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Nutrient Intake and Physical Exercise as Modulators of Healthy Women

Edited by
Sílvia Rocha-Rodrigues, José Afonso and Monica Sousa

Printed Edition of the Special Issue Published in *Nutrients*

www.mdpi.com/journal/nutrients

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Sílvia Rocha-Rodrigu
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Editors

Sílvia Rocha-Rodrigu
Instituto Politécnico de Viana do Castelo, Escola Superior de Desporto e Lazer
Portugal

José Afonso
University of Porto
Portugal

Monica Sousa
Universidade NOVA de Lisboa
Portugal

Editorial Office
MDPI
St. Alban-Anlage 66
4052 Basel, Switzerland

This is a reprint of articles from the Special Issue published online in the open access journal *Nutrients* (ISSN 2072-6643) (available at: https://www.mdpi.com/journal/nutrients/special_issues/Nutrient_Intake_and_Physical_Exercise).

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-0365-6085-4 (Hbk)
ISBN 978-3-0365-6086-1 (PDF)

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About the Editors

Sílvia Rocha-Rodrigues

Dr. Sílvia Rocha-Rodrigues is a full-time assistant Professor at Escola Superior de Desporto e Lazer – Instituto Politécnico de Viana do Castelo, researcher member at Tumor & Microenvironment Interactions Group - i3S and a coordinator for designing and prescribing physical exercise for breast cancer patients. She has a Ph.D in Physical Activity and Health (2017), master degree in Physical Activity and Health (2010) and a graduation in Physical Education and Sport (2007). She is a member of the editorial board of Journal of Endocrinology Research, International Journal of Endocrinology and Diabetes, Nutrients and an active member of academic organizations.

Her research interests are exercise training, obesity, skeletal muscle and adipose tissue physiology, and breast cancer.

José Afonso

Dr. José Afonso is a Full-time Professor at the Faculty of Sport of the University of Porto, with a Ph.D. in Sports Sciences and Post-Doctoral Studies in Exercise Periodization. He is a Review Editor for Frontiers in Psychology, Frontiers in Physiology and Frontiers in Sports and Active Living. He has published more than 150 papers in peer-reviewed journals, as well as several peer-reviewed books and book chapters. He regularly collaborates with the Portuguese Institute for Sport and Youth, as well as the Portuguese Olympic Committee.

Monica Sousa

Dr. Monica Sousa has a bachelor's degree in Nutrition Sciences and a PhD in Sports Sciences. She also holds the IOC Diploma in Sports Nutrition from the International Olympic Committee and the International Accreditation in Anthropometry level 2 from the International Society for the Advancement of Kinanthropometry (ISAK).

She is currently an invited Assistant Professor at NOVA Medical School/Faculdade de Ciências Médicas, NOVA University of Lisbon, in Nutrition and Lifestyle. She is also a researcher at the ProNutri group - Clinical Nutrition & Disease Programming, CINTESIS.

Mónica is the Head of the Nutrition Department of Sporting Clube de Portugal. She has also clinical roles at the Portuguese Athletics Federation and at the Lifestyle Medicine University Unit - José de Mello Saúde by NOVA Medical School. Additionally, Mónica is an external Nutrition Consultant for the Medical & Anti-Doping Commission of the European Athletics.

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Editorial

Nutrition and Physical Exercise in Women

Sílvia Rocha-Rodrigues [1,2,*], José Afonso [3] and Mónica Sousa [4,5]

[1] Escola Superior de Desporto e Lazer, Instituto Politécnico de Viana do Castelo, Rua Escola Industrial e Comercial de Nun'Alvares, 4900-347 Viana do Castelo, Portugal
[2] Tumour & Microenvironment Interactions Group, INEB—Institute of Biomedical Engineering, i3S-Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Rua Alfredo Allen, 208, 4200-153 Porto, Portugal
[3] Centre for Research, Education, Innovation and Intervention in Sport (CIFI2D), Faculty of Sport, University of Porto, Rua Dr. Plácido Costa, 91, 4200-450 Porto, Portugal; jneves@fade.up.pt
[4] Nutrition and Lifestyle, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade Nova de Lisboa, Campo Mártires da Pátria, 130, 1169-056 Lisboa, Portugal; monica.sousa@nms.unl.pt
[5] CINTESIS-Center for Health Technology Services Research, NOVA Medical School, Faculdade de Ciências Médicas, NMS, FCM, Universidade NOVA de Lisboa, 1169-056 Lisboa, Portugal
* Correspondence: silviadarocharodrigues@gmail.com

Citation: Rocha-Rodrigues, S.; Afonso, J.; Sousa, M. Nutrition and Physical Exercise in Women. *Nutrients* **2022**, *14*, 2981. https://doi.org/10.3390/nu14142981

Received: 7 July 2022
Accepted: 13 July 2022
Published: 21 July 2022

While the benefits of nutrition and physical exercise are commonly studied separately, their concomitant integration has the potential to produce greater benefits in women than strategies focusing only on one or the other [1]. Studying the specificities of women in response to interventions is of the utmost importance for providing optimal healthcare and aids specifically designed guidelines that are better suited for women [1]. Women have a number of specificities that differentiate them from men, particularly the variations of sex steroid hormone, especially oestrogens and progestogens, which significantly impact women's physiology [2,3]. Cumulative evidence demonstrates that the combination of healthy nutrient intake and regular physical exercise is a powerful lifestyle strategy that modulates lifelong health through its ability to improve body composition, sex-steroid hormones, and physical performance and prevent chronic diseases across the lifespan [1].

With this Special Issue on "Nutrient Intake and Physical Exercise as Modulators of Healthy Women", we are honoured to contribute with important pieces of evidence for an integrational approach—nutrition and physical exercise—as a potential modulator of lifelong. It includes ten studies: eight articles and two narrative reviews. Please let us introduce the articles with a short summary.

The differences in substrate oxidation between men and women have been a topic of interest in the past few years. In this line, Nosaka et al. [4] performed a randomised, double-blind, placebo-controlled, three-arm, within-participants crossover trial during three 14-day interventions separated by two 14-day washout periods to analyse the impact of medium chain fatty acids supplementation on men and women aged 40–59 years. Women showed an increase in carbohydrate oxidation and oxygen uptake during the exercise trial after the C10R diet, while no changes in fatty acid oxidation were detected compared to men. Of note, the differences between sex on the type of substrate oxidation and on strategies to enhance oxidative pathways, both during exercise, are definitively topics that need further enlightenment.

In elderly women, Amirato et al. [5] found that 30 days of L-glutamine supplementation (10 g/day) and endurance and resistance exercise significantly improved glycemia control, plasma antioxidant capacity, and strength and power of knee muscles. Dechichi et al. [6] focused their studies on a very special phase in a woman's life—menopause. The topic of women's health during menopause has been brought to attention in the past few years, with isoflavones being tested as a substitute for endogenous oestrogens. In non-obese post-menopausal women, Dechichi et al. [6] aimed to study if the isoflavones supplementation

had additive effects on aerobic and resistance exercise on resting and ambulatory blood pressure monitoring, and on blood pressure variability, in non-obese postmenopausal women. The authors reported no additional effects of isoflavone supplementation on these blood pressure-related variables.

In apparently healthy women, Waliko et al. [7] assessed whether rumination (a form of repetitive negative thinking) could lead to changes in eating behaviours (usually in a maladaptive direction). Overall, 188 women were recruited for the study (83% response rate), and rumination was partially associated with uncontrolled eating and emotional eating. The authors propose that targeting rumination could help in reducing uncontrolled and/or emotional eating. However, due to the nature of the study design, no causal inferences should be made. Moreover, the terminology of "predictors" that was used across the manuscript should be best interpreted as "correlates". While it is possible that rumination increases the likelihood of maladaptive eating behaviours, it is also possible that maladaptive eating behaviours stimulate rumination. Still, breaking this potential positive feedback loop could help the subjects return to their healthier eating patterns; additionally, acting on rumination may provide an interesting strategy for achieving this intent.

In overweight or obese women with hypertension, Dos Santos Fechine et al. [8] found that 8-weeks of fibre supplementation (guar gum, NutraFlora, psyllium and microcrystalline cellulose) resulted in differential metabolite response when compared to a control group. Specifically, the experimental group observed an increase in peak intensity values of all three analysed metabolites, accompanied by a decrease in blood pressure. Overall, the results suggest that overweight and obese women with hypertension could benefit from the intake of mixed dietary fibre.

The term normal weight obesity (NWO) was firstly described by Lorenzo et al. [9] and was defined as a body mass index ≤ 25 kg/m^2 but with an increased body fat percentage. The cut-offs used for body fat percentage have varied depending on the study population, sex, and ethnicity. NWO is a widespread public health issue that may be prevalent in up to one-third of individuals [10]. In NWO women, Haghighat et al. [11] reported that six-month of soy-enriched high-protein snack meals containing 50 g of soybean (vs. low-protein snacks containing 3.5 servings of fruits) improved body composition parameters, including increased skeletal muscle mass and reducing body mass, body fat percentage, and appetite levels. These findings underline the clinical applicability that consuming a soy-enriched high protein snack replacement may provide a practical approach to controlling energetic intake and improving body composition in cohorts with NWO.

Attention to preferred physical activities encourages participation in physical activity. Participation in physical activity is associated with a better body size perception but not in a consistent way. In this line, Hubert et al. [12] reported that collegiate women (n = 251; 74% white), who perceived greater body size, reported less liking of physical activity as well as less healthy dietary behaviours. Additionally, women whom both liked and engaged in physical activities had a lower body size perception and healthier diet quality.

In a prospective randomised trial, Bak-Sosnowaska et al. [13] found that all women aged 18 to 65 years with BMI ≥ 30 kg/m^2) presented significant changes in the anthropometric parameters, namely decreased body weight, waist and hips circumference, as well as body mass index (BMI) and waist-to-hip ratio (WHR) after three months of endurance or endurance strength training intervention. Additionally, those alterations were associated with a better perception of the current figure and a lower level of concern about body shape, with more significance for the endurance training group compared to the endurance strength training group.

In Rocha-Rodrigues et al. [14]'s review, an overview of the mechanisms behind the relationship between menstrual cycle, exercise, and nutritional intake in women was made by exploring the roles of oestrogens and progestogens in response to exercise and how exercise impacts their regulation. Although some internal physiological parameters vary across the menstrual cycle, their impact on performance seems to be highly dependent from woman to woman, and the magnitude of effects tends to be residual or trivial at best.

Moreover, the energy demands and nutritional intake in women in relation to hormonal fluctuations face similar difficulties, as higher energy expenditure in some phases of the menstrual cycle tends to be naturally compensated by an increase in dietary intake. Indeed, this is a promising field of research, but one where the search for populational trends may have to be replaced by highly individualised approaches due to the considerable heterogeneity and variability of responses.

The roles of diet and nutrition on gynaecological disorders were reviewed by Afrin et al. [15]. The evidence suggests that nutritional habits play a role in the development of gynaecological disorders, although it is not clear whether this relationship is associative or causative; if causative, it is unclear whether nutritional habits may contribute to causing such disorders or merely aggravate already existing disorders, or even if gynaecological disorders cause changes in nutritional habits. When gynaecological disorders are installed, varied diets (especially rich in fruits and vegetables), green tea, vitamin D, and other resources may help achieve better development of the disorder. Conversely, fat, red meat, alcohol, and coffee may aggravate the condition. As the authors promptly state, all these associations should not be interpreted causally and may be confounded by numerous factors, including social and environmental factors and levels of physical activity. The authors alert that experimental, prospective, randomised studies are required to better understand the relationships between nutritional habits and gynaecological disorders.

Author Contributions: Conceptualisation, S.R.-R. and J.A.; writing—review and editing, S.R.-R., J.A. and M.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The editors would like to thank all participating authors, all the reviewers for their work in evaluating the submitted articles and the editorial staff of nutrients.

Conflicts of Interest: The author declares no conflict of interest.

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Article

Isoflavone Supplementation Does Not Potentiate the Effect of Combined Exercise Training on Resting and Ambulatory Blood Pressure in Non-Obese Postmenopausal Women: A Randomized Double-Blind Controlled Trial-A Pilot Study

Juliene G. C. Dechichi [1], Igor M. Mariano [1], Jéssica S. Giolo [1], Jaqueline P. Batista [1], Ana Luiza Amaral [1], Paula A. B. Ribeiro [2], Erick P. de Oliveira [3] and Guilherme M. Puga [1,*]

[1] Laboratory of Cardiorespiratory and Metabolic Physiology, Physical Education and Physical Therapy Department, Federal University of Uberlândia, 38400-678 Uberlândia, MG, Brazil; julienegoncalves@hotmail.com (J.G.C.D.); igormmariano@gmail.com (I.M.M.); sanjuliaogiolo@hotmail.com (J.S.G.); jaquebpontes@gmail.com (J.P.B.); anaribeiro.am@gmail.com (A.L.A.)

[2] Research Center of University of Montreal Hospital Centre, Montreal, QC H2X 0A9, Canada; paulaabribeiro@gmail.com

[3] Laboratory of Nutrition, Exercise and Health (LaNES), School of Medicine, Federal University of Uberlandia (UFU), 38402-022 Uberlândia, MG, Brazil; erick_po@yahoo.com.br

* Correspondence: gmpuga@ufu.br; Tel.: +55-343218-2965

Received: 6 October 2020; Accepted: 9 November 2020; Published: 13 November 2020

Abstract: Physical exercise and isoflavone supplementation are potential strategies to prevent and treat cardiovascular diseases in postmenopausal women. The aim of this study was to investigate whether there are additive effects of isoflavone supplementation when associated with combined aerobic and resistance exercise on resting and ambulatory blood pressure monitoring (ABPM) and in blood pressure variability (BPV). Thirty-one non-obese postmenopausal women were randomly allocated into two groups: placebo and exercise (Placebo $n = 19$); and isoflavone supplementation (100 mg/day) and exercise (isoflavone $n = 19$). ABPM and BPV were evaluated before and after 10 weeks of moderate combined (aerobic and resistance) exercise training. Generalized Estimating Equation (GEE) with Bonferroni correction and intention-to-treat analysis was used to compare the effects of interventions on resting BP, ABPM and BPV. Combined exercise training decreased resting systolic (SBP) and diastolic blood pressure (DBP) and reduced 24 h and awake ambulatory SBP, DBP and mean blood pressure over time, with no additional effects of isoflavone supplementation. No changes were observed in sleep period, or in BPV indexes (Standard Deviation of 24 h (SD), daytime and nighttime interval (SDdn) and average real variability (ARV) in both groups. We conclude that isoflavone supplementation does not potentiate the effects of combined training on resting and ambulatorial systolic and diastolic blood pressure in non-obese postmenopausal women.

Keywords: soy; ambulatory blood pressure monitoring; aerobic exercise; menopause; blood pressure variability

1. Introduction

Several physiological changes (especially in endocrine system) occur during the transition period of menopause due to the influences of the aging process, lifestyle and hypoestrogenism [1]. These factors can lead to an increase in blood pressure (BP) and body mass index, which can intensify the risk of developing cardiovascular diseases and cardiovascular events [2,3]. The prevalence of hypertension

is significantly higher in individuals older than 55 years, and postmenopausal women are more affected by this disease compared with premenopausal women [2] or men at the same age [4,5].

Non-drug interventions have been sought for the benefits of reduced cost, minimal risk and proven results in reducing BP [6,7]. Among the interventions used, supplementation with phytoestrogens such as isoflavone (ISO; soy-derived compound) have been used for women in the menopause transition as an alternative to the effects of reduced estrogen production [8]. The use of ISO by postmenopausal women may have benefits in reducing cardiovascular diseases, climacteric symptoms [9] and lipid levels [10]. A meta-analysis [11] showed that isoflavone supplementation is beneficial for the reduction of systolic (SBP) and diastolic blood pressure (DBP), −5.9 mmHg and 3.3 mmHg, respectively, in hypertensive individuals; however, the results for normotensive individuals are still controversial. In addition, in rats supplemented with isoflavone for more than six months, there was an increase in endothelial nitric oxide synthase (eNOS)activity and an increase in the increase in nitric oxide (NO)bioavailability [12], which may interfere in blood flow and blood pressure responses.

Another strategy for reducing BP is exercise, which improves the bioavailability of NO [3,7], endothelial function and hemodynamic and cardiovascular parameters such as blood flow and BP [3]. In general, exercise training results in important autonomic and hemodynamic adaptations that influence the cardiovascular system, decreasing resting BP levels and improving ambulatory BP monitoring (ABPM) [13] and its variability (BPV, i.e., Ambulatory Blood Pressure Variability) [14]. These improvements may be important for the prevention and treatment of hypertension and have been shown usually in continuous moderate aerobic or resistance exercise trials [15]. However, there is a lack of studies showing ABPM and BPV responses after combined training (aerobic and resistance in the same session) protocols, especially in postmenopausal women.

We have previously shown that associating isoflavone supplementation with physical training does not modify markers of oxidative stress in postmenopausal women [16]. Further, few studies have investigated the use of isoflavone supplementation therapy associated with exercise in BP responses [17]. These results are still inconclusive, and we do not know any study that has evaluated BPV. Therefore, our hypothesis is that the supplemented group would present a greater hypotensive response to training, i.e., ISO supplementation enhances the training effect, which may be due to an increase in blood flow and endothelium-mediated vasodilation by the NO release [18–20]. Therefore, the aim of this study was to investigate whether there are additive effects of isoflavone supplementation, when associated with combined aerobic and resistance exercise, on resting BP, ABPM, BPV and nitrite in non-obese postmenopausal women.

2. Materials and Methods

2.1. Participants

Inclusion criteria were: age between 50–70 years, having amenorrhea of at least 12 uninterrupted months; body mass index 25–30 kg/m^2; being able to perform treadmill and resistance exercises; no history of cardiovascular disease, stroke or acute myocardial infarction, cancer, kidney disease, diabetes or hypertension; does not use any hormone therapy or soy supplementation; non-smoker; does not use hypoglycemic agents. This study was approved by the Human Research Ethics Committee of the Federal University of Uberlândia under registration number 40622414.9.0000.5152, and all volunteers signed a consent form. The experiments followed the principles of the Declaration of Helsinki and were registered at Clinicaltrials.gov (number: NCT03008785).

This pilot randomized double-blind controlled trial was developed in two stages: baseline and after 10 weeks of ISO supplementation and training with combined exercises (aerobic and resistance). Participants were randomly allocated through an electronic draw program into two groups: exercise with placebo (PLA; n = 14); and exercise with isoflavone supplementation (ISO; n = 17). At the three weekly visits for training, the capsules were monitored and delivered by the researchers containing ISO or placebo (indistinguishable-same shape, size and color) and on the days that participants did not go

to the laboratory, they were advised to take one capsule a day at the same training time. The capsules were previously packed in identical bottles and numerically organized according to the randomization schedule for each patient. The bottles were coded by a blinded researcher, so the content was unknown to researchers and participants. The ISO group ingested a dose of 100 mg of isoflavone (74.4 mg of aglycone equivalent) containing 3.3% genistein, 93.5% daidzein and 3.2% glycitein. The capsules ingested by the placebo group were non-active capsules containing 100 mg of corn starch. A blinded statistician evaluated all the results.

2.2. Outcomes

The primary outcome was blood pressure response assessed by the monitoring of ambulatory blood pressure. The secondary endpoints were blood nitrite concentration, heart rate variability, blood pressure variability, body composition and muscle strength.

2.3. Evaluation of Food Intake and Anthropometry

The food intake evaluation was performed using three 24 h dietary recalls, applied by nutritionists on non-consecutive days. The dietary data analyses were performed using a web-based program (Dietpro® v5.7, Viçosa, MG, Brazil) and the United States Department of Agriculture (USDA) food composition table.

Body mass was measured using an electronic scale (Micheletti, São Paulo, SP, Brazil), and stature was measured via a stadiometer (Sanny, São Paulo, SP, Brazil). An inelastic tape (Sanny, São Paulo, SP, Brazil) measuring 0.5 cm wide was used for waist circumference measurements. Total lean body mass (LBM) and fat mass (FM) were estimated by bioelectrical impedance analysis (Biodynamics model 450c, Biodynamics, Shoreline, WA, USA).

2.4. Physical Activity and Exercise Intensity Evaluation

The physical activity level was assessed using the short version of the International Physical Activity Questionnaire (IPAQ) [21]. The assessment of aerobic capacity was assessed by means of a submaximal incremental test on a treadmill, adapted from Puga et al. [22]. The speed was fixed at 5.5 km/h and the intensity was increased by inclination, at 1% every two minutes until arriving at 85% of the maximum predicted heart rate (HR), or the reporting of subjective perception of effort of a score of 18 on the Borg Scale. A Cosmed Quark CPET gas analyzer (Rome, Italy) was used to record oxygen consumption (VO_2) and carbon dioxide output (VCO_2). The purpose of this test was to identify the ventilatory thresholds (VT1 and VT2) from the ventilatory equivalents of oxygen (VE/VO_2 ratio) and carbon dioxide (VE/VCO_2 ratio) [23]. The prescription of resistance exercise and strength assessment was based on the maximum repetition test (1RM) [24]. This test consisted of the workload performed with no more than one repetition in five tries, with three min of rest between tries [24].

2.5. Resting Blood Pressure and Ambulatory Blood Pressure Monitoring

To assess BP 24 h before and after 10 weeks of intervention, we used the Dyna Mapa + Cardius® device (Cardios °Sistemas, São Paulo, SP, Brazil), programmed with measurements to be taken every 15 min during the day (7 a.m. to 11 p.m.) and every 30 min at night (11 p.m. to 7 a.m.). Together with this device, the volunteers filled out a daily record of activities such as sleep, work, food, or any event that could interfere with BP or measurements. In a standardized manner, the device was set at 7 a.m., and measurements were considered valid when 24 h of monitoring were obtained. Resting BP was measured with the same device after 15 min of rest in a sitting position, and for the analysis of the pressure curve, time 0 was adopted when the monitor was placed. SBP, DBP, mean blood pressure (MBP) and HR in periods of awake, sleep and 24 h were analyzed.

2.6. Blood Pressure Variability

Using ABPM data, we analyzed BPV by three different parameters [25]: standard deviation of 24 h (SD24), the time interval between consecutive readings; the mean daytime and nighttime deviations weighted by the duration of the daytime and nighttime interval (SDdn) and the average real variability (ARV) weighted by the time interval between consecutive readings.

SDdn is the mean of the daytime and nighttime standard deviations corrected for the number of hours included in each period, eliminating the influence of the day-night blood pressure difference on the BPV estimate. ARV averages the absolute differences in consecutive measurements and accounts for the order in which BP measurements are obtained [26].

2.7. Blood Collection and Analysis

For analysis of nitric oxide, 15 mL of blood sample was collected after an overnight fast, following a period of 72 h to five days after the last training session to eliminate any acute effects of exercise. The collection was made by an experienced nurse, and the volunteers were instructed not to exercise or to consume alcohol or caffeinated drinks in the 24 h prior to collection. After collection, the samples were centrifuged at 3000 rpm for 15 min, separated into two microtubes (1.5 mL) and stored at −80 °C until analysis. Serum NO_2^- was analyzed by the total dose of it determined at 570 nm in microplate readers (Molecular Devices, Menlo Park, CA, USA), according to the protocol of Moorcroft et al. [27].

2.8. Exercise Program

The exercise program lasted for 10 weeks containing 30 combined sessions of aerobic and resistance training. Each session lasted 45 min, with 5 min of warm-up, 20 min of resistance and 20 min of aerobic exercise, with the order reversed in each session. Resistance training was performed in two sets of 15 repetitions via seven exercises (60% of 1RM) for large muscle groups: leg press 45°, seated low row, vertical chest press machine, seated lever fly machine, wide grip lat pull-down, swiss ball squat and abdominal crunch. Aerobic exercise was performed on a treadmill with a fixed speed of 5.5 km/h and intensity between ventilatory thresholds 1 and 2 regulated by the inclination of the treadmill. For load readjustment, after 5 weeks of training the 1RM test was performed again, and the intensity of the aerobic exercise was readjusted for a 20% increase (inclination of the treadmill).

