the multivariate analysis showed that weight loss was specifically defined by fat mass. In the analysis after 1 year of LSG, correlations between body fat and body weight, muscle mass, and A1C were found in the multivariate analysis [17]. In contrast, only a correlation between body fat and body weight was found three years after LSG. The current study found that a decrease in body fat was most closely related to weight loss.

In the present study, we discuss the mechanisms underlying decreased FM and MM maintenance. Possible mechanisms for the decrease in FM and MM maintenance are an

increase in appropriate protein intake through nutritional guidance using body composition results in our institution and an overall increase in lower extremity physical leg activity due to weight loss. In fact, the lower MM is better maintained than the upper MM 3 years after LSG.

Many studies indicate that bariatric surgery leads to more sustained weight loss and reduced rebound rates compared to intensive medical therapy alone [31–33]. Surgery for obesity is more successful than traditional medical treatment in managing type-2 diabetes and enhancing life expectancy [29,31,34]. Nonetheless, there are limited studies indicating long-term changes in body composition, including body fat and muscle mass. It is important to examine the long-term changes in body composition after LSG. It has been shown that the body composition of Japanese subjects with obesity differs significantly from that of non-Japanese subjects. Japanese individuals with obesity are more likely to have abdominal fat and develop T2DM than Caucasian individuals [21,22]. It would also be interesting to investigate the association between variations in visceral adipose tissue and subcutaneous adipose tissue and impaired glucose metabolism in Japanese individuals with obesity following LSG.

The current study demonstrated that the impact of weight loss in Japanese individuals with obesity was maintained for an extended duration of three years. Loss of muscle mass and sarcopenia after LSG is an important issue in non-Japanese subjects [35]. In the present study, body composition indicated that a decrease in fat mass and preservation of % muscle mass could be sustained over a three-year period in Japanese subjects. It is a possibility that there are racial differences in MM maintenance.

5. Limitations

Our study had several limitations. Potential statistical overfitting due to small sample size and potential confounders. The first is the observational nature of the small sample size. The number of bariatric surgery cases per year in Japan is very small compared to other developed countries. Hence, large surgery cohorts involving sub-groups are very hard to perform in our institution. Second, this study could not identify the determining factors that influenced body composition, as lifestyle and therapy modifications may have influenced body composition after LSG. Third, the adiposity analysis did not separate subcutaneous and visceral fat in this study.

6. Conclusions

Bariatric surgery has a significant impact on body composition, including FM and MM. Over a 3-year period, we observed improvements in FM, fasting plasma glucose, and HbA1c levels among Japanese patients following LSG. The decrease in FM and the maintenance of %MM following LSG were proposed as potential connections between the impacts of LSG on obesity and diabetes over a period of 3 years. Well-designed studies are required to enhance the strategies for decreasing FM and preserving MM following LSG.

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Institutional Review Board Statement: This study received approval from the Ethics Committee of Oita University (21/July/2023 as protocol code 1761) and adhered to the Declaration of Helsinki.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding authors.

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Conflicts of Interest: The authors state that the research was carried out without any financial relationships that might be seen as possible conflicts of interest.

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