2.9. Statistical Analysis

Sample calculation (n = 30) was performed in G-Power software v3.1 (Universität Düsseldorf, Düsseldorf, Germany) (Bicaudal; α err: 0.05; power: 0.80), adopting a significant expected difference of 22 mmHg in SBP with a standard deviation of 25 mmHg, which are the maximum ceilings [28,29]. Unpaired t-test was used to compare baseline characteristics between groups. BP variation over time was analyzed by area under the curve (AUC) calculated by the trapezoidal method in GraphPad Prism Software v6 (San Diego, CA, USA) Comparison between groups and time of ABPM, AUC of BP and BPV were made by Generalized Estimates Equation (GEE) with Bonferroni correction. The GEE analyzes were performed in two ways: by protocol (including only those who completed the protocol: ISO n = 17; PLA n = 14) and by intention to treat (including those who did not complete the study: ISO n = 19; PLA n = 19) using the last-observation-carried-forward method. NO_2^- were made by ANOVA two way. A p-value of <0.05 was used for statistical significance, and all statistical analyses were performed using SPSS software v20.0 (IBM, New York, NY, USA).

3. Results

A total of 260 postmenopausal women, aged 50–70, were recruited via traditional media (TV, radio and posters) from January to December 2015. From a total of 260 responders, 38 non-obese fulfilled the inclusion criteria and were randomized; 33 completed the 10 weeks of training and 31 performed post-tests (Figure 1).

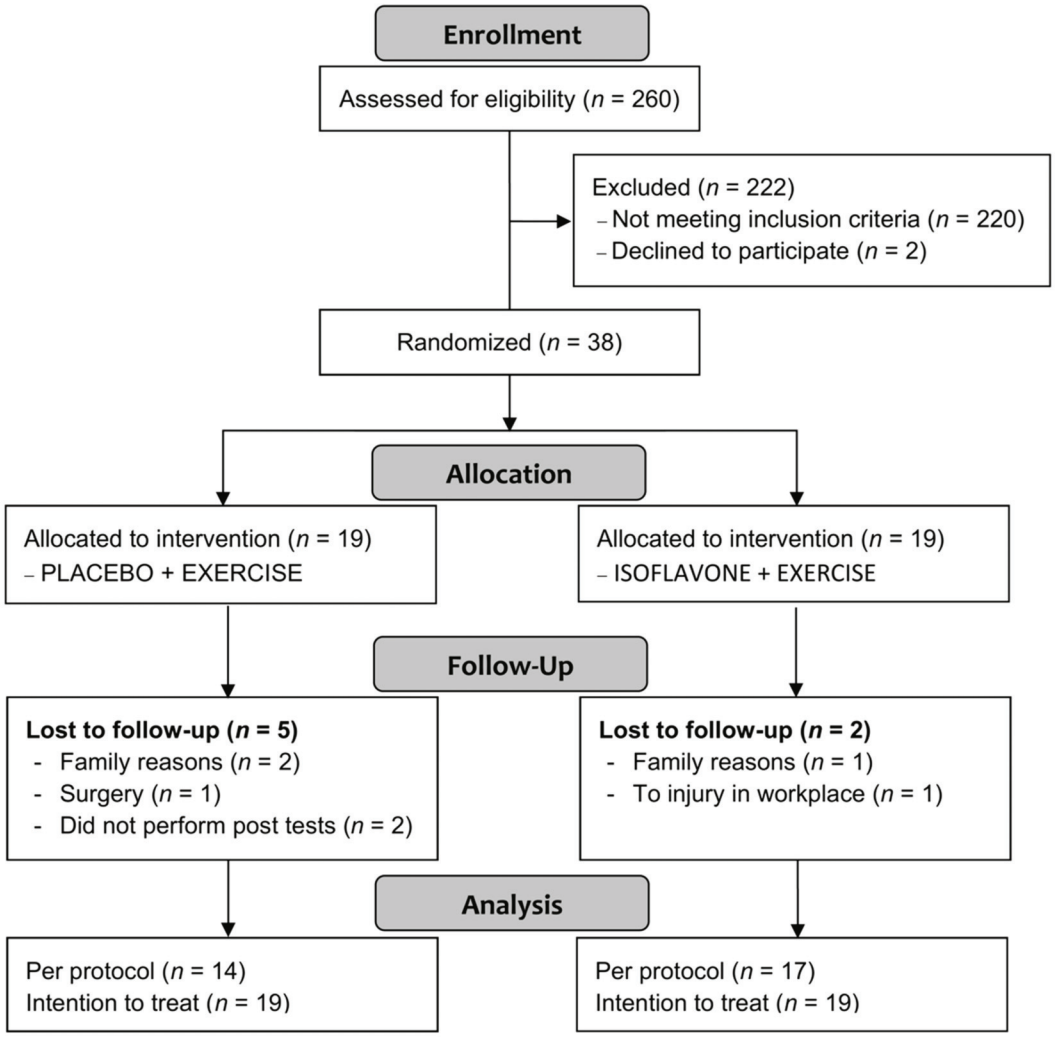

Figure 1. Flowchart of study participants.

Characteristics of participants are listed in Table 1. There was no difference between the PLA and ISO groups in age, time after menopause, body mass index and physical activity level measured by International Physical Activity Questionnaire (IPAQ) and metabolic equivalent of task (MET). Values of maximum strength performed in 1RM and anthropometric measurements are listed in Table 2. There was a time effect in all resistance exercises but no interaction between groups and time effects, so both groups increased their strength as measured by 1RM test. There was also no difference between groups and time in body mass, BMI, total lean mass, waist circumference and fat mass.

Table 1. Characteristics of participants (n = 31).

Clinical Characteristics	PLA (n = 14)	ISO (n = 17)	p
Age (years)	53 ± 5	56 ± 5	0.08
Time after menopause (years)	4 ± 4	7 ± 5	0.14
Body mass index (kg/m^2)	26.9 ± 0.7	26.2 ± 0.2	0.51
Physical activity level-MET (min/week)	954 ± 990	1268 ± 869	0.35

PLA: placebo and exercise; ISO: isoflavone and exercise; MET: metabolic equivalent of task. Unpaired t-test was used to compare groups. Data were described on average e standard deviation.

Table 2. Comparison of clinical, anthropometric and strength characteristics at baseline and after 10 weeks combined exercise training moments between groups using placebo + exercise (PLA − n = 14) and isoflavone + exercise (ISO − n = 17) interventions.

	Baseline Mean ± Standard Error	10 Weeks Mean ± Standard Error	p (Time)	p (Groups)	p (Group*Time)	Change Mean (95% IC)
Body Mass (kg)						
PLA	64.7 ± 2.1	65.7 ± 2.3	0.12	0.91	0.46	1.1 (−1.2 to 3.3)
ISO	65.3 ± 2.0	65.7 ± 2.0				0.4 (−0.6 to 1.4)
Body Mass Index (kg/m^2)						
PLA	26.9 ± 0.7	25.1 ± 1.9	0.28	0.54	0.66	−1.80 (−6.53 to 2.92)
ISO	26.2 ± 0.8	26.5 ± 0.8				0.24 (−0.21 to 0.69)
Total Lean Mass (kg)						
PLA	41.7 ± 1.0	42.6 ± 1.0	0.04	0.99	0.02	0.9 (0.5 to 1.6)
ISO	44.2 ± 0.9	42.2 ± 0.8				−0.6 (−0.5 to 0.4)
Fat Mass (kg)						
PLA	23.8 ± 1.3	23.0 ± 1.4	0.22	0.89	0.13	−0.8 (−1.7 to 0.1)
ISO	23.6 ± 1.5	23.7 ± 1.6				0.1 (−0.6 to 0.8)
Waist Circumference (cm)						
PLA	92.8 ± 1.9	91.9 ± 2.0	0.04	0.96	0.49	0.9 (−2.9 to 0.9)
ISO	93.2 ± 2.3	91.2 ± 1.8				−1.9 (−4.0 to 0.6)
1RM leg press (kg)						
PLA	164.9 ± 8.0	249.7 ± 10.1	<0.01	0.30	0.54	84.8 (69.5 to 100.1)
ISO	154.8 ± 10.3	232.6 ± 12.0				78.5 (65.4 to 91.5)
1RM bench press (kg)						
PLA	27.1 ± 1.0	37.2 ± 1.0	<0.01	0.01	0.06	10.1 (8.2 to 12.0)
ISO	25.0 ± 1.2	32.2 ± 1.3				7.2 (4.8 to 9.5)
1RM lat pull down (kg)						
PLA	31.3 ± 1.7	41.2 ± 2.5	<0.01	0.30	0.28	9.9 (6.0 to 13.7)
ISO	30.1 ± 0.9	37.7 ± 1.5				7.4 (5.3 to 9.6)
1RM peck deck (kg)						
PLA	20.0 ± 1.3	31.1 ± 1.4	<0.01	0.26	0.20	11.1 (9.0 to 13.2)
ISO	19.3 ± 1.0	28.5 ± 1.1				9.2 (7.2 to 11.3)
1RM seated row (kg)						
PLA	57.7 ± 2.1	74.5 ± 1.5	<0.01	0.41	0.36	16.8 (13.7 to 19.9)
ISO	56.5 ± 2.8	71.1 ± 2.1				14.6 (11.0 to 18.2)

PLA: placebo and exercise; ISO: isoflavone and exercise; WT6: 6−minute walk test; 1RM: 1 maximum repetition test. GEE with Bonferroni correction was used to compare groups, time and interaction (group*time). Data were described on average e standard error.

The volunteers trained at an intensity (treadmill inclination) of 5% ± 5%, a rate of perceived exertion of 16 ± 2 and a heart rate (HR) of 158 ± 16 bpm. Although there was no dietary control, the analysis of dietary data did not show significant differences between groups or over time (data published by Giolo et al. [16]). No changes were observed in the consumption of macronutrients (carbohydrates, proteins and lipids) and branched chain amino acids (total BCAA, isoleucine, leucine and valine).

Figure 2 shows intention-to-treat analysis of the BP measures obtained during resting and ABPM in the awake, sleep and 24 h periods. There were no interaction effects (group*time) in daytime (power analysis: Intention to treat: Daytime: SBP = 0.738; DBP = 0.576; MBP = 0.887; nighttime: SBP = 0.984; DBP = 0.952; MBP = 0.986; 24 h periods: SBP = 0.504; DBP = 0.292; MBP = 0.766). On the other hand, SBP, DBP and MBP decreased ($p < 0.01$) in both groups in 24 h and awake times. SBP and DBP, at rest also decreased after the intervention in both groups. No interaction was found in SBP, DBP or MBP AUCs over 24 h or in HR during these periods of the day (data non-shown). These results using per protocol analysis is in the Supplementary Figure S1.

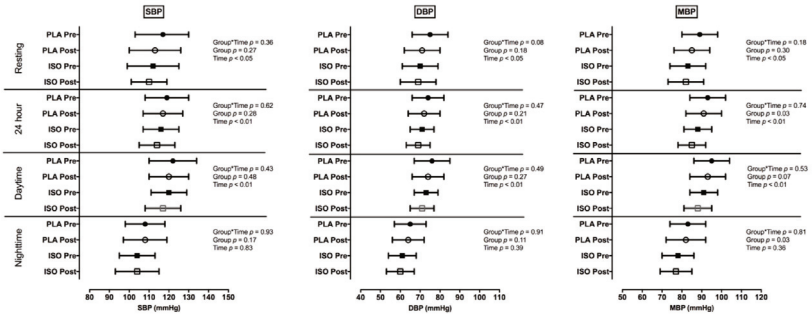

Figure 2. Intention-to-treat analysis of resting and ambulatory blood bressure monitoring AMBP results during 24 h, night-time and daytime periods (mean ± SD). PLA: placebo and exercise group; ISO: isoflavone and exercise group; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; Pre: Measures pre interventions; Post: Measures post interventions.

Table 3 shows intention-to-treat analysis of BPV results. No changes in ARV (Power analysis ARV: SBP = 0.959; DBP = 0.948; MBP = 0.948), SD24h (Power analysis SD24h: SBP = 0.697; DBP = 0.602; MBP = 0.640) and SDdn (Power analysis SDdn: SBP = 0.748; DBP = 0.828; MBP = 0.799.) were found in time or interaction (group*time) effects. These results using per protocol analysis is in the Supplementary'Table S1.

Figure 3 shows serum NO_2^- variation in both PLA and isoflavone ISO groups. There was no interaction (group*time) or difference in group or time (Power analysis of serum NO_2^- = 0.594).

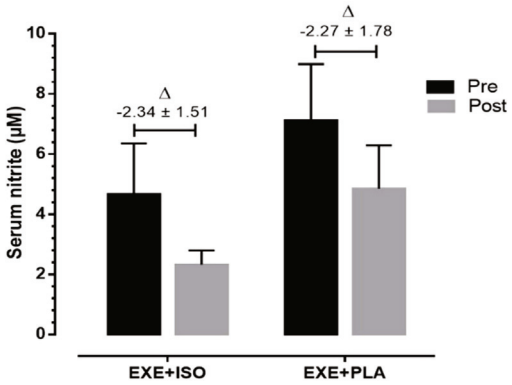

Figure 3. Values of serum NO_2^- variation in both placebo (PLA) and isoflavone (ISO) groups. EXE: exercise; Δ: final minus baseline values. ANOVA two way was used to compare groups and time. Student *t*-test was used to compare delta values.

Table 3. Intention-to-treat analysis of ambulatory blood pressure variability evaluated before (baseline) and after 10 weeks of exercise training in both placebo + exercise (PLA) and Isoflavone + exercise (ISO) groups.

Blood Pressure Variability	Baseline Mean ± SD	10 Weeks Mean ± SD	*p* (Time)	*p* (Groups)	*p* (Group*Time)	Change Mean (95% IC)
ARV SBP (mmHg/min)						
PLA	9.42 ± 0.47	9.45 ± 0.59	0.68	0.06	0.63	0.03 (−0.90 to 0.95)
ISO	10.96 ± 0.62	10.63 ± 0.60				−0.32 (−1.40 to 0.76)
ARV DBP (mmHg/min)						
PLA	6.67 ± 0.32	6.90 ± 0.31	0.83	0.07	0.24	0.23 (−0.35 to 0.81)
ISO	7.77 ± 0.36	7.44 ± 0.45				−0.33 (−1.10 to 0.40)
ARV MBP (mmHg/min)						
PLA	6.46 ± 0.28	6.63 ± 0.29	0.50	0.07	0.94	0.17 (−0.36 to 0.70)
ISO	7.24 ± 0.32	7.38 ± 0.44				0.14 (−0.57 to 0.85)
SD24h SBP (mmHg)						
PLA	12.50 ± 0.98	12.03 ± 0.76	0.61	0.21	0.74	−0.47 (−2.14 to 1.19)
ISO	13.62 ± 0.64	13.53 ± 0.86				−0.10 (−1.53 to 1.33)
SD24h DBP (mmHg)						
PLA	9.56 ± 0.46	9.70 ± 0.48	0.97	0.26	0.69	0.14 (−0.91 to 1.18)
ISO	10.44 ± 0.53	10.28 ± 0.64				−0.16 (−1.23 to 0.90)
SD24h MBP (mmHg)						
PLA	9.61 ± 0.63	9.50 ± 0.54	0.96	0.24	0.83	−1.12 (−1.42 to 1.18)
ISO	10.31 ± 0.48	10.39 ± 0.62				0.07 (−1.06 to 1.21)
SDdn SBP (mmHg)						
PLA	10.52 ± 0.66	10.32 ± 0.58	0.84	0.18	0.73	−0.21 (−1.32 to 0.90)
ISO	11.41 ± 0.54	11.47 ± 0.62				0.06 (−0.96 to 1.08)
SDdn DBP (mmHg)						
PLA	7.69 ± 0.35	8.33 ± 0.47	0.41	0.14	0.29	0.63 (−0.31 to 1.58)
ISO	8.79 ± 0.42	8.71 ± 0.46				−0.08 (−1.01 to 0.84)
SDdn MBP (mmHg)						
PLA	7.70 ± 0.40	8.00 ± 0.44	0.42	0.15	0.96	0.29 (−0.71 to 1.30)
ISO	8.38 ± 0.33	8.63 ± 0.45				0.26 (−0.61 to 1.12)

SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; PLA: placebo group; ISO: isoflavone group; ARV: average real variability; SDdn: standard deviation of daytime and nighttime; SD24h: standard deviation for 24 h. Generalized Estimating Equation (GEE) with Bonferroni correction and intention-to-treat analysis was used to compare groups, time and interaction (group*time). Data were described on average e standard deviation.

4. Discussion

Our study analyzed the effects of isoflavone supplementation associated with combined aerobic and resistance training on resting BP, ABPM, serum NO_2^- and BPV in non-obese postmenopausal women. Our main findings were that no additive effects of isoflavone supplementation on exercise training-mediated hemodynamic responses in non-obese menopause women were found. However, the exercise training improved the resting BP, and 24 h ambulatory BP, but not BPV. Moreover, our volunteers improved their muscle strength and aerobic capacity and no differences were found between anthropometric characteristics for time or group interactions.

Acute and chronic exercises for postmenopausal women have been shown to have beneficial effects on fat and lean mass and also for the prevention and treatment of metabolic and cardiovascular diseases [30]. Changes in body composition are common after menopause, such as reduced lean mass and bone mass, reduced muscle strength and changes in fat deposition with increased visceral fat [31] due to aging and also reduced levels of estrogen [32]. Our study showed that the combination of aerobic and resistance training in the same session was effective in improving strength in both groups, playing an important part in the treatment in decreasing and/or delaying these changes. In our study, these improvements are probably due to exercise training since we were not able to demonstrate any additive effect of isoflavone, as demonstrated in previous studies [33,34].

Exercise training can improve cardiovascular parameters such as resting BP responses in both hypertensive and normotensive patients, including postmenopausal women [35]. These results seem to be greater in aerobic training when compared to resistance training [15]. However, evidence shows that combined exercise training shows a less pronounced reduction in resting BP compared with aerobic or resistance training, but this can be due to the lack of studies using these modalities as comparative groups [15]. Our data showed that combined exercise training could improve both rest and ambulatory SBP, DBP and MBP with no additive effect of isoflavone supplementation, a result which is not well established in normotensive postmenopausal women due to the lack of relevant studies.

ABPM is an important method to demonstrate the behavior of BP [36], which can provide 24 h monitoring with data referring to awake and sleep periods. Moreover, these data allow the analysis of BPV with reduced discomfort during daily routine activities [37]. Some studies show that high values of both ABPM and BPV during 24 h are associated with a higher risk for cardiovascular diseases such as hypertension and cardiovascular events, making this an important parameter for cardiovascular health monitoring [14]. The use of BPV has increased due to the possibility of analysis of fluctuating values over time, which is additional information compared to in-office measurements, and considering milder variations as lower risk [14].

In this study, we did not find differences for any BPV indexes, which corroborates with several studies of healthy men and women in the same age group [38–40]. However, in populations with cardiometabolic dysfunctions, the results appear to be more promising [41–43]. It is worth mentioning that most studies use only aerobic training [38–40], few use dynamic [44] or isometric [45] resistance training, and we are not aware of studies with combined training. In addition, not only the type but the intensity of exercise training seems to be related to variations in BPV, and its route of effect seems to be through adaptations of vascular endothelium and smooth muscle [46]. In view of this, exercise seems to play an important role in modulating BP variations in individuals with dysfunctions, but has little or no influence on healthy subjects undergoing mild to moderate intensity training for a few weeks [42,47].

Importantly, vascular responses are mediated by multiple factors. It has been demonstrated that exercise training improves the bioavailability of NO [48] improving endothelial function, and hemodynamic and cardiovascular parameters such as BP [3,22]. A previous study showed increased serum NO_2^- concentration in postmenopausal women, but the intervention consisted of aerobic training alone, with a longer intervention, and was followed by decreased in resting BP [29,49]. Nevertheless, we were not the first group to find no effect on BP responses after exercise training with

ISO supplementation [50–52]. We believe that training characteristics [53], population [54] and dietary control [55] could play an important role in these responses.

Its chemical similarity to estrogen allows that, although with less affinity than the hormone, isoflavone acts through the stimulating mechanisms involved in the activity of nitric oxide synthase, increasing the production of NO and the control of calcium channels [56], mechanisms that are directly linked to the control of BP. This effect of isoflavone was found in the study carried out by Walker et al., in which the main components of isoflavone (genistein and daidzein) were administered to analyze the vasodilator effect when compared to estrogen. It was found that the acute administration of genistein in the brachial artery produces vasodilation in the forearm vasculature similar to that caused by estrogen, and that doses used compared to that consumed by Asian populations [57].

Chronic physical exercise [7] and isoflavone [12] stimulate the production of NO, which is directly linked to the reduction of BP and vasodilation. Because vascular health is reduced with age and hypoestrogenism, a condition of postmenopausal women [58], this may establish a physiological limit for the activation or production of NO by this route, so that the two isolated interventions can be beneficial; but when associated, they do not result in their potentiated effects, but are neutralized. The post-menopausal normotensive population evaluated in our study may show an absence of effects after isoflavone supplementation, in which the vasculature and absorption are not as efficient for obtaining high results as in pre-menopause; but despite these effects, isoflavone may compensate for the effects of the disease and for hypoestrogenism as found in hypertensive postmenopausal women.

A meta-analysis showed that the duration of supplementation can also interfere with the results, being more effective when interventions last for more than three months in hypertensive patients [59]. Although the total amount of isoflavone used in our study is within the effective range (25–375 mg), the intervention duration may have been insufficient to demonstrate effects. However, our results corroborate those found by Carmignani et al. [60], who used similar amounts of isoflavone (90 mg isoflavone containing 26.5 mg of aglycone: ~8 mg of daidzein, 15 mg of genistein and 3.5 mg of glycitein) and found no effect on BP, even with 16 weeks of intervention. It is noteworthy that this study shows methodological differences, not only in the duration, but mainly in a protocol without exercise intervention, and uses different amounts of types of isoflavone, which limits the comparison of findings between the studies.

In addition, the total amount of isoflavone seems to be less important than the specific amount of its compounds, genistein being the main type of isoflavone. The amount of isoflavone (100 mg) and its compounds (3.3% genistein, 93.5% daidzein and 3.2% glycitein) used in our study may have interfered with the findings obtained, since studies that demonstrated beneficial effects of isoflavone on vasomotor symptoms [61], BP [62] and arterial compliance [63] used amounts of genistein greater than 15 mg. In contrast, studies that used less than 10 mg did not present reduction of symptoms [64].

The main limitation of the study was the sample size, which was estimated using an expected change of 22 mmHg of SBP, resulting in a relatively small number of volunteers per group. Some variables also have a low power of analysis, making it difficult to generalize these results. Another limitation of our study was that, despite the greater amount of daidzein used, it may have failed to convert to equol (a non-steroidal estrogen) and so may not have allowed us to achieve better results. For conversion to take place, a specific intestinal microorganism, present in 30% to 50% of individuals, is essential to achieve the benefits obtained with supplementation [65]. In addition, other factors act as influencers on the complete action of phytoestrogens, affecting their bioavailability and their biological effects, such as a diet rich in carbohydrates that increases fermentation, diseases related to the intestinal tract, parasitosis and use of antibiotics [66], which were not checked in our volunteers. In addition, although our study was designed to assess the ISO additive effect, it has the limitation of not verifying the isolated effect in the ISO supplementation group. The 10-week intervention period may have been insufficient to demonstrate potentiated responses in BP in non-obese postmenopausal women. Thus, additional studies evaluating the possible effects of ISO and its mediators associated

with exercise on hypotension mechanisms are needed, as the current evidence seems to demonstrate a limited impact on healthy subjects.

5. Conclusions

The addition of isoflavone supplementation to combined aerobic plus resistance training does not change training-mediated responses in resting and ambulatory blood pressure and serum nitrite levels. However, since our results suggest that the combined training improved resting and ambulatory blood pressure, it should be considered for interventions focused on reverse menopausal effects in non-obese women.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/11/3495/s1, Figure S1: Per Protocol analysis of resting and AMBP results during 24 h, nigthttime and daytime periods (mean ± SD). PLA: placebo and exercise group (n = 14); ISO: isoflavone and exercise group (n = 17); SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure; Pre: Measures pre interventions; Prost: Measures post interventions, Table S1: Per Protocol analysis of ambulatory blood pressure variability evaluated before (baseline) and after 10 weeks of exercise training in both placebo + exercise (PLA n = 14) and Isoflavone + exercise (ISO n = 17) groups.

Author Contributions: Data collection and analysis, statistical analysis, writing the manuscript: J.G.C.D. Data collection and analysis, statistical analysis, and contributed to the revision of the manuscript: I.M.M. Study design, data collection, revision of the manuscript for intellectual content: J.S.G. Data collection and revision of the manuscript for intellectual content: J.P.B. Data collection and revision of the manuscript for intellectual content: A.L.A. Interpretation of the data, English review and revision of the manuscript for intellectual content: P.A.B.R. Interpretation of the data, English review and revision of the manuscript for intellectual content: E.P.d.O. Conception and design of the study, interpretation of the data; Revision of the manuscript: G.M.P. All authors have read and agreed to the published version of the manuscript.

Funding: Authors disclosed receipt of the following financial support for the research, authorship, and publication of this article: This work was funded by the Minas Gerais Staten Research Foundation (FAPEMIG: APQ-00750-14), National Council for Scientific and Technologic and Development (CNPq: 456443/2014-2 and 794078/2013) and Coordination for the Improvement of Higher Education Personnel (CAPES: Finance Code 001).

Acknowledgments: Acknowledgment of the Coordination for the Improvement of Higher Education Personnel (CAPES) for the post-graduate scholarship.

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

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Effect of Ingestion of Medium-Chain Triglycerides on Substrate Oxidation during Aerobic Exercise Could Depend on Sex Difference in Middle-Aged Sedentary Persons

Naohisa Nosaka [1,*], Shougo Tsujino [1], Kazumitsu Honda [2], Hiromi Suemitsu [3], Kazuhiko Kato [3] and Kazuo Kondo [4,5]

[1] Central Research Laboratory, The Nisshin OilliO Group, Ltd., Kanagawa 235-8558, Japan; s-tsujino@nisshin-oillio.com
[2] The Nisshin OilliO Group, Ltd., 1 Shinmori-Cho, Isogo-ku, Yokohama, Kanagawa 235-8558, Japan; k-honda@nisshin-oillio.com
[3] Kato Clinic, 1-1-1 Nakaizumi, Komae, Tokyo 201-0012, Japan; kato.kaz@me.com (H.S.); kato-kaz@db3.so-net.ne.jp (K.K.)
[4] Ochanomizu University, 2-1-1 Ohtsuka, Bunkyo-ku, Tokyo 112-8610, Japan; k24kondo523@gmail.com
[5] Toyo University, 1-1-1 Izumino, Itakura-machi, Ora-gun, Gunma 374-0193, Japan
* Correspondence: n-nosaka@nisshin-oillio.com; Tel.: +81-45-757-5461

Abstract: Fat oxidation (FAO) during aerobic exercise and whole-body FAO via lipid intake are thought to be important for the maintenance of health, such as the prevention of type 2 diabetes and obesity in sedentary persons in their 40s and 50s. Medium-chain triglycerides (MCTs) ingestion has been attracting attention. However, the effects of difference of sex and the composition of medium-chain fatty acids (MCFAs) are unclear, so we examined the effects of these factors on FAO during aerobic exercise. We conducted a randomized, double-blind, placebo-controlled, 3-arm, within-participants crossover trial. FAO during low- to moderate-intensity exercise was compared when octanoate-rich MCTs (C8R), decanoate-rich MCTs (C10R), or carbohydrate (control) was ingested. Three 2-week interventions were separated by two 2-week washout periods. An increase of FAO during exercise after the C8R diet was found in males, but not in females. An increase of carbohydrate oxidation (CAO) and oxygen uptake during exercise after the C10R diet was found in females, but not in males. In a pooled estimate of the effect of MCTs (C8R and C10R) in women and men, FAO increased during exercise. In conclusion, short-term ingestion of MCTs by middle-aged sedentary persons could increase FAO during aerobic exercise compared to carbohydrate ingestion, but the enhancing effect of MCTs on substrate utilization and oxygen uptake might vary, depending on sex and the composition of MCFAs.

Keywords: sedentary; sex difference; aerobic exercise; exercise intensity; ventilation threshold; fat oxidation; octanoate; decanoate

Citation: Nosaka, N.; Tsujino, S.; Honda, K.; Suemitsu, H.; Kato, K.; Kondo, K. Effect of Ingestion of Medium-Chain Triglycerides on Substrate Oxidation during Aerobic Exercise Could Depend on Sex Difference in Middle-Aged Sedentary Persons. *Nutrients* **2021**, *13*, 36. https://dx.doi.org/10.3390/nu13010036

Received: 30 November 2020
Accepted: 21 December 2020
Published: 24 December 2020

1. Introduction

Fat intake is considered to be important for health, such as prevention of type 2 diabetes and obesity. Fat intake enhances fat oxidation (FAO) during exercise and results in whole body FAO via increased mitochondrial biogenesis and enzyme activity [1,2]. A high-fat diet and ketone compounds, which are degradation products of fats, have been reported to increase FAO [3–5]. However, a high-fat diet causes restriction of carbohydrate intake, which has negative effects such as suppressing glycogen accumulation and increasing fatigue [6]. The use of ketone compounds has not been widespread due to their poor acceptance in taste and gastrointestinal distress [4].

Medium-chain triglycerides (MCTs) have been reported to increase FAO at relatively low doses (6 g/day) [7], and are tasteless, odorless, and as acceptable as regular fats and oils [8,9]. Animal studies have shown that the combination of aerobic exercise and MCTs

ingestion increases the duration time of moderate-intensity exercise and activity of the ketone body oxidation enzyme in muscle tissue compared to the combination of aerobic exercise and long-chain triglycerides (LCT) ingestion [10]. In a human study, continuous ingestion of MCTs in recreational athletes was found to increase FAO [7] and exercise time to exhaustion [7,11].

A previous study using animals reported that MCTs ingestion without exercise also increased the duration time of moderate-intensity exercise associated with enhancement of fat utilization [10,12]. However, little is known about studies that examine the effects of FAO during aerobic exercise after ingestion of MCTs in persons without exercise habits. Furthermore, sex differences in FAO during exercise have been revealed [1,13,14], but the effects of MCTs ingestion on sex differences have not been clarified. Furthermore, a study using cultured cells examining changes in the activity of mitochondrial enzymes has reported that decanoate (C10) enhanced their activity, while octanoate (C8) did not affect it [15,16]. On the other hand, it has been reported that β-hydroxybutyrate (β-HB), which is one of the ketone bodies, increases the activity of mitochondrial enzymes and that C8 increases blood ketone bodies more than C10 after ingestion [17]. Therefore, although differences in the length of carbon chains of medium-chain fatty acids (MCFAs) that consist of MCTs may affect FAO during exercise, little is known about studies that have compared the effect of MCTs with different compositions of MCFAs.

In the present study, we examined whether there were sex differences in the effect of FAO during exercise between sedentary men and women after ingestion of MCTs. In addition, we examined the effects of ingestion of MCTs with different compositions of MCFAs.

2. Materials and Methods

2.1. Ethics

The present study was conducted in accordance with the Declaration of Helsinki (2013) and with the approval of the Ethics Committees of Nihonbashi Egawa Clinic (Ethical approval code: RD07001KW04). In advance, the study protocol was registered (UMIN000033886) with the University Hospital Medical Information Network Center (UMIN-CTR).

2.2. Participants

Seventeen Japanese females (Body Mass Index (BMI) 18.8–26.0) and 13 males (BMI 20.7–27.5) aged 40–59 years volunteered for the study. Participants had to have no chronic diseases, not be pregnant or lactating, and be non-smoking. Participants also had to be sedentary (less than 60 min of exercise per week and without high-labor occupational activity of 10 or more hours per week) and without contraindications for intense exercise and with a stable weight (within 10 kg for the previous 12 months). The power calculation was based on results from a previous MCT study [7]. From the result of FAO during exercise, the mean difference was 1.6 and the SD was 2.7. From crossover design power analysis, required number of participants was calculated as 13 (the following coefficient was input: alpha = 0.05, power = 0.80, theta = 1.0). An independent clinical research organization managed the participants during the intervention period and measured exercise trials. After their informed consent was obtained, screening of the participants (interview, physical measurement, blood collection and analyses, and a stepping exercise using a platform and electrocardiography) occurred as described previously [18]. To select eligible participants, the participants were screened based on the following inclusion and exclusion criteria, which were registered with UMIN-CTR (https://upload.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000038644 [19]).

The inclusion criteria were: (1) Japanese males and females aged 40 years or older and under 60 years at the time informed consent was obtained, and Japanese males and females who met the following inclusion criteria for protection of human rights and who did not conflict with the exclusion criteria and could comply with the management requirements

during the study period; (2) subjects with a BMI of 20.7 to 27.5 in males and 18.8 to 26.0 in females (to recruit Japanese subjects with average BMI); (3) nonsmoker; (4) persons who have received sufficient explanation on the purpose and content of the research, who have the ability to give consent, who voluntarily volunteer to participate in the research with good understanding, and who have given written consent to participate in the research.

The exclusion criteria were: (1) currently receiving any medication or ambulatory treatment; (2) have a history of or complication of serious heart, liver, kidney, cardiovascular system, or blood disorders; (3) have experienced chest pain or vein abnormalities at rest; (4) frequent shortness of breath, light-headedness, dizziness, and loss of consciousness; (5) have a history of drug allergy, food allergy, or allergy to raw materials (milk protein, etc.) used in test foods; (6) have a family member who died suddenly for unknown reasons; (7) diagnosed with lumbar foot disorders; (8) taking health foods, supplements, or drugs that may affect fatigue relief; (9) eat extremely unbalanced meals; (10) extremely irregular lifestyle habits such as diet and sleep; (11) suspected of having insomnia (insomnia, sleep apnea syndrome, etc.); (12) present or past history of psychiatric disorder (depression, etc.); (13) drug dependence, present illness of alcoholism or previous history; (14) currently participating in other clinical trials or have participated in other clinical trials within the past 3 months; (15) irregular working hours, such as working at night; (16) feels an effect or pain in the lower back, knee, or body during ascent or descent of stairs, etc.; (17) receiving treatment for rheumatoid arthritis; (18) have had surgery or disease of the knee or routinely use a walking cane; (19) body weight fluctuates by $\pm$ 10 kg or more within 1 year; (20) wishing to become pregnant or are pregnant or lactating during the study period; (21) difficulties in observing records on various questionnaires; (22) exercising to maintain or improve physical fitness for at least 60 min per week; (23) engaged in physical labor for 10 h or more per week; (24) scheduled to donate blood or receive a vaccination or wishing to donate blood or receive a vaccination during the study period; (25) other, persons who are judged by medical doctor to be inappropriate for the study.

2.3. Randomized Allocation

The study participants were allocated by independent assignment–organization to one of three allocation arms by stratified randomization, with sex and age as a stratified factor. The results of the allocation showed that sex and age as a stratified factor were almost balanced in the three allocated groups. In three allocations, ingestion of test drink with oil was conducted in the following order (i, ii, iii); allocation 1: (i) octanoate-rich MCTs (C8R), (ii) control (carbohydrate), and (iii) decanoate-rich MCTs (C10R); allocation 2: (i) C10R, (ii) C8R, and (iii) control; allocation 3: (i) control, (ii) C10R, and (iii) C8R. The assignment information was not disclosed to the participants or the practitioner until the study was completed and the statistical analysis protocol was prepared. The study was conducted in a randomized, double-blind, and within-participants crossover manner.

2.4. Test Drink

The test drink provided to participants is shown in Table 1. C8R and C10R were comprised of MCTs comprising 75% of C8 and 25% of C10 (C8R) or 30% of C8 and 70% of C10 (C10R). The study participants were asked to ingest the test drink (0 g or 6 g per day of MCTs) each day. The test drinks were provided to the participants, and neither the participants nor the experiment staff were aware of the content of the drinks.

Table 1. Test drinks in the present study.

Nutrition	Control	C8R	C10R
Energy, kcal	200	200	200
Protein, g	1.4	1.4	1.4
Fat, g	0	6.0	6.0
(C8R MCTs, g)	(-)	(6.0)	(-)
(C10R MCTs, g)	(-)	(-)	(6.0)
Carbohydrate, g	48.0	34.6	34.6

C8R: octanoate-rich MCTs; C10R: decanoate-rich MCTs; medium-chain triglycerides (MCTs); (-): not contained.

2.5. Intervention

This study included three 14-day interventions separated by two 14-day washout periods (Figure 1). The details of the interventions were described previously [18]. In brief, the 14-day interventions consisted of a 13-day life survey, 5-day food survey, and 1-day exercise trial. Nutritional calculations were performed by the managing registered dietitian to obtain daily intakes of energy, protein, lipid, carbohydrate, and MCFAs (C8 and C10) from the dietary records (photographs, with a ruler and food survey sheet) on the basis of the standard tables of food composition in Japan, 2015 (seventh revised edition) [20].

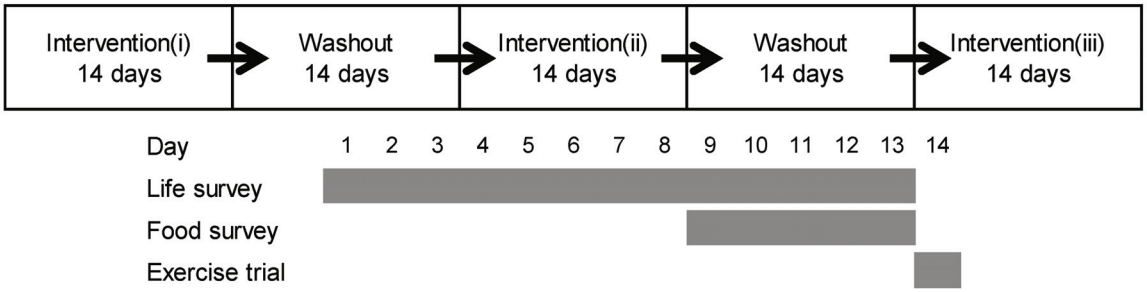

Figure 1. The protocol of the present study. This study was carried out in crossover manner. The subjects were examined on 3 interventions separated by a washout period of 14 days. In the intervention, the participants were asked to ingest test drink (containing 0 g of medium-chain triglycerides (MCTs) as control or 6 g of octanoic-acid rich MCTs or 6 g of decanoic-acid rich MCTs) and to record their consumption each day for 13 days. They were instructed to maintain their physical activity at a fixed level and to record their activity time for 13 days. They were also asked to record all meals consumed from days 9 to 13 and to abstain from exercise and alcohol on day 13. On day 14, exercise trial was conducted in each intervention. Intervention (i); the first period of intervention; intervention (ii): the second period of intervention; intervention (iii): the third period of intervention.

2.6. Exercise Trial

On day 14 the participants were asked to visit the laboratory and underwent an exercise trial. The details of the procedure of the exercise trial were previously shown [18]. In brief, the participants were asked to measure oxygen uptake (VO2) and carbon dioxide excretion (VCO2) in a sitting posture for five minutes before the experimental trial (rest), using a respiratory gas analyzer (AE-310S; Minato Medical Science, Osaka, Japan). The participants wore the mask used for the expired gas measurement in such a way that no expired air leaked from the mask. The participants were first required to exercise on a stationary bicycle at a pedaling frequency (cadence) of 50−60 rpm and at a fixed workload of 20 watts (20-watt fixed-load exercise (20Ex)) for 3 min. The workloads were then incrementally increased by 13 watts (male) and 10 watts (female) per min (incremental load exercise (InEx)) until their ventilation threshold (VT) was reached. The appearance of VT was defined as the point when the respiratory exchange ratio (RER) rapidly exceeded 1.00 by the V-slope method [21].

2.7. Calculation

From respiratory measurements (VO2 and VCO2), RER, FAO, and carbohydrate oxidation (CAO) rates were calculated as follows [22,23].

$$\text{RER} = \text{VCO2}/\text{VO2} \quad (1)$$

$$\text{FAO rate} = 1.695\ \text{VO2} - 1.701\ \text{VCO2} \quad (2)$$

$$\text{CAO rate} = 4.585\ \text{VCO2} - 3.226\ \text{VO2} \quad (3)$$

The cumulative value of FAO and CAO (cFAO and cCAO) was calculated from the FAO and CAO rates during 20Ex, for 2 min (from 2 to 3 min after starting the exercise), and during InEx until the appearance of VT. The maximal FAO rate (mFAO) was determined during 20Ex, for 2 min (from 2 to 3 min after starting), and during InEx. The RER at mFAO was determined during 20Ex (RER at mFAO during 20Ex (RER@20Ex), (@, at time of)) and during InEx (RER at mFAO during InEx (RER@InEx)).

Power outputs at VT (PO@VT) during InEx were determined. VO2 at VT (VO2@VT) during InEx were determined. Ventilation volume per VCO2 (VE/VCO2) at VT (VE/VCO2@VT) during InEx were calculated as the ventilation volume (unit: ml/min) divided into the VCO2 volume (unit: mL/min). Values of FAO, power outputs (PO), and RER were determined as moving averages (every 1 min) calculated from the expired gas measurement value taken every 10 s. The average and variance of cadence (unit: rpm) were calculated every minute, from 20Ex to InEx. To estimate the exercise intensity of the present study, the ratio of energy expenditure before (while resting) and during exercise was calculated. Abbreviations are described in the Abbreviations Section.

2.8. Statistical Analyses

A statistical analysis protocol was prepared before the disclosure of allocation information. After obtaining their informed consent, qualified participants were determined by screening test. The qualified participants who did not withdraw their consent before the start of the intervention became the study participants, and the data were made into a full analysis set (FAS).

Of the FAS, participants disqualified by analysis were determined using the following criteria: (i) those whose consent was withdrawn after starting the intervention; (ii) those whose consumption of the test food did not meet the following criteria: in each intervention, those who had taken fewer than two packages of the test food for two consecutive days, or those who had not consumed fewer than two packages of the test food for three days; (iii) those who had not sufficiently provided the records requested during the intervention; (iv) in the exercise trial, those who did not attend for personal reasons; (v) those who stopped pedaling or whose cadence dropped below 50 rpm three times during the exercise trial; and (vi) those who discontinued pedaling before the appearance of VT.

In the event of any participants disqualified by analysis, the data of analysis-qualified participants would be made per protocol set (PPS). The study participants were divided into two sex groups, and the following statistical analysis was conducted on FAS and PPS.

The bodyweight data in the intervention and for the data obtained from the exercise trial (cadence, cFAO, cCAO, mFAO, RER@20Ex, RER@InEx, PO@VT, VO2@VT, and VE/VCO2@VT) were calculated as the difference, (C8R diet or C10R diet) minus (control diet), as intervention effect. The intervention effects were confirmed by the Shapiro–Wilk test for normality of distribution. When the normality of the data was confirmed, the data were compared using a linear mixed model for repeated measures in which allocation and intervention period were set as fixed effect and participants were set as a random effect, and when normality was not confirmed, the Bartlett's test was used to confirm equal variance. When equal variance was confirmed, then Dunnett's method was performed. When equal variance was not confirmed, Steel's test was performed.

Regarding the mean values of nutritional daily intake during intervention (energy, protein, fat, carbohydrate, and MCFAs (C8 and C10)), multiple comparisons were performed

to compare the control group with the test groups. When equal variance was confirmed by Bartlett's test, then Dunnett's method was performed. When equal variance was not confirmed, Steel's test was performed.

All statistical measurement values were expressed as mean and standard deviation or standard error or 95% confidence intervals. Calculation of basic statistics of the data was performed using Microsoft Excel 2010 for Windows (Microsoft Japan Co., Ltd., Tokyo, Japan). All statistical analyses were performed using R statistical software, v3.4.3 for Windows (R Core Team, Vienna, Austria) [24]. The level of significance for all comparisons was set at $p < 0.05$.

An exploratory group analysis was conducted to estimate the overall pooled effect and its confidence of cFAO. The pre-specified group was sex and diet. The mean difference and 95% confidence interval of cFAO in intervention effect during overall (20Ex and InEx; VT) exercise for each group, and the pooled estimate of cFAO, were calculated by a random effects model with a restricted maximum likelihood method.

3. Results

Part of the data (unstratified data and age-stratified data) obtained in the present study has been published previously [18], and analyses of the sex-stratified data on sex difference were conducted in the present study.

3.1. Participants

Figure 2 is a flowchart of study participants. Of the 13 male and 17 female eligible participants who were enrolled, no participants dropped out before or after randomization. All study participants completed the study, and there were no analysis-disqualified participants. Hence, the data were analyzed using the FAS. The characteristics of the present study participants are shown in Table 2.

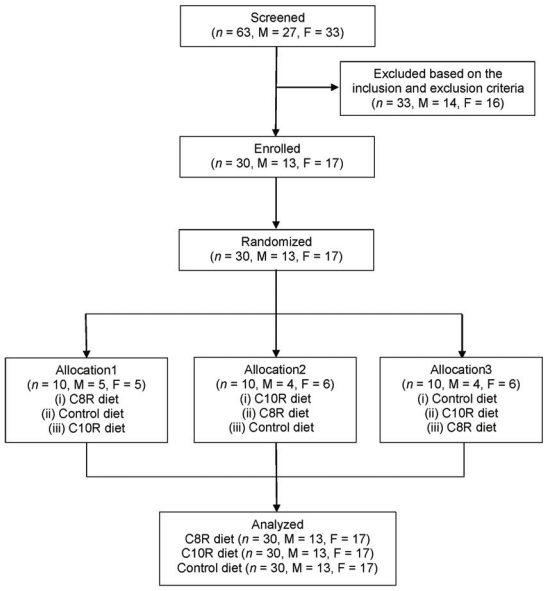

Figure 2. Flowchart illustrating the selection and participation of study participants in the present study. Thirty-six female and 27 male participants underwent screening, which included a medical history, physical, and blood tests. Of the 63 participants, 19 female and 14 male participants were not assigned. The remaining 17 female and 13 male participants were randomly allocated to 3 groups to receive the intervention (control diet: 0 g of MCTs; C8R diet: 6 g of octanoic-acid rich MCTs; C10R diet: 6 g of decanoic-acid rich MCTs). No participants dropped out and were excluded. The data from 17 female and 13 male participants were analyzed. F: females; M: males; C8R: octanoate-rich MCTs; C10R: decanoate-rich MCTs.

Table 2. Characteristics of the participants in the present study.

Characteristics	Sex	Value
Number, *n*	Female	17
	Male	13
Age, year	Female	48.2 ± 3.7
	Male	48.8 ± 3.6
Height, cm	Female	157.8 ± 5.0
	Male	169.1 ± 8.8
Body weight, kg	Female	55.4 ± 5.6
	Male	67.8 ± 9.0
Body mass index, kg/m^2	Female	22.2 ± 1.7
	Male	23.6 ± 1.1

Values are means ± standard deviations.

3.2. Dietary Intake

Mean average dietary intakes per day for the five days before each exercise trial are shown in Table 3. In both males and females, there were no significant differences in energy, protein, carbohydrate, and fat intakes in the C8R and C10R diet, compared to the control diet. MCFAs (C8 and C10) intakes were significantly greater in the C8R and C10R diet than in the control diet.

Table 3. Means of dietary intake per day, during the five days before each exercise trial in the present study.

Nutritional Indices	Control Diet	C8R Diet	C10R Diet
Female, *n* = 17			
Energy, kcal	1758 ± 337	1755 ± 252	1775 ± 349
Protein, g	56.6 ± 13.0	57.9 ± 9.7	58.3 ± 10.6
Fat, g	58.9 ± 17.3	63.6 ± 12.6	67.9 ± 14.8
Octanoic acid, mg	124.8 ± 118.5	3790.8 ± 78.8 *	1623.4 ± 67.1 *
Decanoic acid, mg	211.5 ± 147.6	1506.2 ± 142.4 *	3724.0 ± 133.2 *
Carbohydrate, g	240.5 ± 47.6	225.5 ± 35.9	223.5 ± 52.9
Male, *n* = 13			
Energy, kcal	1978 ± 342	2034 ± 412	1936 ± 430
Protein, g	69.6 ± 19.2	70.9 ± 17.2	64.8 ± 12.4
Fat, g	63.2 ± 16.5	69.8 ± 20.5	67.0 ± 22.0
Octanoic acid, mg	88.5 ± 116.4	3748.8 ± 101.7 *	1585.4 ± 84.0 *
Decanoic acid, mg	167.3 ± 227.8	1402.6 ± 121.2 *	3657.9 ± 150.5 *
Carbohydrate, g	268.5 ± 49.9	259.7 ± 51.8	253.2 ± 55.8

Values are means ± SD. * Statistically significant difference from the value in the control diet ($p < 0.05$). If equality of the 3 variances was hypothesized when performing Bartlett's test, Dunnett's test was performed. If the hypothesis was rejected, Steel's test was performed.

3.3. Exercise Trial

The means and variances of body weights during the intervention, and of cadence during the exercise trials, were compared between the diet groups. No significant difference was found. The ratios of 20Ex and VT to rest were 2.2 ± 0.1 times and 4.5 ± 0.9 times (mean ± SD) in female participants.

The intervention effect of ingestion of MCFAs, which expresses values (C8R or C10R diet) minus (control diet), is shown in Table 4. The normality distribution on the data of intervention effect is shown in Table A1. For female participants ingesting the C8R and C10R diet, there were no changes to cFAO, mFAO, and RER during 20Ex, InEx, and overall (20Ex and InEx until VT); there were changes to cCAO during InEx and overall, but not 20Ex. When ingesting the C10R diet, PO@VT, VO2@VT, and overall elapsed time were all greater, but not when consuming the C8R diet (Table 4).

Table 4. Cumulative values of fat and carbohydrate oxidation, maximal fat oxidation rate, respiratory exchange ratio, power output, oxygen uptake, and ventilation volume per VCO2 volume during the experimental trial after ingestion of a control or C8R or C10R diet for two weeks, in the present study.

Intervention Effect, Δ	Diet	Female, *n* = 17	*p*	Male, *n* = 13	*p*
Cumulative fat oxidation, mg					
# 20Ex	C8R	12.1 ± 16.2	0.46	84.0 ± 28.2	0.007
	C10R	6.5 ± 16.2	0.69	37.1 ± 28.2	0.20
# InEx (~VT)	C8R	82.6 ± 55.0	0.14	118 ± 139	0.41
	C10R	108 ± 55.0	0.06	−28.6 ± 139	0.84
# overall (20Ex and InEx, ~VT)	C8R	94.7 ± 65.8	0.16	202 ± 151	0.20
	C10R	115 ± 65.8	0.09	8.5 ± 151	0.96
Cumulative carbohydrate oxidation, mg					
# 20Ex	C8R	9.1 ± 38.8	0.82	−286 ± 81.6	0.002
	C10R	−9.8 ± 38.8	0.80	−46 ± 81.6	0.58
# InEx (~VT)	C8R	775 ± 373	0.046	−188 ± 770	0.81
	C10R	825 ± 373	0.03	366 ± 770	0.64
# overall (20Ex and InEx, ~VT)	C8R	784 ± 381	0.048	−474 ± 803	0.56
	C10R	815 ± 381	0.04	320 ± 803	0.69
Maximal fat-oxidation rate, mg/min					
# 20Ex	C8R	11.6 ± 8.7	0.19	38.2 ± 17.4	0.04
	C10R	1.8 ± 8.7	0.84	22.0 ± 17.4	0.22
# InEx	C8R	16.6 ± 9.3	0.08	22.4 ± 21.2	0.30
	C10R	12.3 ± 8.1	0.20	9.2 ± 21.2	0.67
Respiratory exchange ratio					
# 20Ex (at mFAO)	C8R	−0.009 ± 0.008	0.28	−0.05 ± 0.02	0.007
	C10R	−0.004 ± 0.008	0.63	−0.02 ± 0.02	0.30
# InEx (at mFAO)	C8R	−0.007 ± 0.008	0.38	−0.03 ± 0.01	0.02
	C10R	−0.006 ± 0.008	0.48	−0.01 ± 0.01	0.27
Power output, W					
# InEx (at VT)	C8R	2.5 ± 2.0	0.23	4.5 ± 4.1	0.28
	C10R	5.8 ± 2.0	0.008	4.3 ± 4.1	0.30
Elapsed time, sec					
overall (20Ex and InEx, ~VT)	C8R	15.2 ± 12.3	0.22	22.0 ± 19.1	0.26
	C10R	34.8 ± 12.3	0.008	21.1 ± 19.1	0.28
Oxygen uptake, ml/min					
# InEx (at VT)	C8R	28.8 ± 31.2	0.36	−31.2 ± 78.6	0.70
	C10R	60.5 ± 27.2	0.009	67.9 ± 78.6	0.40
Ventilation volume per VCO2 volume, mL/mL					
# InEx (at VT)	C8R	0.01 ± 0.43	0.99	0.56 ± 0.42	0.20
	C10R	−0.39 ± 0.43	0.37	0.77 ± 0.42	0.08

Values are expressed as intervention effect, Δ value ((C8R or C10R diet) minus (Control diet)). Values are least squares means ± SE. 20Ex: 20-watt fixed-load exercise; InEx: incremental load exercise; (#) overall: from 20Ex to InEx until VT; VT: ventilation threshold; mFAO: maximal fat oxidation.

For male participants ingesting the C8R diet, cFAO during 20Ex and PO@VT were significantly greater, and cCAO during 20Ex, RER@20Ex, and RER@InEx were significantly less; there were no changes to PO, elapsed time, VO2, and VE/VCO2; there were no changes when ingesting C10R (Table 4).

For female and male participants, the intervention period effects of the measures that were significant for the allocation (diet group) were not significant.

In the pooled analysis of cFAO in intervention effect during overall exercise (20Ex and InEx, ~VT), the mean difference and 95% confidence interval (CI) for the pooled estimate were 90.92 mg (11.19 to 170.64 mg, 95% CI, $p < 0.05$) (Figure 3). I^2 (degree of heterogeneity) was 0.00%, and the Q test (degree of freedom = 3) was 1.5587 ($p = 0.669$) for the test of heterogeneity. In the funnel plot analysis, z was 0.178 ($p = 0.859$) in the test for funnel plot asymmetry. Kendall's tau was 0.333 ($p = 0.750$) for the rank correlation test for funnel plot asymmetry.

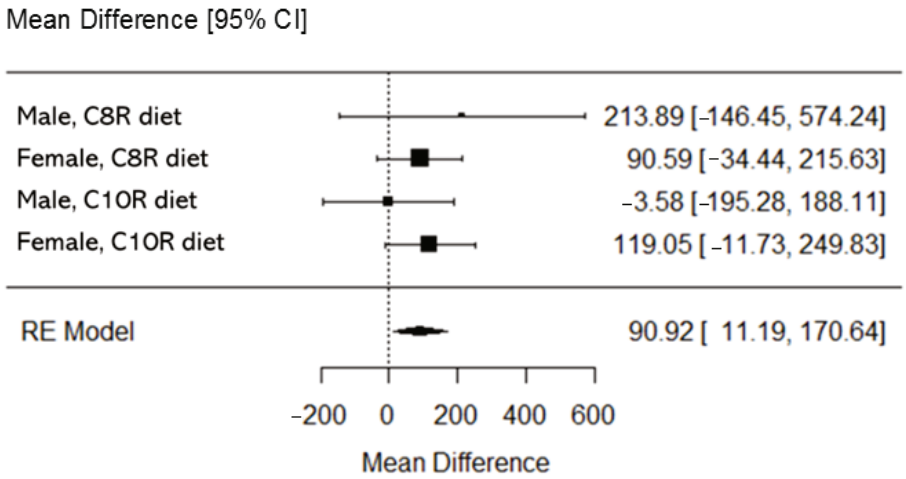

Figure 3. Pooled analysis of cumulative value of fat oxidation during overall (20Ex and InEx, ~VT) exercise in the present study. Values are mean difference and 95% coefficient interval. Random effect model with restricted maximum likelihood method was performed.

4. Discussion

MCTs have been used to enhance FAO during exercise because they are rapidly metabolized after ingestion and are hardly stored [8,25]. Previous studies in which MCTs were ingested before exercise showed negative results [10,26,27]. In a study in which MCTs enhanced fat utilization during exercise, it was effective up to one hour after ingestion, but not two hours [28]. Studies that combined pre-exercise MCTs ingestion and intermittent MCTs ingestion during exercise increased FAO during exercise [22,29] and improved performance during moderate-intensity exercise followed by high-intensity exercise [29]. However, in several studies FAO and performance did not improve during high-intensity exercise [23,30] and resulted in gastrointestinal distress [23]. A study investigating FAO during exercise with intravenous octanoate at moderate and high intensities showed that oxidation of MCFAs in peripheral blood was not inhibited regardless of intensity [31], suggesting that FAO ability during exercise affected the oxidation of MCFAs. It is well known that training improves FAO capacity [32], and it is believed that MCTs ingestion in trained people with high FAO capacity may be more useful than LCT ingestion in supplying fat fuel. However, MCTs intake before and during exercise in people who are without exercise habits with low FAO capacity is unlikely to be useful. Therefore, MCTs ingestion methods that enhance FAO capacity are crucial for people without exercise habits. Short-term continuous ingestion of MCTs did not enhance FAO in high-intensity exercise [33,34] but did enhance FAO in low- to moderate-intensity exercise [7,18]. It has also been reported that gastrointestinal discomfort symptoms should be noted when MCTs intake exceeds 17 g at a time [33], but 6 g intake has little effect in middle-aged sedentary persons [18]. The present study aimed to evaluate people without exercise habits on substrate oxidation during exercise with a fixed load of 20W after continuous MCTs ingestion. The 20-watt exercise was approximately 2.2 times the metabolic rate when at rest and was lower than 3.0–5.9 times, which is the definition of moderate-intensity exercise [35], so it was considered to correspond to low-intensity exercise. Furthermore,

the incremental-load exercise until VT (to resting, approximately 4.5 times at VT) was used because of the low aerobic-exercise capacity in people without exercise habits and the variability in the appropriate exercise load for each individual in aerobic exercise [1].

An exploratory pooled analysis to estimate the influences of all MCTs (C8R and C10R) and sex (female and male) resulted in a positive effect of cFAO during low- to moderate-intensity aerobic exercise in middle-aged sedentary persons who do not exercise habitually. Previous study results [7,18] and the result of the present study support the proposal that enhancement of FAO during aerobic exercise by short-term MCTs ingestion could not depend on exercise habit. However, the results of the sex-specific evaluation showed that there was a sex difference in the increase of FAO and CAO during aerobic exercise after continuous ingestion of MCTs between women and men, as women were presumed to promote both FAO and CAO via increased oxygen uptake, while men were presumed to inhibit CAO instead of promoting FAO. When women consumed MCTs, they showed a trend toward increased FAO during InEx (p = 0.06, for the C10R diet) without affecting FAO during 20Ex. This result indicates that the effect of MCTs ingestion may not have been observed in women due to their higher daily FAO, which may be related to the higher daily fat utilization in women than in men, as pointed out in previous studies [1,13]. Furthermore, the results of the evaluation using incremental exercise until VT showed an increase in oxygen uptake in women, and this increase in oxygen uptake was associated with a significant increase in carbohydrate oxidation. A previous human study has reported an increase in blood triglycerides and an increase in RER during exercise after the 2-week MCTs ingestion [34], indicating that MCTs intake may promote both carbohydrate assimilation and oxidation. Furthermore, studies using animals have reported inhibition of the increase in pyruvate dehydrogenase kinase 4 protein, which suppressed the glycolytic system on a ketogenic MCTs diet [36] and increased blood glucose uptake on a high-fat MCTs diet [37]. The increase in carbohydrate oxidation obtained in the women in the present study may have been due not only to the increase in oxygen uptake by continuous MCTs ingestion but also to the enhancement of glucose metabolism shown in previous studies. When men consumed MCTs, an increase in FAO and mFAO and a decrease in RER were observed during 20Ex, and a decrease in RER was observed during InEx, indicating a possible increase in FAO during aerobic exercise. We also observed a decrease in CAO during 20Ex, but no change in oxygen uptake and CAO during InEx. The lower daily FAO in men as compared to women may be related to substrate oxidation during exercise [1].

In the present study, we examined the effects of MCTs consisting of different compositions of MCFAs on FAO during exercise. Our results indicate that the ingestion of both MCTs may increase FAO in women. On the other hand, in males, C8R ingestion clearly had a greater effect, while C10R intake might not have an effect. Since the C8/C10 ratio of commonly used MCTs (60/40) [8,9] is close to that of C8R (75/25) in this study, the increase in FAO by C8R intake observed in males might be no different from previous studies examining the effects of MCTs ingestion. In addition, regarding the effect on carbohydrate oxidation, C8R ingestion was suppressed in men, and C10R did not have an effect, but both MCTs ingestions increased in women. These results indicate that C8R ingestion may be more potent in increasing FAO during exercise than C10R ingestion and that C10R ingestion may increase CAO via increased oxygen uptake during exercise. Therefore, it was shown that substrate oxidation during exercise might be differentially affected via differences in daily substrate oxidation between men and women [1] and the composition of MCFAs in MCTs.

There are several limitations to the present study. First, the lack of a prior power analysis may have resulted in a sample size that was less than what would have enabled us to draw clear and valid conclusions in the study outcomes. In addition, the study was conducted on the average Japanese BMI level (BMI 22–24), and it is unclear whether the effect would be similar for the average BMI level in other countries. Finally, in the present study, the measurement of substrate oxidation is affected by the exercise modality, as the

exercise trials were performed on a bicycle ergometer with a combination of fixed and incremental loads, and it is not clear whether our results can be translated to other exercise modalities such as running and swimming.

Before starting exercise in a person who does not habitually exercise, a self-check questionnaire and medical check are considered to be important to maintain health [38]. In addition, food-dependent exercise-induced anaphylaxis (FEIAn) is known to be an exercise-induced allergy [39]. In general, FEIAn is induced by high-intensity exercise after ingestion of foods that are wheat products or crustaceans [40].

5. Conclusions

An interventional study showed that short-term ingestion of MCTs by sedentary participants aged in their 40s and 50s could increase FAO during aerobic exercise compared to carbohydrate ingestion, but the enhancing effect of MCTs on substrate utilization and oxygen uptake might vary, depending on sex and the composition of MCFAs.

Author Contributions: K.H. and N.N. conceived and designed the present study. H.S. and K.K. (Kazuhiko Kato) supervised the present study. S.T. and N.N. performed the trials. N.N. wrote the manuscript, and S.T., K.H., and K.K. (Kazuo Kondo) critically revised the manuscript. All authors approved the final manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The present study was funded by the Nisshin OilliO Group, Ltd., Tokyo, Japan.

Institutional Review Board Statement: The study was conducted to the guidelines of the Declaration of Helsinki (2013), and approved by the Ethics Committees of Nihonbashi Egawa Clinic (Ethical approval code: RD07001KW04; date of approval: 10 Aug 2018).

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

Data Availability Statement: Data not available due to commercial restrictions.

Acknowledgments: The authors acknowledge all participants in the present study.

Conflicts of Interest: The authors N.N., S.T., and K.H. are employees of The Nisshin OilliO Group, Ltd.

Abbreviations

The abbreviation list of measurement in exercise trial.

FAO	Fat oxidation
CAO	Carbohydrate oxidation
RER	Respiratory exchange ratio
VT	Ventilation threshold
cFAO	Cumulative value of FAO
cCAO	Cumulative value of CAO
mFAO	Maximal FAO rate
PO	Power output
VE/VCO2	Ventilation volume per VCO2
@	At time of

Appendix A

Table A1. Statistical analyses of normality distribution on the data of intervention effect.

Intervention Effect, Δ	Diet	Female, *n* = 17W	*p*	Male, *n* = 13W	*p*
Cumulative fat oxidation, mg					
20Ex	C8R	0.98	0.95	0.95	0.55
	C10R	0.97	0.74	0.93	0.37
InEx (~VT)	C8R	0.96	0.57	0.94	0.46
	C10R	0.95	0.39	0.83	0.02
# overall (20Ex and InEx, ~VT)	C8R	0.95	0.53	0.96	0.73
	C10R	0.92	0.14	0.93	0.31
Cumulative carbohydrate oxidation, mg					
20Ex	C8R	0.93	0.21	0.96	0.69
	C10R	0.96	0.67	0.93	0.33
InEx (~VT)	C8R	0.97	0.85	0.96	0.72
	C10R	0.93	0.18	0.91	0.18
# overall (20Ex and InEx, ~VT)	C8R	0.97	0.89	0.97	0.86
	C10R	0.90	0.06	0.92	0.22
Maximal fat-oxidation rate, mg/min					
20Ex	C8R	0.96	0.65	0.94	0.51
	C10R	0.98	0.98	0.92	0.22
InEx	C8R	0.95	0.53	0.94	0.51
	C10R	0.88	0.03	0.93	0.36
Respiratory exchange ratio					
20Ex (at mFAO)	C8R	0.97	0.89	0.97	0.85
	C10R	0.97	0.78	0.93	0.30
# InEx (at mFAO)	C8R	0.99	0.99	0.97	0.93
	C10R	0.96	0.67	0.97	0.92
Power output, W					
InEx (at VT)	C8R	0.97	0.82	0.93	0.29
	C10R	0.93	0.21	0.96	0.81
Elapsed time, sec					
overall (20Ex and InEx, ~VT)	C8R	0.97	0.83	0.93	0.39
	C10R	0.92	0.17	0.97	0.83
Oxygen uptake, ml/min					
InEx (at VT)	C8R	0.96	0.57	0.87	0.05001
	C10R	0.87	0.02	0.96	0.73
Ventilation volume per VCO2 volume, mL/mL					
InEx (at VT)	C8R	0.94	0.36	0.96	0.79
	C10R	0.92	0.16	0.99	1.00

20Ex: 20-watt fixed-load exercise; InEx: incremental load exercise; (#) overall: from 20Ex to InEx until VT; VT: ventilation threshold; mFAO: maximal fat oxidation.

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Review

Bidirectional Interactions between the Menstrual Cycle, Exercise Training, and Macronutrient Intake in Women: A Review

Sílvia Rocha-Rodrigues [1,2,3,*], Mónica Sousa [4,5], Patrícia Lourenço Reis [6], César Leão [1,2], Beatriz Cardoso-Marinho [7], Marta Massada [8,9] and José Afonso [8]

1 Escola Superior de Desporto e Lazer, Instituto Politécnico de Viana do Castelo, Rua Escola Industrial e Comercial de Nun'Álvares, 4900-347 Viana do Castelo, Portugal; ces.leao@gmail.com
2 Research Centre in Sports Sciences, Health Sciences and Human Development (CIDESD), Quinta de Prados, Edifício Ciências de Desporto, 5001-801 Vila Real, Portugal
3 Tumor & Microenvironment Interactions Group, i3S, Rua Alfredo Allen, 208 4200-135 Porto, Portugal
4 Nutrition & Metabolism, NOVA Medical School, Faculdade de Ciências Médicas, Universidade NOVA de Lisboa, Campo dos Mártires da Pátria, 1169-056 Lisboa, Portugal; monica.sousa@nms.unl.pt
5 CINTESIS, NOVA Medical School, NMS, Universidade Nova de Lisboa, Campo dos Mártires da Pátria, 1169-056 Lisboa, Portugal
6 Hospital da Luz Arrábida, Luz Saúde, Praceta Henrique Moreira, 150, 4400-346 Vila Nova de Gaia, Portugal; patricialoureis@hotmail.com
7 Oporto Sports Medicine Center, R. António Pinto Machado, 4100-068 Porto, Portugal; beatriz.marinho@fpf.pt
8 Centre for Research, Education, Innovation and Intervention in Sport, Faculty of Sport of the University of Porto. Rua Dr. Plácido Costa, 91, 4200-450 Porto, Portugal; martamassada@gmail.com (M.M.); jneves@fade.up.pt (J.A.)
9 St. Mary's Hospital of Porto, Rua de Camões, 906, 4049-025 Porto, Portugal
* Correspondence: silviadarocharodrigues@gmail.com

Citation: Rocha-Rodrigues, S.; Sousa, M.; Lourenço Reis, P.; Leão, C.; Cardoso-Marinho, B.; Massada, M.; Afonso, J. Bidirectional Interactions between the Menstrual Cycle, Exercise Training, and Macronutrient Intake in Women: A Review. *Nutrients* **2021**, *13*, 438. https://doi.org/10.3390/nu13020438

Academic Editor: Sadia Afrin
Received: 30 December 2020
Accepted: 25 January 2021
Published: 29 January 2021

Abstract: Women have a number of specificities that differentiate them from men. In particular, the role of sex steroid hormones and the menstrual cycle (MC) significantly impact women's physiology. The literature has shown nonlinear relationships between MC, exercise, and nutritional intake. Notably, these relationships are bidirectional and less straightforward than one would suppose. For example, the theoretical implications of the MC's phases on exercise performance do not always translate into relevant practical effects. There is often a disconnect between internal measures (e.g., levels of hormone concentrations) and external performance. Furthermore, it is not entirely clear how nutritional intake varies across the MC's phases and whether these variations impact on exercise performance. Therefore, a thorough review of the existing knowledge could help in framing these complex relationships and potentially contribute to the optimization of exercise prescription and nutritional intake according to the naturally occurring phases of the MC. Throughout this review, an emerging trend is the lack of generalizability and the need to individualize interventions, since the consequences of the MC's phases and their relationships with exercise and nutritional intake seem to vary greatly from person to person. In this sense, average data are probably not relevant and could potentially be misleading.

Keywords: women; sex hormones; menstrual cycle; exercise performance; nutritional intake; macronutrients; interindividual variability

1. Introduction

The biological differences between men and women contribute to several sex-specific features. Many of those differences are largely due to sex steroid hormone fluctuations, especially estrogens and progestogens [1]. Both endogenous estrogens and progestogens fluctuate predictably across the menstrual cycle (MC) in naturally eumenorrheic women [2].

Beyond reproductive function, both hormones have a huge impact on many tissues, including skeletal muscle, cardiac tissue, bone, connective tissues, and the central and peripheral nervous system, among others [3–5]. An important question that is unexplored is whether exercise training changes the endogenous production of these two hormones during the MC, or if there is an adaption to the demands of training and/or competition [6,7].

As we will attempt to establish, the most up-to-date evidence supports the notion that a complex and bidirectional relationship exists between MC and exercise training, with reciprocal influences and adaptations as well as important inter- and intra-individual variations [8,9]. For example, one study reported that alterations in strength, metabolism, body temperature, fluid balance and injury risk are concomitant with hormonal fluctuations throughout the MC and can affect approximately 75% of athletic women [10], pointing out that the way which MC affects exercise performance is highly individual. In addition, both the MC and the responses to exercise training may be mediated by nutritional status. Furthermore, nonlinear dose-response relationships suggest the adoption of equally nonlinear periodization and programming strategies to better account for inter- and intraindividual variation, establishing an ongoing dialogue between plan and process instead of relying on pre-determined plans [11].

In this vein, the relevance of individualized dietary advice in women is becoming increasingly recognized, with dietary strategies varying according to women's physiology, especially due to the different sexual hormone concentrations during the MC [12–14]. Indeed, levels of endogenous estrogens and progesterone affect the proportions of macronutrients used as fuel not only at rest, but also during exercise training. To counteract these imbalances, an adjustment in nutritional intervention to the MC phase in eumenorrheic women may be required. Accumulated evidence has suggested that estrogens improve exercise performance by varying carbohydrate (CHO), lipids and protein metabolism, although progesterone commonly acts antagonistically [15–17]. Despite inconsistent findings being reported, a thorough knowledge of complex and bidirectional interaction between MC, exercise training and macronutrient intake is highly important to further optimize exercise training effects in athletic women, in both health and exercise performance contexts.

Therefore, in the present review, we focused on the current evidence of the complex and bidirectional interactions between MC, exercise training and macronutrient intake in both health and exercise performance contexts, providing an overview of the effects of exercise training and macronutrient intake in the underlying MC-related mechanisms in women. Briefly, in the first part of this review, we will examine the bidirectional interaction of endogenous estrogens and progestogens as well as MC phases in women's physiology and exercise performance. In the second part of this review, we will explore the reciprocal interaction of energy availability, macronutrient intake and MC phases in pre-menopausal women. Two special boxes will address issues pertaining to the menopause and the intake of combined oral contraceptives.

2. State of Art

2.1. Bidirectional Relationships between Exercise Training and Sex Steroid Hormones

There are six families of steroid hormones: androgens (e.g., testosterone, androstenedione), estrogens (e.g., estradiol, estrone), progestogens (e.g., progesterone, 17-hydroxyprogesterone), glucocorticoids (e.g., cortisol), mineralocorticoids (e.g., aldosterone), and vitamin D [2,3]. In the present review, we will focus on estrogens and progestogens. Together with androgens, these are classified as sex steroid hormones [1]. As sex steroids, these hormones play a crucial role in physiological functions, including reproduction, mainly by the hypothalamic–pituitary–gonadal axis, sexual differentiation (secondary sex characteristics) and sexual behavior patterns as well as metabolic processes in adipose tissue, skeletal muscle and connective tissues, among others [3–5].

Women have a 20- to 25-fold lower circulating concentrations of androgens compared with men [18]. As precursors of estrogens synthesis, androgens play a key role in the maturation processes of ovarian follicles in women [19]. However, little is known about the

interaction of the androgens and exercise training in women [20]. Although the additional biological role of testosterone in women remains unclear, a therapeutic role for androgens in the functional limitations of ageing has arisen [20], but this is out of the scope of this review.

Circulating estrogen and progestogen concentrations induce well-known effects in cardiovascular [21,22], respiratory [23] and metabolic processes [24,25] with subsequent implications for strength and aerobic and anaerobic performance. However, much less is known about the effects of androgens in women engaging in exercise [26]. Thus, the sex steroid-related hormonal pathway's response to exercise training in health and exercise performance contexts should be reviewed.

2.1.1. Estrogens

Estrogens are a class of sex steroid molecules produced from cholesterol and secreted by the ovaries and placenta [2,25], of which the three major forms in women are estrone (E1), estradiol (E2) and estriol (E3) [27]. While circulating in the blood, estradiol is bound to a protein carrier, known as sex hormone binding globulin (SHBG), which is produced in the liver; another 30% is loosely bound to albumin, leaving only about 1% unbound and free. The biologic effects of the major sex steroids are largely determined by the unbound and free hormones [19]. Estrogens are primarily involved in the development and maintenance of normal sexual and reproductive function in women [4,5].

In women, circulating estrogens result from the ovarian secretion of estradiol and estrone, and peripheral conversion of its precursors occur in fat tissue, the skin, in muscle, and in the endometrium [19]. Estrogens fluctuate naturally throughout a woman's life [3]. As steroid hormones, they act by binding to steroid receptors through a classical pathway where they regulate gene expression [3]. Additionally, sex steroid receptors can be found outside the nucleus, including in the mitochondria, the endoplasmic reticulum, and the plasma membrane, where they activate different signaling cascades, exerting their action through a nonclassical pathway [3].

As steroid hormones, estrogens can freely pass through the plasma membrane and move into the nucleus, binding to its nuclear receptors: classical nuclear estrogen receptors (ERα and β) and novel cell surface membrane receptors (GRP30 and ER-X) [3]. Therefore, the post-transcriptional effects of estrogens include cell redox state regulation [28,29], mitochondrial function [3] and direct interference with the activity of specific enzymes, such as aromatase [30]. For example, aromatase is the enzyme responsible for the biosynthesis of estradiol and estrone from androgens, while, in postmenopausal women, this reaction usually occurs in white adipose tissue, where aromatase activity is augmented [30].

The accumulated evidence has shown that exercise training has a substantial impact on several female sex steroid-related hormonal pathways in both health and exercise performance contexts [6,7]. As recently reviewed by Rocha-Rodrigues [31], some mechanisms have been proposed for the protective role of exercise training through estrogen-mediated signaling in improving health, such as cancer and the menopause (which will be the focus of a special box). Randomized controlled trials (RCTs) conducted among healthy women demonstrated a significant decrease in total and free circulating estradiol concentrations induced by physical activity [32,33]. A clinical study [34] involving sedentary premenopausal women reported that a four-cycle intervention of moderate-intensity aerobic exercise combined with energy restriction resulted in significant decreases in serum estradiol and urinary estrone-1-glucuronide and pregnanediol glucuronide levels.

In contrast, Smith et al. [35] found no alterations in estradiol, estrone or SHBG levels after 16 weeks (4 MCs) of aerobic exercise (150 min per week at 65–70% of the maximum age-predicted heart rate) in sedentary, healthy, young and eumenorrheic women. Although studies demonstrated the effect of exercise training on free estradiol, a small part of those reporting on total estradiol revealed that the effect of exercise training was more obvious for free estradiol than for total estradiol [36,37]. As reported by Ennour-Idrissi et al. [36], total estradiol levels decreased in response to intervention-induced weight loss, but the

low levels of free estradiol observed were not attributed only to exercise training-mediated weight loss, but also to increased levels of binding proteins, as in the case of SHBG levels. These findings are interesting, as increased SHBG was associated with decreased amounts of unbound, active estrogens and androgens in circulation, which have been linked to a protective phenotype in women [38].

Estrogens exert important biological functions in the development, maturation and aging in extragonadal tissues, including in the bone [39], cardiac [28,29] and skeletal muscles [40], as well as connective tissues [41]. It is important to note that estrogen may play distinct roles in different tissues, mainly owing to intricate crosstalk between circulating and tissue-specific estrogens; therefore, their beneficial or harmful effects remain under debate. The powerful antioxidant capacity of estrogen to protect cardiac muscle [28,29] through its antioxidant and membrane-stabilizing properties is well documented, but this effect in skeletal muscle is not completely understood. Recent reports have suggested that estrogen has a noticeably positive effect on skeletal muscle mass and strength, albeit in animal models [40,42,43], suggesting that in the absence of estrogen, skeletal muscle is more prone to injury, thereby limiting regrowth. Therefore, it is hypothesized that estrogen could stabilize the extracellular matrix or act as an antioxidant to decrease muscle injury; however, the physiological significance of this effect on human skeletal muscle has not been clearly defined because of estrogen fluctuations and/or confounding factors such as age, fitness levels, exercise type and/or intensity.

There is evidence to support that both estrogens and ERs play a crucial role in the musculoskeletal tissues, such as the bone, ligaments, and tendons through regulation of CHO and lipid metabolism. Moreover, the data support the hypothesis that the ablation of ERα in skeletal muscle results in muscle mass loss, suggesting that the beneficial effects of estradiol on muscle strength might be receptor dependent [42]. In vitro and in vivo assays of skeletal muscle-specific ERα knockout mice showed that muscle contractility was impaired. These results support the hypothesis that a primary mechanism through which estradiol elicits its effects on strength is mediated by ERα. Evidence has been presented in support of the belief that estradiol signaling through ERα appears to modulate force at the molecular level via posttranslational modifications of the myosin regulatory light chain.

A controversial topic concerns the hypothetic estrogen-mediated mechanism concerning ligament laxity. In fact, ERs are expressed in the anterior cruciate ligament (ACL) and the inhibitory effect of estrogen on fibroblast proliferation and collagen production in human ACL has been reported [44]. From a clinical point of view, alterations in ACL in association with estrogen concentrations likely provides it with a greater susceptibility to injury. This issue will be explored further in Section 2.2.

In summary, the mechanisms by which estrogens interact with exercise performance in women is still unclear. There are complex mechanisms of action at play; therefore, more research is needed in order to explore the pathways by which estrogens could act on skeletal muscle in premenopausal and postmenopausal women. Furthermore, it is highly possible that considerable interindividual variation exists, which will also be analyzed in Section 2.2.

2.1.2. Progestogens

Progesterone is the major and most important progestogen in the body, and it is an endogenous steroid hormone primarily synthesized by the ovarian corpus luteum (which produces the majority of progesterone), adrenal and mammary glands [2,45]. Progesterone has functions in maintaining pregnancy (secreted by the ovarian corpus luteum during the first 10 weeks of pregnancy and by the placenta in the later phase of pregnancy), but also in other phases of the MC [19]. Moreover, progesterone has several biological activities in the human body, especially within the reproductive system, including the facilitation of implantation, and the maintenance of pregnancy due to its promotion of uterine growth and suppression of myometrial contractility, as well as the development of functional milk-producing alveolar lobules in the mammary gland [45].

Primarily recognized as a hormone of the reproductive system, progesterone also plays a functional role in the neuroendocrine axis, as well as in the musculoskeletal system, in both men and women, throughout life [46,47]. Its neuroprotective role has been demonstrated in both the central and peripheral nervous system, influencing the control of myelination and myelin plasticity by astrocytes [48]. Generally, progesterone likely interacts with estrogen to induce concerted functional and metabolic effects [49]; however, some effects can antagonize each other's effects [50]. The isolated effect of progesterone on skeletal muscle function and growth has been poorly described [51], as the roles of progesterone receptors—commonly found as three isoforms of progesterone (PR-A, PR-B, and PR-C)—in skeletal muscle are not as clear as those of estrogen receptors [52]. In fact, Greeves, Cable, and Reilly [53] found that increased quadriceps strength was associated with progesterone concentration. However, Janse De Jonge et al. [51] found that skeletal muscle contractile properties (e.g., isometric quadriceps strength with superimposed electrical stimulation) were not affected by the fluctuations in progesterone levels throughout the MC in healthy women with regular MCs. These observations have been reported by others [54,55].

Furthermore, treatment with progesterone seems to have no effect on the maximal activity of several key enzymes of lipid oxidation in both red and white rat skeletal muscle cells [56]. In the same study, the combination of estradiol and progesterone induced similar fat oxidation enzyme activities to those of ovariectomized rats, demonstrating that progesterone inhibited estradiol in physiological concentrations. Similarly, other studies found that progesterone antagonizes the stimulation of hepatic triglyceride secretion [57] and fatty acid oxidation [15] induced by estradiol in nonovariectomized female rats. Biochemically, the anti-estrogenic actions of progesterone (and also progestins) in women is mediated by the synergetic actions of estradiol (E2) and progesterone, in which a decrease in the estradiol (E2) receptor along with the synthesis of 17-hydroxysteroid dehydrogenase result in an increase in the conversion of estradiol (E2) into a less active estrone (E1) in the target tissues [58,59].

Despite the high level of interest in the effects of the female sex steroid hormones on both health and exercise performance, there remains considerable controversy in the literature regarding the effects of exercise training on progesterone. One study [60] found no changes in progesterone levels after incremental exercise on a cycle ergometer in top five basketball players, whereas others [61] reported progressive increases in progesterone levels with an incremental exercise in the luteal phase (LP) in four teenage swimmers. In healthy and untrained women (n = 10), two hours of running at 70% of maximal oxygen consumption ($\dot{V}O_{2max}$) decreased progesterone levels but increased the metabolic clearance rate of this hormone [62]. In fact, during moderate-to-high intensity exercise training, with a generalized increase in body metabolism, the metabolic clearance rate of progesterone (and also estrogen) increases, which may contribute to the decrease in hormone levels. Nonetheless, the degradation of sex steroid hormones depends on their metabolism in the splanchnic area, which decreases during high-intensity exercise training sessions, thus resulting in increased post-exercise sex steroid hormone levels [63].

2.1.3. Estrogens, Progesterone and Exercise Training

Mechanisms have been proposed by which both ovarian sex steroid hormones, estrogen and progesterone, affect women's physiology and consequently their exercise performance [17]. Specifically, estrogens are thought to have an anabolic effect on skeletal muscle [64] with a crucial role in substrate metabolism changes through increased muscle glycogen storage capacity, free fatty acid availability and the use of oxidative pathways [15,17,64]. Therefore, this mechanism decreases the dependence on anaerobic pathways for ATP production, and consequently lowers blood lactate levels, thus resulting in less fatigue [16]. This metabolic hormonal action perhaps contributes to improving the ultra-endurance exercise capacity in women vs. men. It should be noted that this oxidative energy-dependent pathway may occur at certain specific exercise intensities, and

at a higher relative effort, dependent, of course, on increased blood glucose and muscle glycogen stores [16,64].

In turn, endogenous progesterone and the synthetic progestins have a central thermogenic effect that is responsible for the increase in the basal body temperature in LP of the cycle [65]. Hence, an increase in basal body temperature is reported to increase the subjective feeling of greater exertion or strain when exercising, decreasing athletic performance, especially in hot and/or humid environments [66]. In the same study, progesterone induced an increased ventilation and maximal exercise response during the LP of the MC. As mentioned above, progesterone has the ability to antagonize estrogen actions and, thus, high levels of progesterone can constrain the increased CHO metabolism induced by estradiol [67]. Known as the catabolic breakdown effect hormone, progesterone can also reduce the muscle protein synthesis [68].

It should be noted that, during exercise training, ovarian sex steroid hormones may have indirect effects on substrate metabolism through interactions with other hormones, particularly catecholamines [69]. These findings suggest that the fluctuations in ovarian sex steroid hormones during the MC have the potential to interfere with exercise performance in women.

2.2. Bidirectional Relationships between Exercise Training and the Menstrual Cycle

After the menarche, women experience MCs, i.e., naturally occurring, hormone-dependent cycles deeply related to the female reproductive system, and in particular to levels of estradiol, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormones (LH) [16,70]. In some cases, hormonal secretion can vary from 10- to 100-fold during the MC [47]. The nonpathological MC can vary between 26 and 35 days [71], and is associated with variations in sex hormone levels, which have been hypothesized to affect neuromuscular performance and the likelihood of musculoskeletal injury [16,72,73]. In the study of Ekenros et al. [72], fifteen healthy women with regular MCs volunteered for biopsies from the vastus lateralis at three verified time points: follicular phase (FP), ovulatory phase (OP) and LP. During the MC, significant variation was found for mRNA and protein levels of estrogen ERα, which were highest in FP, while progesterone levels were highest in the LP. Incidentally, no significant fluctuations were found for androgen receptors. The authors postulated that these fluctuations may have an impact on responses to exercise training and on the risk of injury, but these were not assessed during the study, and therefore remain speculative.

In a study with fifteen eumenorrheic sedentary women aged 22.1 $\pm$ 1.0 years, not taking oral contraceptive drugs for at least 6 months prior to the experiment [74], the participants performed exercise on a cycle ergometer at 60% of $\dot{V}O_{2max}$ during 45 min, followed by exercise of progressive intensity until 80% of $\dot{V}O_{2max}$ until exhaustion. The cross-over protocol was conducted in the early FP and also in the mid LP, after a 6-month period of monitoring the MC and basal body temperature every morning of the previous 2 months. In the LP period, women had significantly lower serum total carnitine and free carnitine, but blood levels of estradiol, progesterone and acylcarnitine were not significantly different. Interestingly, one group had superior endurance performance in the LP, while the other group had superior performance in the FP. Therefore, and against the authors' conclusions, data showed differences in only two of the five blood markers, and this was not correlated with endurance performance. In a similar vein, a narrative review speculated that MCs could increase the production of hepcidin, preventing macrophages from releasing iron and reducing the intestinal absorption of dietary iron, thereby interfering with iron regulation [75]. However, the authors concluded that these relationships were still unclear, and the relationships with exercise performance were entirely hypothetical.

A systematic review with a meta-analysis (SRMA) of 21 studies and 68,758 participants aimed to identify the relationships between the MC phase and the utilization of oral contraceptives on the laxity of the ACL and noncontact injuries [76]. The data suggested a link between the phases of the MC and the likelihood of a noncontact ACL injury, and

also that oral contraceptives could reduce this risk by up to 20%. However, the authors emphasized the low quality of the evidence, advising against more definitive conclusions. Another systematic review including 17 studies [77] showed increased ACL injury risk during the pre-OP, especially in women with ACL laxity, due to kinematically altered patterns, as assessed through functional activities, including greater dynamic knee valgus and external rotation of the tibiae.

A study of 13 women with regular MCs, eight of which had premenstrual syndrome (PMS), showed that women with PMS had greater postural sway and greater threshold for detecting passive knee motion than women without PMS [78]. The authors of this study speculated that this could explain the increased incidence of exercise-related injuries in the LP. Postural sway in a static standing posture was also assessed in 18 healthy women (19.11 $\pm$ 0.9 years-old) [79]. The task was performed 1–3 days after menstruation and repeated 13 days after menstruation. In the second moment, velocity moments of postural sway were significantly higher, suggesting that the MC affects the static balance of healthy women. The authors further speculate that balance exercises could therefore relate to injury prevention. However, this study presented no investigation or demonstration that balance training could alter this MC-related dynamic in static balance. In line with the small sample size and the absence of the calculation of effect sizes, the authors' conclusions may have been premature. Unfortunately, unsupported speculation is common in this field of research. A study with 30 sedentary healthy women aged between 18 and 25 years old with a regular MC [80] assessed static and dynamic balance and stated that both had better scores in the OP in comparison to early FP, and on that basis suggested the inclusion of balance-based exercise programs. However, no effect sizes were reported, and the effectiveness of such programs in changing these fluctuations over the course of the MC were not assessed.

Research has further explored the relationships between the MC's phases and flexibility. In a study with 20 women aged between 18 and 35 years old, engaged in gymnastics classes at fitness centers, and not taking oral contraceptives, Melegario, Simão, Vale, Batista, and Novaes [81] assessed flexibility at three points in time (FP, LP and OP) using goniometry to assess eight movements across five joints (shoulder, elbow, lumbar column, hip, knee). There were no significant differences in flexibility across the three phases. Consistent with these findings, a study with 20 women not using hormonal contraceptives (HCs) and 24 women using HCs [73], flexibility was assessed in FP, OP and LP using the sit-and-reach test and, again, no significant differences were found between groups and between MC phases. Therefore, the hormonal fluctuations associated with the MC do not seem to alter flexibility levels in young, healthy women.

Beyond objective measures, however, it may also be relevant to consider the perceptions of athletic women with regard to the relationships between MC and sport performance. Interviews with fifteen international rugby players (24.5 $\pm$ 6.2 years) showed that >90% of athletes reported symptoms related to the MC [82]. Almost 70% reported that the symptoms during menstruation interfered negatively with their performance. Regardless of whether this effect on performance was real or merely perceived, it may still lead to important challenges for the athlete and the technical staff. For example, in the above-mentioned study with rugby players, common symptoms during menstruation included reduced energy levels, worry, distraction, impaired motivation and fluctuating emotions.

An RCT with healthy but sedentary women aged between 18 and 45 years-old analyzed 73 women not taking hormonal contraceptives (HCs) and a control group taking HCs [83]. The women performed a treadmill exercise at 65% of $\dot{V}O_{2max}$ in different stages of the MC (or equivalent days, in the case of women undertaking HCs), after which perceived exertion and pain were self-reported using Borg's Rating of Perceived Exertion (RPE) and the Borg Category Ratio-10 (CR-10), respectively. Women in the early FP not taking HCs presented with significantly greater increases in RPE and pain, in comparison with the late FP and LP. In a similar vein, a study with 9 elite and 21 non-elite athletic women monitored the assessed salivary testosterone levels before breakfast during the FP, OP and LP of the

MC, followed by two questions related to competitive desire and training motivation [84]. The OP was associated with the highest concentrations of salivary testosterone, with a significantly more accentuated response in the elite group. This was accompanied by an equivalent increase in competitiveness ratings. Therefore, in this study, an objective and a subjective measure peaked during the same phase of the MC, and this relationship was stronger in elite athletes. However, training factors such as modality, intensity and volume were not monitored.

Establishing causal relationships is a complex venture, usually dependent on well-designed and implemented RCTs [85]. Furthermore, most interventions tend to be multimodal, which precludes a more thorough understanding of the effects of unimodal interventions and limits our understanding of physiological responses to a given exercise protocol [86]. Despite some evidence suggesting that estrogen may increase performance in endurance exercise through a change in macronutrient metabolism [16], perhaps it is naïve to expect that MCs interfere linearly with exercise performance. MCs promote hormonal and mechanical changes that could theoretically have an impact on exercise performance, but these hypothetical links are not necessarily backed up by research. Indeed, the relationships between MCs and exercise performance have presented contradictory evidence [16]. Often, hormonal fluctuations do not reflect changes in muscle contractility, lactate kinetics, bodyweight or heart rate, among other relevant variables [9].

Additionally, *p*-values only inform about the probabilities of an event, but it would be relevant to understand the magnitude of the effects, which is precisely what Pereira, Larson, and Bemben [87] attempted to do in a recent review. Analyzing studies with eumenorrheic women, the authors included 46 studies reporting on motor output in the FP and LP of the MC. Only 15 of the 46 studies showed statistically significant differences between FP and LP, which, again, denotes that MC-related hormonal changes do not translate linearly into assessments of motor performance. Equally importantly, effect sizes varied widely in terms of magnitude and direction, i.e., they expressed both quantitative and qualitative differences. The authors present potential confounding factors, such as upper versus lower limbs, isometric versus dynamic contractions, single limbs versus full body, as well as how the phase of the MC was assessed. However, in our opinion, the results highlighted by this review reinforce the notion that there may be high interindividual variability in how the MC affects exercise performance. Moreover, adherence rates may influence the effects of any exercise program, and so this factor should also be considered [86].

In this vein, a recent SRMA analyzed the relationships between strength-related variables and the phases of the MC in eumenorrheic women [88]. Twenty-one studies permitted a comparison between early FP, OP, and mid-LP in relation to isokinetic peak torque, explosive strength, and maximal voluntary contraction. Beyond the effects being nonsignificant, again, effect sizes varied in magnitude (i.e., quantitatively) and in direction (i.e., qualitatively), reflecting the uncertainty associated with the relationships between the MC phase and performance in strength tests. Notably, the authors reported a high risk of bias in the study design. In fact, the authors reported a high level of bias, but the assessments report a risk of bias that might or might not translate into actual bias. Therefore, in line with Cochrane's recommendations, we prefer the term risk of bias [89]. Furthermore, Hayashida, Shimura, Sugama, Kanda, and Suzuki [90] found an increase in the levels of two inflammatory markers (IL-6 and calprotectin) in all three MC phases after 60 min of cycling at 75% of the anaerobic threshold. These findings suggest that exercise training at high intensity increases stress and inflammation regardless of the phase of the MC, even though the authors have concluded that this was more apparent in the menstrual phase. Conversely, low intensity exercise does not seem to have a large effect on inflammation or cell-mediated immune function [91].

The relationships between MCs and exercise performance should probably be analyzed bidirectionally, with reciprocal influences and adaptation [8,92], and subject to inter- and intraindividual variation [9]. These relationships may even be traced to before the establishment of regular MCs. Sports that promote a low body mass are associated

with menstrual dysfunction and may also be associated with delayed puberty [93]. Does intensive exercise delay the menarche, however, or do girls with delayed menarche benefit from this in some types of exercises or sports? In general, this question remains up for debate. Through regular exercise, it is possible that women learn how to maximize their performance regardless of their MC phase, especially for high-intensity exercise bouts [9]; therefore, exercise performance may be minimally affected by the phases of the MC [88]. Moreover, energy demands and nutritional status should be considered important confounding variables [16], which is why they are the subject of Section 2.3.

This section can perhaps be best summarized through the findings of a recent SRMA assessing the effects of the MC phase on exercise performance in eumenorrheic women [94]. The authors found that exercise performance could only be trivially reduced during the early FP in comparison to the other phases of the MC. They further reported large between-study variations in responses and the overall poor quality of the studies, concluding that generic guidelines on exercise performance across the phases of the MC could not be established. Finally, the authors suggest a personalized approach, considering each woman's response to exercise across the MC. We fully agree with the authors' conclusions Box 1.

2.3. Bidirectional Relationships between Macronutrient Intake and Sex Steroid Hormones

The relevance of individualized dietary advice in women is becoming increasingly recognized, with dietary strategies varying according to the health status, physical condition and endogenous estrogen and progestogen variations during the MC [12–14,108]. Overall, the concentrations of estrogen and progesterone have an impact on the utilization of macronutrients not only at rest, but also during exercise. Therefore, there might be a need to adjust the nutritional interventions during the MC phase in eumenorrheic women, especially because nutritional habits may change during such phases [13].

Nevertheless, to the best of our knowledge, few studies have investigated the hypothetic impact of diet manipulation, e.g., the percentage of endogenous sex steroid hormonal levels over the course of the MC in relation to the total energy value of various macronutrients. Furthermore, the changes that are observed throughout the MC in terms of the metabolism of macronutrients, particularly the levels of circulating hormonal concentrations, will have implications for strategies that could potentially work to increase the performance of athletic women [16]. For this reason, there is a need to design study interventions that take these changes into account and that can help define which strategies may actually be successful. The proposed nutritional strategies should be applied according to the menstrual phase for eumenorrheic and pre-menopausal women.

Box 1. Special box—Menopause and exercise training.

Menopause is a physiological process characterized by a reduction in circulating estrogen as an effect of the reduced sensitivity of the ovary to circulating gonadotropins—follicle-stimulating hormone (FSH) and luteinizing hormone (LH)—caused by a significant decrease in available binding sites due to the reduction in follicle numbers [95]. Thus, this lower sensitivity results in decreased estrogen synthesis and increased anovulatory cycles. In postmenopausal women, daily physical activity levels were inversely associated with circulating concentrations of estradiol and estrogen, but positively associated with SHBG levels [96,97]. These observations were accompanied by weight loss and decreased abdominal adipose tissue mass [98,99], the main source of estrogen synthesis after menopause.

Recently, a meta-analysis comprising 6 RCTs [100] has shown that combined intervention, including low calorie intake and exercise training, with durations ranging from 16 to 52 weeks, had a positive impact on estrone, total and free estradiol, and SHBG levels in healthy postmenopausal women. The increase in SHBG was also observed by McTiernan et al. [99] after 12-week moderate-intensity aerobic exercise, which likely resulted in decreased amounts of unbound, active estrogen. Similar effects were observed by van Gemert et al. [101] in response to 14 weeks of intensive combined aerobic and resistance exercises (4 h/week) with additional reduced fat mass and increased fat-free mass (lean mass). Taken together, these data suggest that exercise training was relatively effective in decreasing circulating estradiol levels independent of weight loss, which highlights the benefits of regular exercise training for women.

In fact, a loss of estrogen may result in greater aging-related strength loss and a reduced rate of strength gain [102]. After menopause, the rapid decline in muscle mass may be explained by the increase in the protein synthesis rate, which is counteracted by a greater increase in protein breakdown or by the fact that the proteins synthesized are not myofibrillar proteins, but rather those needed for injury repair [47]. Furthermore, postmenopausal women show reduced sensitivity to anabolic stimuli when compared to age-matched men, which may suggest that a chronic decline in estrogen decreases the response to anabolic stimuli [103]. Myofibrillar protein synthesis in women taking estrogen replacement therapy (ERT) increased in response to strength training, but not in postmenopausal women who did not take ERT [104], which emphasize the role of estrogen in determining the sensitivity of the muscle to anabolic signaling. A well-designed study dealing with monozygotic twin pairs (n = 16) who were discordant for hormone-replacement therapy (HRT) use (one twin was on HRT while the other was not), the thigh muscle cross sectional area and relative muscle area were greater in the twins taking HRT than their sisters [105]. Similarly, Sipilä et al. [106] found that the muscle cross-sectional area and knee extension torque increased in exercised postmenopausal women taking HRT. Based on compelling data from studies in postmenopausal women, exercise training in combination with HRT is more effective in fostering skeletal muscle performance and mass than either HRT or exercise alone in postmenopausal women.

However, the discussion is still ongoing. Recently, an SRMA [107] verified the effects of HRT for protection against the age-related loss of lean body mass in women aged 50 years and older. Only randomized trials were included. Twelve studies comprising 4474 participants afforded an analysis of 22 intervention arms using only estrogen, and 15 using estrogen and progesterone. Controls either received no HRT or a placebo. While 14 studies showed a protective effect of HRT with regard to not losing lean body mass, seven studies still showed a significant loss. More importantly, the difference between interventions and controls was not significant, and the absolute mean difference was of a mere 0.06 kg (95% CIs: −0.05 to 0.18) in favor of the interventions. Stratification based on the type and dosage of treatment, duration of follow-up, time since menopause, study quality and the specific protocol for assessing lean body mass provided similar results. The evidence was considered low quality based on GRADE. Therefore, perhaps there is still a considerable amount of work to be conducted within this field.

2.3.1. Energy

In order for all the systems to work properly, a sufficient amount of energy must be ingested. The energy availability is the difference between the energy intake and energy exercise expenditure in relation to fat-free mass (FFM) and is considered to be optimal for women when it is $\geq$45 kcal^{-1}·kg^{-1}·FFM^{-1}·day [109]. A recent SRMA [110] found that the MC exerted a small but statistically significant effect on the resting metabolic rate (RMR) in women, with higher values during the LP compared to the FP. The difference between the two phases is estimated to be between 100 and 300 kcal [111]. Curiously, this increased energy expenditure seems to be naturally compensated by an increase in energy intake during the LP. This was shown by the study of Barr, Janelle, and Prior [112], where women

consumed around 301 kcal more in the LP compared to the FP. This might be mediated by progesterone since this hormone can increase the appetite and food intake in the presence of estrogen [113]. In conclusion, the energy expenditure seems to be higher in the LP compared to the FP (100–300 kcal), and this difference seems to be naturally compensated by an increase in the energy intake during this phase of the MC.

2.3.2. Carbohydrates

Women rely less on CHO oxidation to support fuel requirements compared to men [12]. Carter, Rennie, and Tarnopolsky [114] investigated the effect of 7 weeks of endurance training using a cycle ergometer on whole body substrate, glucose, and glycerol utilization during 90 min of exercise at 60% peak O_2 consumption in men (n = 8, healthy) and women (n = 8, healthy, tested in the early to mid FP of the MC). Considering the lower respiratory exchange ratio, glucose metabolic clearance rate, glucose rate of appearance and disappearance, and the higher exercise glycerol rate of appearance and disappearance, the authors concluded that women oxidize proportionately more lipids and less CHO during exercise compared with men. In turn, women may favor the utilization of lipids in moderate-intensity exercise training of long duration, in comparison with men of equivalent training and nutrition status [115].

Additionally, exercise training-induced glycogen reduction is attenuated in women in comparison with men, especially in type I muscle fibers [116], and there are sex-related differences in the amounts necessary to obtain maximal glycogen reserves [108]. Devries et al. [12] observed that women (n = 13, healthy, recreationally active) in the LP, but not in the FP, used less glycogen during a 90 min bike ride at 65% peak oxygen uptake compared to men (n = 11). Additionally, the authors found that, in the LP, there was a lower glucose rate of appearance, rate of disappearance and metabolic clearance rate at 90 min of exercise compared with the FP. Estrogen leads to a decrease in muscle glycogen use in the LP compared to the FP, while also decreasing CHO use during exercise [12]. At rest, glucose levels were also lower at the FP compared to the LP, as shown by McLay et al. [14] in a study with nine moderately trained women. Due to this, and compared to men, an adjustment of the CHO intake might be postulated throughout the MC, with a decrease in the CHO requirements in the LP and possibly also in the FP. Moreover, estrogen seems to mediate a favorable effect on insulin sensitivity in women compared to men [117]. Due to this, an adjustment of the CHO intake might be postulated for athletic women throughout the MC, with a decrease in the CHO needs at rest and during exercise. These changes seem to be more pronounced in the LP, although they can also occur in the FP.

For CHO consumption during exercise, Wallis, Yeo, Blannin, and Jeukendrup [118] demonstrated that, in endurance-trained women (n = 8), the highest rates of exogenous CHO oxidation and greatest endogenous CHO sparing were observed when CHO was ingested at a rate of 60 g/h during a 2 h cycling exercise bout (≈60% $\dot{V}O_{2max}$), with no further increases at an ingestion rate of 90 g/h. The general recommendations for CHO intake during exercise are 60 $g.h^{-1}$ for exercise with a 2–3 h duration and only 90 $g{\cdot}h^{-1}$ when the exercise event is >2.5 h [119]. Therefore, the results of Wallis et al. [118] are in line with general recommendations since, for a 2 h exercise event, the CHO intake recommendation would be 60 $g{\cdot}h^{-1}$.

Regarding CHO loading, an increase in energy intake (≈30%) might be needed to achieve a significant increase in CHO intake, at least in the FP. In a study [108] comparing the different energy levels for CHO requirements to maximize glycogen storage in both female (n = 7) and male (n = 6) endurance-trained athletes, the authors found sex-related differences in the required energy levels. Indeed, athletic women were able to significantly increase their muscle glycogen concentration only when the increase in CHO intake (from 5.1 $g{\cdot}kg^{-1}{\cdot}day^{-1}$ to 8.8 $g{\cdot}kg^{-1}{\cdot}day^{-1}$) was combined with a 34% increment of the total energy intake. In the trial where only CHO was increased, the total daily amount was 6.4 $g{\cdot}kg^{-1}.day^{-1}$. In contrast, men endurance-trained athletes could increase the total glycogen concentration during both of the trials: (1) when only CHO increased (from

6.1 g·kg^{-1}.day^{-1} to 7.9 g·kg^{-1}.day^{-1}) and also (2) when both energy and CHO increased (+34% of energy and 10.5 g CHO·kg^{-1}·day^{-1}).

In this line, a study with nine athletic women, McLay et al. [14] submitted to 3 days of CHO loading (8.4 g·kg^{-1}·day^{-1} CHO) and 3 days of an isoenergetic normal diet (5.2 g·kg^{-1}·day^{-1} CHO) showed a significant increase (27%) in the resting muscle glycogen concentration in the mid FP, but not in the mid-LP. However, performance was not affected by diet or MC phase, and the MC phase had no effect on substrate utilization during exercise. In another study with six well-trained athletic women in the mid LP [120], muscle glycogen concentration showed only a modest increase (13%) with a CHO loading dose around 8.2 g·kg·day^{-1}, compared with a moderate CHO diet of around 4.7 g CHO g·kg·day^{-1}. Therefore, the impact of a CHO loading protocol on muscle glycogen concentration seems lower if an athletic woman is in the LP (0%–13%) compared to the FP (17–31%) and compared to athletic men (18–47%) [121].

These findings support previous observations of increased resting muscle glycogen concentration in the mid-LP than mid-FP, proposing that lower glycogen storage in the mid FP can be overcome by CHO loading [12]. Moreover, there is evidence that ingestion of CHO in the LP may help mitigate some of the negative effects observed in this period, such as the decrease in blood glucose to normal levels, as well as helping to support immune function [122]. Taken together, these data suggest that, in athletic women, a lower total daily CHO amount might be needed compared to men; this decrease might be more pronounced in the LP of the MC. Additionally, a CHO loading protocol of >8 g·kg^{-1}·day^{-1} might be useful to increase the glycogen concentrations in women in the FP, and an increase in energy intake might be needed to achieve this total daily amount of CHO; however, this protocol might not be needed or, at the very least, a vast difference should not be expected if the athlete is in the mid LP.

2.3.3. Lipids

Women oxidize proportionately more lipids than men at all exercise intensities [123,124]. Additionally, findings suggest that women have a greater content of intramyocellular lipids and a greater capacity to utilize these lipid stores [124,125], with the possibility that sex-based differences are more apparent with increasing exercise training duration [124]. On the other hand, the higher capacity to oxidize lipids during exercise may indicate that women perhaps require lower amounts of CHO during exercise compared to men. Additionally, and considering that estrogen, which is higher in the mid LP, increases fat oxidation and decreases CHO dependence [126], during exercise, an even lower CHO intake, both daily and during exercise, might be considered.

Concomitantly, and as mentioned above, during exercise training, the differences in preferences for energetic substrates [108,115], e.g., fat oxidation and CHO, seem to be related to the levels of estrogen and, eventually, progesterone [12]. However, even when taking into account these differences, there seems to be no advantage to applying certain strategies that aim to take advantage of this increased fat oxidation during exercise performance, such as the ketogenic diet [127]. Furthermore, existing studies do not seem to consider this difference in the oxidation of substrates throughout the MC. Taking all of these data into account, in regard to daily lipid intake, the general population recommendation of 20–35% of the total energy value [128] might be recommended for athletic women.

2.3.4. Protein

Regarding protein, the LP seems to be more catabolic than the FP, with the LP showing a higher leucine flux and oxidation, leading to an increase in resting energy expenditure [129]. Additionally, Sawai et al. [130] also demonstrated that, during the LP, the plasma concentration of several free amino acids was lower compared to the FP, suggesting an accelerated protein catabolism during the LP. There is also evidence of a greater protein catabolism during the LP, with the differences being smaller when a CHO supplement (0.6 g CHO kg·h^{-1}, in a total of around 35 g·h^{-1}) was ingested during endurance exercise

(cycling at 70% peak $\dot{V}O_2$ until exhaustion) compared to a placebo drink [131]. This suggests a greater amino acid catabolism at rest and during exercise [16], and that the intake of CHO during exercise might attenuate the amino acid catabolism. Progesterone seems to be the hormone responsible for the increased catabolism in the LP [132], while estrogen may help to reduce protein catabolism [133], both at rest and during exercise [126]. Given this, the need for a greater amount of protein during the LP might be considered [121], along with the intake of protein during exercise. However, it is also possible that these higher levels of catabolism seem to correspond to changes in the amount of protein ingested by athletic women, with an increase in the amount ingested at the end of this phase [134].

A recent systematic review [135] aiming to determine the protein requirements of premenopausal (18–45 years) athletic women concluded that the requirements are similar to recreational and/or competitive women undertaking aerobic endurance (1.28–1.63 $g \cdot kg^{-1} \cdot day^{-1}$), resistance (1.49 $g \cdot kg^{-1} \cdot day^{-1}$) and intermittent exercise (1.41 $g \cdot kg^{-1} \cdot day^{-1}$) of ~60–90 min duration. These requirements are aligned with the current sports nutrition guidelines for all athletes (1.2–2.0 $g \cdot kg^{-1} \cdot day^{-1}$) [136]. Additionally, since anabolic sensitivity seems to be similar between men and women [137], a similar amount of daily protein might be suggested, with a range of 1.2–2.0 $g \cdot kg^{-1} \cdot day^{-1}$ being recommended for the majority of situations regarding athletic performance. Unfortunately, in a systematic review [135], the influence of the MC phase on protein requirements could not be determined. The authors also concluded that 0.32–0.38 $g \cdot kg^{-1}$ pre- and post-exercise demonstrated beneficial physiological responses in recreational and competitive female athletes completing resistance and intermittent exercise. This is within the upper range of the general amount of protein recommended per meal, which is 0.25–0.4 $g \cdot kg^{-1} \cdot meal^{-1}$ [138] Box 2.

Box 2. Special box—Methodological considerations: Hormonal contraceptive effects on the MC and its impact on sport performance.

Combined oral contraceptives (COC) might provide a more controlled and stabilized hormonal profile as they play a dual role: downregulation of endogenous concentrations of estrogen and progesterone, whilst simultaneously providing daily supplementation of exogenous estrogen and progesterone [16,139]. This altered hormonal milieu differs significantly from that of eumenorrheic women and might impact exercise performance due to changes in ovarian hormone-mediated physiological processes [16,139,140]. The endogenous hormonal profile of an COC user, i.e., low levels of estrogen and progesterone, is comparable to the profile observed during the early FP of the MC [141]. COC are commonly used by athletic women, primarily to alleviate symptoms of dysmenorrhea and menorrhagia, reduce the occurrence of premenstrual tension, and other clinical conditions [142]. However, information on menstrual manipulation practices in young physically active women is sparse, with its use being estimated to be similar between athletes and the general population [16,17,143]. The potential side effects of COC impacting performance (or not) are in agreement with the newer progestins, lower dose triphasic pills or continuous pills [139].

Multiple variables may be considered in research on sports performance and COC, such as: training status (untrained, trained, elite); number of participants; variety of testing protocols; intensity of the exercise; circadian fluctuation in hormonal secretion; nutrition [16,144]. Physical fitness is generally defined in terms of aerobic and anaerobic power and capacity, as well as muscle strength and flexibility. However, sports performance is a broader and more complex concept. It encompasses neuromuscular, cognitive and psychological functions, with both nature and nurture (genetic factors and training) combined determining athletic prowess [144,145].

A meta-analysis from 2020 with 590 participants [141] was the first to used robust assurance tools, aiming to select information from early research that has had its share of methodological inaccuracies. Overall, the use of COC might result in a slightly inferior exercise performance, which may be due to an individual variability in the response of different parameters, particularly changes in substrate metabolism and heat stress response, although any group-level effect was most likely to be trivial [141,146]. However, in elite sports, it must be emphasized that even nonsignificant changes can make the difference in terms of winning a gold medal, and so an individualized approach should be adopted.

3. Concluding Remarks

This review has taken rather a long journey, in which the literature has been scrutinized in the search for knowledge regarding the relationships between MC, exercise and nutritional intake in women. Beyond an overview of the mechanisms behind these phenomena, the intention was to also deliver some concrete guidelines for translating theory into practice. In the end, the major take-home message is that relevant interindividual variation exists; therefore, any generic guidelines are prone to a lack of generalizability and may fail to provide much-needed guidance. With regard to sex steroid hormones, we explored the roles of estrogens and progestogens. In both cases, their complex mechanisms are still being unraveled by research. Moreover, their relationships with exercise, either how they impact exercise and/or how exercise impacts their regulation, remain the subject of ongoing research, which has had its fair share of controversy. In general, even the hormonal fluctuations during the phases of the MC cannot be easily correlated with exercise performance. While some internal physiological parameters indeed vary across the MC, their impact on performance seems to be highly variable from woman to woman and the magnitude of effects tends to be residual or trivial at best. The same complex and heterogeneous relationships can be observed between exercise and menopause symptoms. Before the menopause, intake of OCs may at least bring comfort to the women taking them, as they do not have to fear inopportune bleeding and pain, with the impact on exercise performance appearing to be minimal.

The interpretation of research findings concerning energy demands and nutritional intake in women in relation to hormonal fluctuations face similar difficulties, as a higher energy expenditure in some phases of the MC tends to be naturally compensated by an increase in nutritional intake. Therefore, even if an increase in energy is required, it will likely occur naturally. Likewise, strategies aiming to increase fat oxidation do not seem to bring any advantages in terms of exercise performance, and the management of protein intake during the different MC phases is also poorly understood. Overall, this is a promising field of research, but one where the search for populational trends may have to be replaced by highly individualized approaches, due to the considerable heterogeneity and variability of responses.

Author Contributions: Substantial contributions to the conception of the work: S.R.-R., M.S., P.L.R., C.L., and J.A., S.R.-R., M.S., P.L.R., C.L., B.C.-M., M.M., and J.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

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Article

L-Glutamine Supplementation Enhances Strength and Power of Knee Muscles and Improves Glycemia Control and Plasma Redox Balance in Exercising Elderly Women

Gislene R. Amirato [1,†], Juliana O. Borges [1,†], Daniella L. Marques [1], Juliana M. B. Santos [2], Carlos A. F. Santos [3], Marilia S. Andrade [4], Guilherme E. Furtado [5], Marcelo Rossi [6], Lais N. Luis [1], Raquel F. Zambonatto [1], Eliane B. da Silva [1], Sarah O. Poma [1], Mariana M. de Almeida [1], Renato L. Pelaquim [1], Laiane C. dos Santos-Oliveira [1], Vinicius L. Sousa Diniz [1], Maria E. P. Passos [1], Adriana C. Levada-Pires [1], Renata Gorjão [1], Marcelo P. Barros [1,*,‡], André L. L. Bachi [6,7,‡] and Tania C. Pithon-Curi [1,‡]

[1] Interdisciplinary Post-Graduate Program in Health Sciences, Institute of Physical Activity Sciences and Sports (ICAFE), Cruzeiro do Sul University, São Paulo, SP 01506-000, Brazil; prof.gislene@hotmail.com (G.R.A.); julili_borges@hotmail.com (J.O.B.); daniella.m.lima.dl@gmail.com (D.L.M.); laisnascimento_pa@hotmail.com (L.N.L.); zambonattoraquel@gmail.com (R.F.Z.); eliane_borges91@hotmail.com (E.B.d.S.); sarah.poma@hotmail.com (S.O.P.); maribonnes@gmail.com (M.M.d.A.); renato.pelaquim@gmail.com (R.L.P.); laiane.cris@hotmail.com (L.C.d.S.-O.); viniciusleo_diniz@hotmail.com (V.L.S.D.); melizabethpp@gmail.com (M.E.P.P.); adriana.pires@cruzeirodosul.edu.br (A.C.L.-P.); renata.gorjao@cruzeirodosul.edu.br (R.G.); tania.pithon-curi@cruzeirodosul.edu.br (T.C.P.-C.)

[2] Post-Graduation Program in Science of Human and Rehabilitation, Federal University of São Paulo (UNIFESP), Santos, SP 11015-020, Brazil; juliana-mbs@hotmail.com

[3] Department of Medicine (Geriatrics and Gerontology), Federal University of São Paulo (UNIFESP), São Paulo, SP 04020-050, Brazil; freitas.carlos@uol.com.br

[4] Department of Physiology, Federal University of São Paulo (UNIFESP), São Paulo, SP 04023-901, Brazil; marilia.andrade@unifesp.br

[5] Health Sciences Research Unit: Nursing (UICISA:E), Nursing School of Coimbra (ESEnfC), 3000-232 Coimbra, Portugal; guilhermefurtado@esenfc.pt

[6] Post-Graduation Program in Health Sciences, Santo Amaro University (UNISA), São Paulo, SP 04829-300, Brazil; mrossi@dim.fm.usp.br (M.R.); allbachi77@gmail.com (A.L.L.B.)

[7] ENT Lab, Department of Otorhinolaryngology, Federal University of São Paulo (UNIFESP), São Paulo, SP 04023-062, Brazil

* Correspondence: marcelo.barros@cruzeirodosul.edu.br; Tel.: +55-11-3385-3103

† These authors contributed equally to this work.

‡ These authors contributed equally to this work.

Citation: Amirato, G.R.; Borges, J.O.; Marques, D.L.; Santos, J.M.B.; Santos, C.A.F.; Andrade, M.S.; Furtado, G.E.; Rossi, M.; Luis, L.N.; Zambonatto, R.F.; et al. L-Glutamine Supplementation Enhances Strength and Power of Knee Muscles and Improves Glycemia Control and Plasma Redox Balance in Exercising Elderly Women. *Nutrients* **2021**, *13*, 1025. https://doi.org/10.3390/nu13031025

Academic Editors: Sílvia Rocha-Rodrigues, José Afonso and Monica Sousa

Received: 31 January 2021
Accepted: 16 March 2021
Published: 22 March 2021

Abstract: We investigated the effects of oral L-glutamine (Gln) supplementation, associated or not with physical exercises, in control of glycemia, oxidative stress, and strength/power of knee muscles in elderly women. Physically active (n = 21) and sedentary (n = 23) elderly women aged 60 to 80 years were enrolled in the study. Plasma levels of D-fructosamine, insulin, reduced (GSH) and oxidized (GSSG) glutathione, iron, uric acid, and thiobarbituric acid-reactive substances (TBARs) (lipoperoxidation product), as well as knee extensor/flexor muscle torque peak and average power (isokinetic test), were assessed pre- and post-supplementation with Gln or placebo (30 days). Higher plasma D-fructosamine, insulin, and iron levels, and lower strength/power of knee muscles were found pre-supplementation in the NPE group than in the PE group. Post-supplementation, Gln subgroups showed higher levels of GSH, GSSG, and torque peak, besides lower D-fructosamine than pre-supplementation values. Higher muscle average power and plasma uric acid levels were reported in the PE + Gln group, whereas lower insulin levels were found in the NPE + Gln than pre-supplementation values. TBARs levels were diminished post-supplementation in all groups. Gln supplementation, mainly when associated with physical exercises, improves strength and power of knee muscles and glycemia control, besides boosting plasma antioxidant capacity of elderly women.

Keywords: physical exercise; aging; oxidative stress; antioxidant; muscle contraction performance; diabetes; sarcopenia

1. Introduction

Aging is a natural process associated with a dynamic and progressive dysfunction of many physiological systems in living organisms [1]. Morphological, functional, biochemical, and psychological alterations result from co-acting variables, such as genetic factors, lifestyle, environmental issues, and diseases [2,3].

According to the document "World Population Ageing 2019—Highlights" (presented by the Department of Economy and Social Affairs of the United Nations Secretariat), by 2050, one in six people in the world will be 65 years of age or older, which will input hard pressure on global health and economy [4]. While developed countries have long lived with a large contingent of elderly people, developing countries began to face this new situation mainly from the 20th to 21st-century transition and beyond [5].

The search for healthy aging has long been the goal for many people worldwide [6]. In this sense, it is essential to point out that healthy aging is vital for the individual to perform his/her daily activities in an independent and safe way [7]. This ability is directly dependent on the efficiency of the neuronal, cardiovascular, and musculoskeletal systems and, on the other hand, is adversely affected by chronic diseases [8]. There is convincing evidence that sedentarism predisposes the individual to higher risks of chronic diseases, age-related comorbidities, and premature death [8].

Regular physical exercises can undoubtedly mitigate the harmful effects of a sedentary lifestyle on people's health. At this point, it has been highlighted that combined exercise training (associating both aerobic and resistance exercises) is more effective than any other forms of physical exercises performed alone [1]. In older people, combined exercises optimize the acquisition, maintenance, and recovery of the lost physical abilities [1,9].

Beyond the regular practice of physical exercises, efficient nutritional interventions should be applied to minimize the harmful impact of aging [10,11]. Malnutrition due to an inadequate intake of calories or protein plays a role in some of the worst endpoints of aging, e.g., muscle weakness, sarcopenia, frailty, and premature death [12]. Limited access to healthy foods, malabsorption, anorexia, and depressive behavior are among the leading causes of aging malnutrition [12].

A low energy intake may result in the depletion of muscle mass, leading to impaired musculoskeletal system function and physical disability [13]. Age-related skeletal mass loss closely associates with reducing intracellular protein content, especially key metabolic crosslink/anaplerotic amino acids, such as L-glutamine (Gln) [14]. In this sense, supplementation with protein or even isolated L-amino acids can be useful to circumvent this depletion [15]. Although there is no consensus on the ability of Gln to induce muscle hypertrophy [16], some studies reported that supplementation with this non-essential amino acid minimizes the loss of muscle mass [17]. In this respect, our research group reported that Gln supplementation for 15 consecutive days not only increased the expression of signaling factors for protein synthesis, but also reduced the expression of those involved in the protein degradation pathway in soleus muscle of diabetic rats [18]. Corroborating these findings, we also reported lower intracellular levels of Gln associated with increased protein breakdown, whereas oral Gln supplementation attenuates skeletal muscle atrophy induced by fasting [17]. In vitro, Gln improves skeletal myocyte differentiation and prevents myotube apoptosis [19]. Gln also attenuates inflammation and oxidative stress-mediated muscle proteolysis [20].

The information above led us to investigate whether Gln supplementation, associated or not with a regular practice of combined exercise training (CET), could improve glycemia control (as an antidiabetic effect), restrain oxidative stress, and enhance knee muscle strength and power in elderly women.

2. Materials and Methods

2.1. Subjects and Study Design

This was a pre/post-interventional double-blind randomized study with two major endpoints: (i) to evaluate whether Gln supplementation is able to improve plasma antioxi-

dant capacity when associated with regular exercises (focusing on glutathione metabolism); and (ii) whether Gln supplementation could enhance muscle strength and better control glycemia in exercising elderly women. As shown in the flowchart diagram (Figure 1), 44 elderly women (age between 60 to 80 years, average 69.2 $\pm$ 4.5 years) participated voluntarily in this study. All volunteers were recruited from the Primary Health Care Program of the Department of Geriatrics and Gerontology, Medical School, Federal University of São Paulo (UNIFESP), São Paulo, Brazil. The same geriatric physician, coordinator of this Primary Health Care Program at UNIFESP and coauthor of this study, carried out the clinical and physical examinations. The inclusion criteria for volunteers' selection and recruitment were: elderly women 60 to 85 years old; with clinical and medical authorization to perform the regular program of physical exercises; and with complete registration in the Primary Health Care Program (UNIFESP), including the contact of a responsible person for any emergency. The exclusion criteria were: pre- or current diagnosis of asthma; type-1 diabetes mellitus; neoplastic, renal, or liver diseases; dementia; thrombosis; or angina—diets with more than 4000 cal/day or a protein intake >1.75 g/kg body mass at the time of the study—volunteer taking any antioxidant or multivitamin supplements—use of anti-inflammatory drugs in the last two months. The weekly protein consumption (from main sources: poultry, fish, beef, and pork) of both NPE and PE volunteers is presented as Supplementary Materials.

After exclusion criteria, the volunteers were split into two groups: (i) exercising elderly women (PE, n = 21); and non-exercising elderly women (NPE, n = 23). Then, each group was subdivided according to L-glutamine (Gln) or placebo supplementation (details ahead), thereby, composing a four-group pre/post-interventional intercrossed study.

Volunteers from the PE group attended the Primary Health Care Program (UNIFESP) for, at least, 24 months, performing their regular physical exercise program at the same place and supervised by the same experienced instructor throughout the study.

Non-exercising volunteers (NPE group) were also attending the Primary Health Care Program (UNIFESP) for appointments and psychological support during identical $\geq$ 24 months. Although independent and active, they were not involved in any regular exercise program during this period. These volunteers were oriented to maintain their routine during the study.

The Ethics Committee of Cruzeiro do Sul University approved this study (protocol number #213/2014), and it is in agreement with both the Ethical Standards and Declaration of Helsinki. All volunteers were informed of the possible risks involved in the study and all of them signed written consent forms.

2.2. Glutamine Supplementation

As aforementioned, PE and NPE volunteers were randomly separated into two subgroups: (i) L-glutamine group (Gln), who ingested 10 g of Gln + 10 g of maltodextrin diluted in 250 mL water (PE + Gln, n = 11; NPE + Gln, n = 12); and (ii) placebo group (PL), which ingested 20 g of maltodextrin also diluted in 250 mL water (PE, n = 10; NPE, n = 11), per day. The Gln supplement was purchased from Tongliao Meihua Biological Sci-Tech Co. Ltd. (QiXu, China) and maltodextrin from PR Netto Indústria e Comércio de Alimentos Ltd.a., (São Paulo, SP, Brazil). Supplementation was conducted for 30 days. The experimental design is in Table 1.

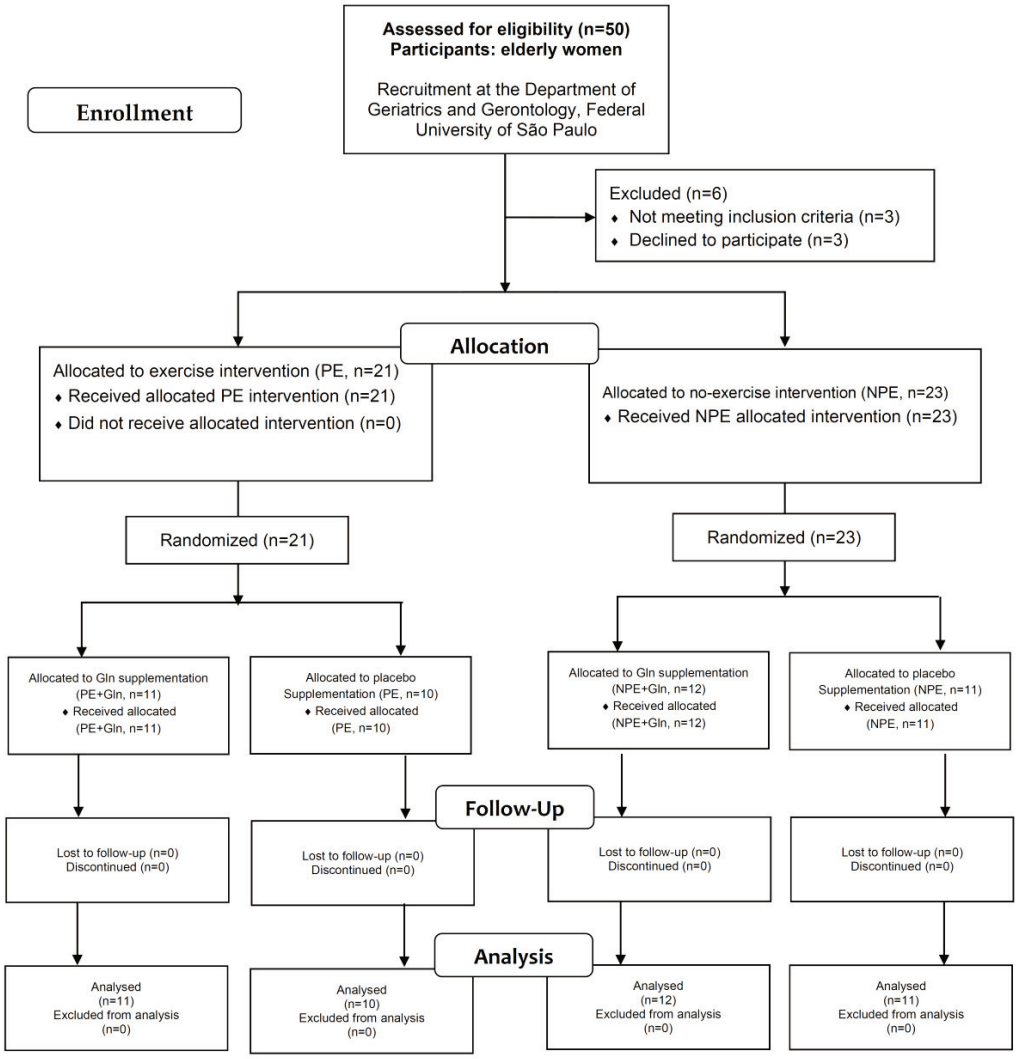

Figure 1. Flowchart of study participants.

Table 1. Experimental design of the study.

	Pre	Exercise Intervention	Post
	Before supplementation period	30 days (between sampling)	After supplementation period
Blood sampling	X		X
Isokinetic Test	X		X
Gln or placebo supplementation		X	

It is of utmost importance to clarify that the supplement randomization was carried out following this process: First, we created (computer-generated) a random list of numbers for sequential groups: PE + Gln (n = 11), NPE + Gln (n = 12), PE (n = 10), and NPE (n = 11). Each volunteer was then anonymously listed in the experimental groups (in the order as mentioned earlier) according to their recruitment ordering number in the program. After that, the volunteers received a package with 30 sachets of Gln or placebo supplement as designed by the random distribution above. The use of maltodextrin (placebo) was here

recommended since it provides the same taste, color, and texture of Gln as a supplement without any visual distinction between them. The volunteers were oriented to ingest the supplement 1× per day, during lunchtime. Dosages of Gln supplement above 30 g/day have been avoided since volunteers have attested stomach discomfort, flatulence, or even more rare, diarrhea under those circumstances (personal communications).

2.3. Body Composition

The body composition of volunteers was measured by dual X-ray absorptiometry (DXA—GE Healthcare Lunar, Madison, WI, USA). The DXA scanner was calibrated daily, as instructed by the manufacturer's guidelines. We used the software (12.3, Lunar DPX, Madison, WI, USA) to estimate body composition.

2.4. Determination of Daily Physical Activity

We assessed the daily physical activity of volunteers using the International Physical Activity Questionnaire (IPAQ) according to Craig et al. [21], adapted and validated for the Brazilian population [22]. In agreement with the World Health Organization (WHO), an individual can be considered "active" with a physical activity level above 150 min/week, while levels < 150 min/week classify the subject as "sedentary" [23].

2.5. Exercise Program

The exercise regime performed by elderly women from PE-groups involved endurance (aerobic) and resistance (strength training) physical exercises. The combined exercise program followed the guidelines for exercise prescription recommended by the American College of Sports Medicine [1,9] and performed moderately.

Daily exercises were conducted for 60–75 min, three times a week, on non-consecutive days. The aerobic exercises were performed during the first 30 min of sessions, between 60 to 70% of the maximal heart rate reserve, calculated by equation [24]:

$$HR_{max} = 208\ bpm - (0.7 \times age), \quad (1)$$

Aerobic regimes included exercises in step platforms, jump, coordination, and rhythmic movements, all performed in a low impact mode. After the aerobic exercises, the volunteers were submitted to 30–45 min of resistance exercises that involved, at least, five different exercises for different muscle groups: upper and lower limb muscles, abdomen, gluteus, and muscles related to core/postural stabilization, including dorsal and lumbar muscles. All resistance exercises were performed slowly in two series of 10–20 repetitions each, between 50 to 60% of 1-RM (repetition maximum). The resistance exercises compiled different combinations of two muscle groups (described above) and in four consecutive sessions during 30–45 min. Soreness and physical effort were estimated by the post-session Borg Scale, which was also used for monthly weight load adjustments. Monthly cardiac control was also applied for endurance/aerobic adjustments (Polar, FT1, Helsinki, Finland).

2.6. Isokinetic Strength Testing

Before the isokinetic testing, the volunteers performed a 5-min warm-up on a cycle ergometer (Cybex Inc., Ronkonkoma, NY, USA) at a resistance level of 25 W, followed by low intensity dynamic stretching exercises for the hamstrings and quadriceps (to avoid stretching influence in strength values). Following the warm-up period, volunteers performed the isokinetic concentric strength test of both lower limbs on a calibrated isokinetic dynamometer (Biodex Medical Systems Inc., Shirley, NY, USA) in a random order. Peak torque (PT) and the average power (AP) of knee flexor and extensor muscles (dominant and non-dominant) in concentric activity were measured. The concentric activity was evaluated at 60°/s and 240°/s. All volunteers completed three submaximal trials before each velocity-test for equipment familiarization and five maximal repetitions to test strength at 60°/s and 240°/s.

2.7. Functional Fitness Tests

Functional fitness results were all expressed in seconds in the timed up-and-go test (TUGT), in the 5-times chair stand test (5XST), and in the number of full steps completed in the 2-min step test (2 min step). These tests were specifically recommended for fitness evaluation of older adults [25].

2.8. Biochemical Assays

2.8.1. Sample Collection

Blood samples were collected in EDTA-containing Vacutainer® tubes at 8:00 a.m. after a 12 h fast on two different occasions: before (Pre) and 30 days after (Post) supplementation with Gln or placebo. The PE group performed its last exercise training session at least 24 h beforehand. Plasma samples (500 µL) were obtained after centrifugation of blood samples (400× *g*, 10 min) and stored at −80 °C until laboratory analysis.

2.8.2. Determination of Plasma D-Fructosamine and Insulin Concentrations

Plasma insulin concentration was determined by the ELISA technique using a commercial kit (mU/L, Mercodia, Uppsala, Sweden), and the plasma D-fructosamine concentration was determined by a colorimetric commercial kit (µmol/L, Labtest, Minas Gerais, Brazil). All the evaluations were performed to monitor anti-diabetic responses in volunteers, following manufacturers' instructions.

2.8.3. Determination of Reduced/Oxidized Glutathione (GSH/GSSG)

Reduced (GSH) and oxidized (GSSG) glutathione contents in plasma were determined using colorimetric kits (Bioassay System, Hayward, CA, USA) following the manufacturer's instructions. The reducing power of plasma was calculated based on the ratio between reduced (GSH) and total glutathione (GSH + GSSG) in the plasma of volunteers [26].

2.8.4. Determination of Iron, Uric Acid, and Lipid Oxidation Indexes

Commercial kits purchased from Bioclin-Quibasa (Belo Horizonte, Brazil) were used to quantify total iron (#K017-1), and uric acid (#K139-1) concentrations. Lipid peroxidation was assayed as thiobarbituric acid-reactive substances (TBARs) in plasma [27]. The concentration of TBARs in plasma was measured after sample treatment with 4% butylated hydroxytoluene (BHT, in ethanol) and further reaction with 0.375% thiobarbituric acid in 0.25 M HCl and 1% Triton X-100 (15 min, at 100 °C). Malondialdehyde equivalents (µmol MDA/mg protein) were calculated based on absorbance at 535 nm against blanks lacking TBA and using 1,1,2,2-tetroxyethylpropane as standard.

2.9. Statistical Analysis

All data obtained in this study were initially analyzed for adherence to Gaussian distribution by the Shapiro–Wilk test. Homogeneity of variance was further evaluated by the Levene test. Values with adherence to the normality hypothesis (parametric variables) were presented as mean and standard deviation (x ± SD) and the Student's *t*-test was used to assess significant differences for the anthropometric, IPAQ, and functional fitness data between (NPE versus PE) groups. In addition, the Chi-square test was used to determine associations between categorical variables in the clinical conditions. The acceptance of the null hypothesis was also tested between pre- and post-supplementation periods in order to evaluate possible skewness or multiple modes from the differences. In this sense, to identify significant differences between the two experimental groups (NPE versus PE), supplemented with L-glutamine or placebo in the pre- and post-supplementation periods, we used a two-way ANOVA test for repeated measures with Student–Newman–Keus post-hoc test in the parametric variables, which were presented as mean and standard error (x ± SE, bars graphic). In addition, Kruskal–Wallis test, combined with the Muller–Dunn post-hoc test, was used to assess the TBARs results since we observed deviation from

normality for this parameter (a non-parametric variable, which is presented as a median and interquartile range, box plot graphic).

The effect size (ES) for intragroup analysis was calculated using Cohen's coefficient and values: (i) between 0.2 and 0.49 indicated a small effect; (ii) between 0.5 and 0.79 indicated a moderate effect; and (iii) higher than 0.8 indicated a large effect [28].

The significance level was set to 5% ($p < 0.05$).

3. Results

Table 2 shows anthropometric parameters, clinical conditions, physical activity levels, and performance scores of the functional fitness tests in exercising (PE) and non-exercising (NPE) groups, regardless of Gln supplementation. No differences were found between the groups concerning age or anthropometric characteristics. Only two significant differences were observed regarding clinical conditions: occurrence of arthritis and depression in the NPE group. By IPAQ evaluation, the PE group showed higher physical activity time (+78% and +244% for low and moderate levels, respectively) and also shorter scores (−33%) of sitting time than the NPE group. A significantly lower time to perform TUGT was observed in the PE group than NPE for functional fitness tests performance (−19%; Table 2). No other significant differences were found between the experimental groups. All volunteers were instructed to return the sachets after the supplementation period in order to evaluate their adherence to the supplementation schedule.

Table 2. Anthropometric parameters, clinical conditions, scores of International Physical Activity Questionnaire (IPAQ), and physical fitness tests in non-exercising (NPE) and exercising (PE) older women pre-supplementation with L-glutamine or placebo for 30 days. (* $p < 0.05$).

Characteristics	NPE (n = 23)	PE (n = 21)	p Value
Age (year)	68.6 ± 4.5	69.8 ± 4.8	>0.05
Height (cm)	155 ± 6.1	155 ± 6.5	>0.05
Weight (kg)	64.1 ± 8.1	59.6 ± 10.5	>0.05
Body mass index (kg/m^2)	26.5 ± 3.4	24.6 ± 3.1	>0.05
Total body fat (%)	40.3 ± 7.3	43.8 ± 6.8	>0.05
ASM/height (kg/m)	4.4 ± 0.6	6.1 ± 0.3	>0.05
Clinical conditions (based on medications use)			
Diabetes mellitus, n(%)	3(13)	2(10)	>0.05
Dyslipidemia, n(%)	6(26)	6(29)	>0.05
Hypertension, n(%)	9(39)	8(38)	>0.05
Coronary heart diseases, n(%)	1(4)	1(5)	>0.05
Osteoarthritis, n(%)	4(17)	0(0) *	<0.05
Depression, n(%)	6(26)	0(0) *	<0.05
Hypothyroidism, n(%)	4(17)	3(14)	>0.05
IPAQ			
Low level	497 ± 83	886 ± 175 *	<0.05
Moderate level	413 ± 112	1006 ± 263 *	<0.05
High level	318 ± 70	409 ± 121	>0.05
Sitting time (min/wk)	1745 ± 242	1169 ± 90 *	<0.05
Functional Fitness Tests			
TUGT [a] (s)	8.4 ± 1.0	6.8 ± 1.2 *	<0.05
5X ST [b] (s)	11.0 ± 2.3	11.0 ± 1.5	>0.05

[a] TUGT, timed up-and-go test; [b] 5XST, 5-times chair stand test.

Figure 2 shows D-fructosamine (a) and insulin (b) levels in volunteers before (pre) and after (post) the supplementation period. Regarding placebo volunteers, the intergroup analysis showed that NPE subgroups showed approximately +30% higher D-fructosamine and +92% higher insulin than PE subgroups before (pre) the supplementation period. Similarly, Gln-supplemented volunteers also showed approximately +30% higher D-fructosamine and +107% insulin levels than PE at baseline. In the intragroup analysis, both subgroups supplemented with Gln reduced the D-fructosamine levels (Figure 2A)

post-supplementation compared to baseline values (pre): −28% (effect size, ES = 0.93) and −16% (ES = 0.81) for NPE and PE, respectively. However, it is possible to observe in Figure 2B that only the NPE + Gln subgroup presented lower insulin levels (−23%, ES = 0.92) than corresponding values before (pre).

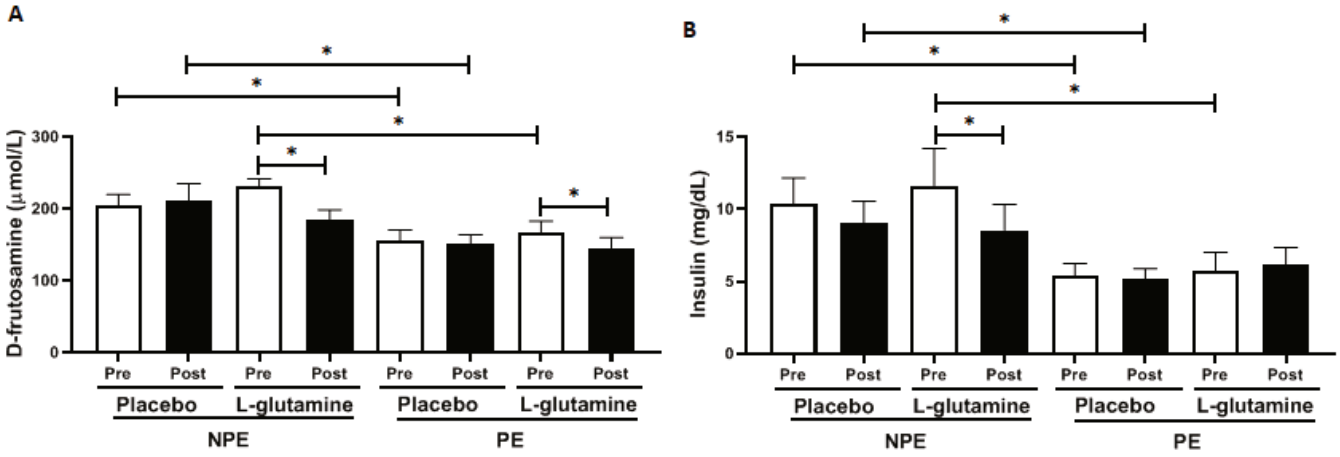

Figure 2. Plasma concentration of (**A**) D-fructosamine (µmol/L) and (**B**) insulin (mg/dL) in non-exercising (NPE) and exercising (PE) older women supplemented with L-glutamine or placebo for 30 days. * $p < 0.05$.

Figure 3A–F shows the oxidative stress indexes before (pre) and after (post) Gln supplementation in NPE and PE groups: reduced (GSH) and oxidized glutathione (GSSG); the reductive power (ratio between GSH and GSSG); and plasma concentrations of uric acid, iron, and TBARs (index of lipoperoxidation). Intergroup analysis did not show any significant differences in GSH or GSSG concentrations between subgroups, but the intragroup analysis revealed that NPE + Gln and PE + Gln samples contain higher concentrations of GSH [respectively, 2.1-fold (ES = 0.92) and 2.0-fold (ES = 1.08); Figure 3A] and GSSG [respectively, +71% (ES = 1.01) and +56% (ES = 1.09); Figure 3B] post-supplementation in comparison to (pre) baseline values. No differences were observed in the reduction power though (Figure 3C). Plasma acid uric levels did not vary significantly between groups, except for a slight increase of 7% (ES = 0.72) observed in the pre/post variation of PE + Gln group (Figure 3D). On the other hand, iron content in plasma was significantly lower in exercising (PE) individuals than in NPE (Figure 3E). Interestingly, in the placebo group, the exercise practice was responsible for an average reduction of almost −68% in plasma iron content (no pre/post effect). A much lower decrease was observed in the PE + Gln group (average of −48%; Figure 3E). Data of lipid peroxidation in plasma (TBARs levels) showed non-normalized distribution within the sample population, and therefore, TBARs levels were presented as box plots (Figure 3F). Pre/post variations were observed for TBARs values in all groups: NPE (−34%, ES = 1.07), NPE + Gln (−45%, ES = 0.99), PE (−58%, ES = 0.51), and PE + Gln (−59%, ES = 0.71; Figure 3F).

Figure 4 shows concentric peak torque and average power of extensor and flexor knee muscles obtained by isokinetic evaluations of the elderly women groups. Throughout intergroup analysis, PE subgroups showed higher peak torque and power of extensor knee muscle values than NPE subgroups, both pre- (peak torque: PE = +24.5% and PE + Gln = +27.3%; extensor knee average power: PE = +27.5% and PE + Gln = +32%] and post-supplementation (peak torque: PE = +24.6% and PE + Gln = +22.5%; extensor knee average power: PE = +32% and PE + Gln = +39.2%; Figure 4a,b). PE volunteers also showed higher peak torque of flexor knee muscle than NPE before supplementation (PE = +32.7% and PE + Gln = +29.2%; Figure 4c). However, after (post-) supplementation, only placebo PE volunteers sustained the pre-observed increased peak torque (+ 32.7%; Figure 4c). No differences were found on the average power of flexor knee muscle between subgroups, either intergroup or intragroup analysis considered (Figure 4d). The intragroup

analysis showed an increase in the peak torque of extensor and flexor knee muscles for both post-Gln supplemented subgroups: +20.6% (ES = 0.91) of extensor and +47.6% (ES = 1.21) of flexor in NPE + Gln, and +16.1% (ES = 1.31) and +21.4% (ES = 0.78), respectively, in PE + Gln subgroups (Figure 4a,c). Figure 4b shows that only the PE + Gln subgroup presented an increase of +12.7% (ES = 0.98) in the average power of extensor knee muscle.

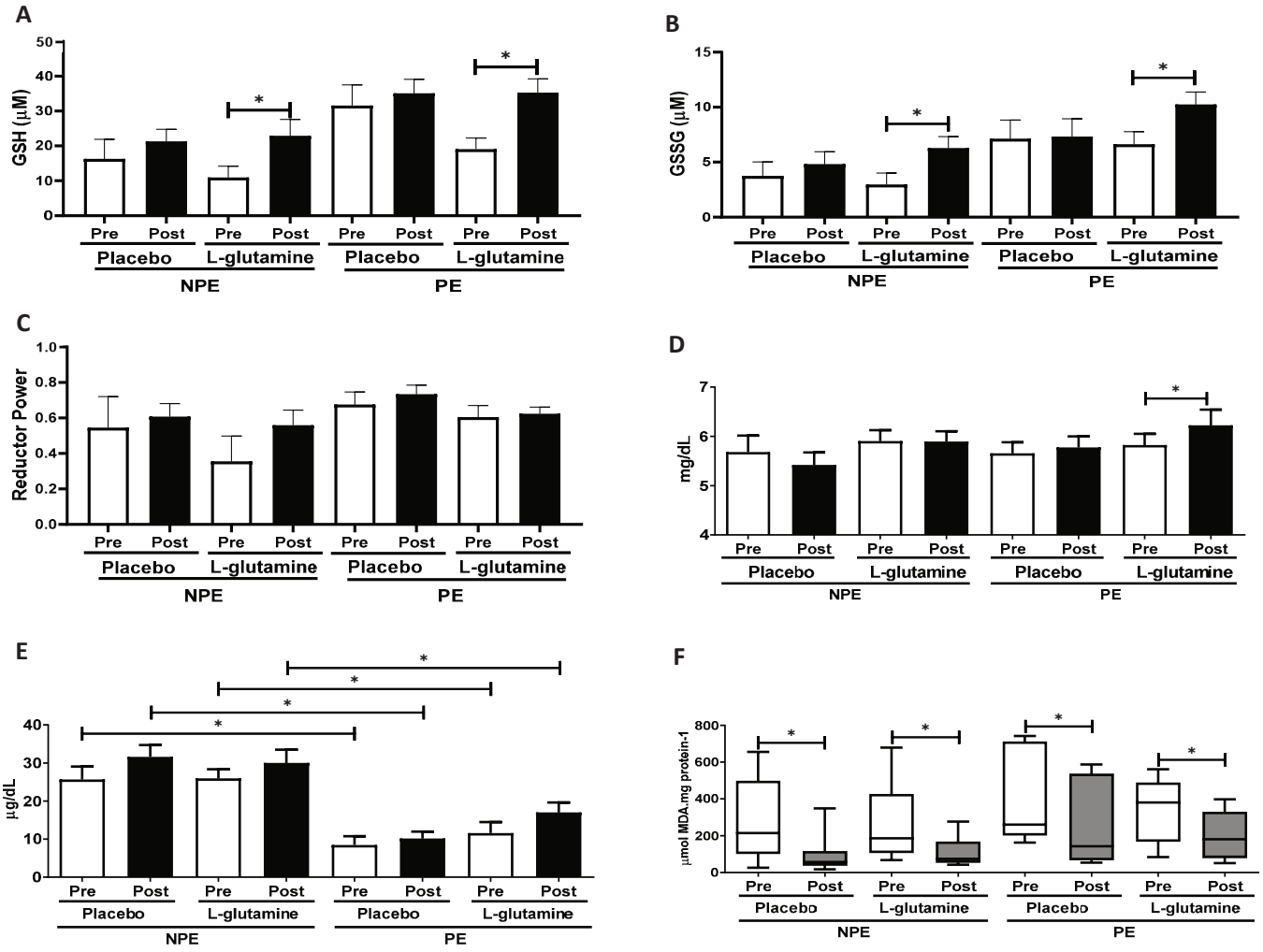

Figure 3. Plasma concentration of: (**A**) reduced glutathione (GSH) (μM), (**B**) oxidized glutathione (GSSG) (μM), (**C**) reductive power (dimensionless), (**D**) uric acid (mg/dL), (**E**) iron ions (mg/dL), and (**F**) thiobarbituric acid-reactive substances (TBARs) (μmol MDA.mg protein^{-1}) in non-exercising (NPE) and exercising (PE) older women supplemented with L-glutamine or placebo for 30 days. * $p < 0.05$.

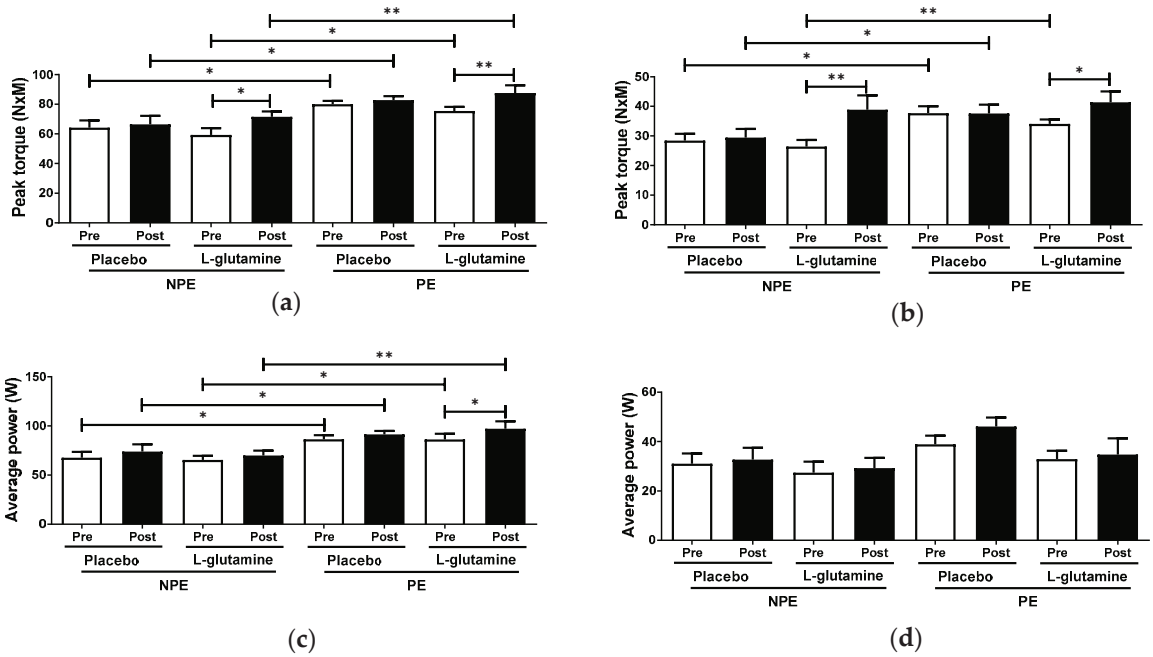

Figure 4. Peak torque and average power of extensor (respectively, **a**,**c**) and flexor knee muscles (respectively, **b**,**d**) in non-exercising (NPE) and exercising (PE) older women supplemented with L-glutamine or placebo for 30 days. * $p < 0.05$; ** $p < 0.01$.

4. Discussion

All data considered, we believe that the major findings of this study are: (i) combined exercise regime was able to improve not only glycemia control (as indicated by plasma insulin and D-fructosamine levels), but also restrained prooxidant free iron in the plasma and increased musculoskeletal strength (evaluated by isokinetic tests); and (ii) L-glutamine supplementation had anti-diabetic effects, and improved redox balance and skeletal muscle functions of elderly women, especially in association with an exercise program.

An active lifestyle is undoubtedly vital to hamper the fast progression of degenerative processes associated with aging [29]. The combination of aerobic and resistance exercises has been proven to provide health benefits to the older population by merging gains in muscle strength, neuromuscular control, cardiovascular capacity, endocrine/metabolic rebalances, and cognitive capacity maintenance [30,31]. Interestingly, clinical evidence from our exercising elderly women here (PE subgroup) reinforces that hypothesis, particularly in terms of the reduced use of medicines related to osteoarthritis and depression treatments (Table 2).

Regarding physical conditions, neuromotor activity is often compromised during aging, causing significant reductions in muscle power [32]. Compromised neuromotor connections present both qualitative and quantitative alterations, mainly characterized by a reduction in the conduction speed of the nervous impulse, lower neurotransmitter responsiveness, and progressive reduction in muscle mass, varying with the type of muscle fiber involved (mostly in type II fibers) [33]. Taken together, these alterations promote a decline of elderly physical condition, which is associated with higher risk and occurrence of dangerous falls, as often reported in advanced aging individuals [34]. In this respect, there is a consensus that the regular practice of physical exercises can mitigate the neuromotor alterations and maintain older adults' functional physical capacity, allowing these subjects to perform their routine activities safely and independently [35].

Many tests are available in the literature to evaluate physical fitness, such as time-up and go test (TUGT), 2-min step in-place test, and 5x sit-to-stand test [36]. Although we did not find differences in the 2-min step in-place and 5x sit-to-stand tests between the elderly women groups, TUGT test revealed that the PE group exhibited a better performance than

the NPE group. However, the absolute TUGT values found in both groups (most < 10 s) indicated that all volunteers preserved their functional capacities during the intervention period. The TUGT is an efficient test to assess a whole set of skills, such as balance/strength transitions for sitting–standing positions, stability and speed in walking, and marching direction changes without using compensatory strategies [37]. We examined the benefits of regular combined exercises in older women's physical capacity, of most, by evaluating the pre/post gains in skeletal muscle strength and power (by isokinetic tests). In healthy elderly people, a progressive loss of muscle power truthfully occurs, in a general way, faster than the strength loss [38].

Although we observed differences between groups in strength of both knee extensor and flexor muscles (peak torque; Figure 4), the average power was unexpectedly different only in the knee extensor muscle. At least part of this fact could be explained by the exercise training program applied, which combined aerobic and resistance training to improve cardiovascular capacity and muscle strength, among other benefits. On the other hand, the combination of aerobic and resistance exercises in the same session may limit the expected gains from each session applied separately [39]. As a hallmark of the aging process, extensor muscles lose around 3.5% of muscle power per year between the ages of 60 and 89, which is associated with alterations in the muscle architecture and reduction of the number and size of type II fibers [40,41]. However, the overall benefits provided by combined exercise programs to elderly people have been extensively reported in the last decades, and it is the best non-pharmacological intervention to avoid most of the neuromuscular and cognitive dysfunctions associated with the aging process [42].

Corroborating this fact, lower plasma levels of insulin and D-fructosamine (a glucose metabolism product) were found in the elderly exercised volunteers (PE) than in non-exercised ones (NPE). Glycemic control is one of the signatures of exercise training in the metabolism, which is particularly important when considering the comorbidities regularly associated with aging, such as metabolic syndrome and type-2 diabetes [43–45].

Since it is of utmost importance to control glycemia and to prevent diabetes during aging (for health purposes), there are many studies proposing the use of supplements and phytochemical medicines to accomplish this goal, but aiming the lowest possible side-effects. In this sense, the oral supplementation of L-Glutamine (Gln) has already been demonstrated to control glycemia in diabetic patients via stimulation of glucagon-like peptide 1 (GLP-1) secretion [46]. Additionally, Gln supplementation improves insulin signaling in muscles and liver. Gln is considered the main substrate for hepatic gluconeogenesis, especially in older people [47]. This fact may explain how oral Gln supplementation here (for 30 days) was able to promote a significant reduction of plasma D-fructosamine levels in both PE and NPE old women, together with a significant decrease of insulin levels in NPE. The 30-day placebo supplementation (maltodextrin, a carbohydrate with a high-glycemic index) did not alter the glycemic levels.

Based on recent evidence, at least part of the physiological observations post-Gln supplementation might be related to Gln-mediated adjustments in the redox metabolism [48,49]. Interestingly, Gln was also associated with efficient mitochondrial activity in muscles, whereas Gln depletion impaired myoblast proliferation, differentiation, and the heat-shock response [50]. Since exercises also input additional oxidative challenges (by overproducing reactive oxygen and nitrogen species, ROS/RNS) on activated muscles, cells, tissues, and blood/plasma, it is not surprising to observe inter- and intragroup differences on the monitored redox biomarkers here. Regular exercise causes key (redox) metabolic adaptations in skeletal and cardiac muscles to cope with the oxidative challenges imposed during practices, which are obviously also reflected in plasma. Chronically exercised young and elderly subjects obtain long-term health benefits exactly from the physiological redox stimulus imposed by exercise, demanding proper (and proportional) metabolic, immunological, neuromotor, respiratory, and cardiovascular adaptations [51].

Exercise, any type, causes iron homeostasis disruption, and its extension is apparently dependent on the duration, type (aerobic/resistance), and intensity of exercise, as well

as the regularity and previous experience of the tested subjects [52]. Ferrous ions (Fe^{2+}) are notorious prooxidants that can catalyze the formation of aggressive ROS/RNS in biological systems, imposing a physiological condition called "oxidative stress" [53]. The exercise-related iron overload is mostly originated from the iron-stocking proteins ferritin and transferrin in the bloodstream or in specific organs, such as the spleen and liver, and also in heme-iron forms from erythrocytes (hemolysis) and contractile muscle fibers (rhabdomyolysis) [54,55]. Therefore, as a product of long-term metabolic adaptations, frequent exercising subjects usually present lower background iron content in plasma, as well as lower peaks of iron release immediately after exercise sessions, compared to sedentary or less-physically active ones [56]. Similar results were also found here, since no apparent effect of Gln was observed on volunteers' background iron levels, before or after interventions. Background uric acid content in plasma was unaltered among the experimental subgroups, which was not unexpected, since most detectable uric acid variations in plasma were often observed in pre/post analysis of single (intense) exercise bouts [55].

Regarding antioxidant defenses, it has been extensively shown that Gln supplementation restores plasma and muscle Gln levels, leading to adjustments in plasma/muscle redox status (reducing power, or GSH/GSSG ratio), lower indexes of oxidative stress in erythrocytes and skeletal muscle, and even attenuation of pro-inflammatory pathways (e.g., NF-kB), mediated by TNF-α and/or IL-6 [57]. However, it is not clear if Gln supplementation quickly induces GSH biosynthesis in different tissues [58] or activates GSH transport/traffic from stocking organs (like the liver and spleen) [59]. Nevertheless, our results are clearly in agreement with these reports, as Gln supplementation significantly increased GSH levels in both exercised and non-exercised older women here (Figure 3a). Although GSSH levels were apparently unaltered by exercise, the Gln effects were reported in both PE and NPE groups, mainly when observed as pre/post changes (Figure 3b).

According to the literature, the general improvement of antioxidant status and also glycemia control can positively impact muscle function, thus, upgrading its capacity to respond to daily life activities [60]. It is paramount to understand that optimized muscle contraction, obtained during the isokinetic test, requests an adequate energy input and efficient redox balance during the contractile activity. In this sense, the capacity of Gln to serve as a substrate for liver gluconeogenesis could be understood as an additional energy supply for the active muscles during fiber contractions. Such an endergonic effect is obviously extended to sustain muscle contraction during the peak torque test. Concomitantly, the GSH-boost effect of Gln supplementation could also be responsible for an upgrade of plasma and muscle antioxidant defensive systems [48], which could better cope with the oxidative challenged imposed by exercise or intense effort performed during the aforementioned physical/strength tests [49].

Although it was possible to see evidence here for both metabolic (redox balance) and physiological benefits (strength of knee muscles and better glycemic control) induced by 30-day Gln supplementation in exercising elderly women, we could not fully cover the proper molecular mechanism involved. For example, there is a clear link between higher nutritional Gln provision and higher circulating levels of GSH (as well its oxidized form, GSSG). However, we could not determine whether this effect was related to increased GSH biosynthesis or simply enhanced GSH translocation from stocking organs, such as the liver or spleen. In addition, the proper signaling pathways, e.g., Keap1-NRf2, NF-kB, mTOR, etc.—need to be elucidated to explain the results reported here. Nevertheless, the limitations of our work truthfully open new questions and possibilities for further studies.

5. Conclusions

In conclusion, we demonstrated, for the first time, that an oral supplementation of L-glutamine for 30 days improves strength and power of knee muscles in association with improved glycemia control and concomitant boost of plasma antioxidant capacity of exercising elderly women.

Supplementary Materials: The following are available online at https://www.mdpi.com/2072-6643/13/3/1025/s1.

Author Contributions: G.R.A., J.O.B., M.P.B., A.L.L.B. and T.C.P.-C. conceived and supervised the study, analyzed the data, wrote and reviewed the initial draft of the manuscript. C.A.F.S., M.S.A., R.G., G.E.F. and A.C.L.-P. participated in the planning and development of this study with further participation in the reviewing process. D.L.M., J.M.B.S., L.N.L., R.F.Z., E.B.d.S., S.O.P., M.M.d.A., R.L.P., L.C.d.S.-O., V.L.S.D. and M.E.P.P. participated in logistic, measuring anthropometric parameters, collecting blood samples, processing, storing, and further laboratory analysis. M.R. participated in the data and statistical analysis. T.C.P.-C. was head of funding acquisition for this study. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the São Paulo Research Foundation (FAPESP) grants: #2017/06032-2; #2010/50025-1, and #2019/14115-0. G.R.A., J.O.B., and A.L.L.B. received a fellowship from CAPES Foundation, Brazil. The authors M.P.B., A.L.L.B., and R.G. are fellows of the Brazilian National Council for Scientific and Technology Development (CNPq; M.P.B. = PQ-2 #305818/2018-0; A.L.L.B. = PQ-2 #307674/2017-7, Brazil; and R.G. = PQ-2 #310711/2020-7).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Cruzeiro do Sul University (protocol number #213/2014).

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

Data Availability Statement: Original data is available with the corresponding authors M.P.B. and A.L.L.B. but not archived in databases elsewhere.

Acknowledgments: The authors are indebted to the graduate students J.C.A.P., G.L.T., and B.P. for technical support during experiments and to Rafael H. Lambertucci for a critical review on statistical analysis and its description.

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

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Choline Metabolites, Hydroxybutyrate and HDL after Dietary Fiber Supplementation in Overweight/Obese Hypertensive Women: A Metabolomic Study

Carla Patricia Novaes dos Santos Fechine [1,*], Mussara Gomes Cavalcanti Alves Monteiro [1], Josean Fechine Tavares [2], Augusto Lopes Souto [2], Rafaella Cristhine Pordeus Luna [1], Cássia Surama Oliveira da Silva [1], Jairo Alves da Silva [1], Sócrates Golzio dos Santos [2], Maria José de Carvalho Costa [1] and Darlene Camati Persuhn [1]

[1] Postgraduate Program in Nutrition Sciences, Federal University of Paraíba, João Pessoa 58059-900, Brazil; mussara.monteiro@hotmail.com (M.G.C.A.M.); rafaellacristhine@yahoo.com.br (R.C.P.L.); suramajpa2@hotmail.com (C.S.O.d.S.); jairoalvess28@gmail.com (J.A.d.S.); mjc.costa@terra.com.br (M.J.d.C.C.); darlenecp@hotmail.com (D.C.P.)

[2] Department of Pharmaceutical Sciences, Federal University of Paraiba, João Pessoa 58059-900, Brazil; josean@ltf.ufpb.br (J.F.T.); augustosouto@gmail.com (A.L.S.); socratesgolzio@ltf.ufpb.br (S.G.d.S.)

* Correspondence: carlafechine@hotmail.com; Tel.: +55-(83)-999841715

Citation: dos Santos Fechine, C.P.N.; Monteiro, M.G.C.A.; Tavares, J.F.; Souto, A.L.; Luna, R.C.P.; da Silva, C.S.O.; da Silva, J.A.; dos Santos, S.G.; de Carvalho Costa, M.J.; Persuhn, D.C. Choline Metabolites, Hydroxybutyrate and HDL after Dietary Fiber Supplementation in Overweight/Obese Hypertensive Women: A Metabolomic Study. *Nutrients* **2021**, *13*, 1437. https://doi.org/10.3390/nu13051437

Academic Editors: Sílvia Rocha-Rodrigues, José Afonso and Monica Sousa

Received: 23 March 2021
Accepted: 21 April 2021
Published: 24 April 2021

Abstract: Metabolomics has been increasingly used to evaluate metabolic changes associated with morbidities. The objective of this study is to assess the metabolic profile before and after intervention with mixed dietary fiber in overweight and obese hypertensive women. This is an intervention study, and the sample consists of 14 women aged 28 to 58 years. An intervention with 12 g of mixed soluble and insoluble fiber is performed for a period of eight weeks. Serum metabolites are identified using a Bruker ^{1}H NMR spectrometer at 400 MHz. Multivariate data analysis, including principal component analysis (PCA), is used to differentiate the two groups. After supplementation with dietary fiber, there is a significant increase in the peak intensity values of the metabolites HDL-C (0.0010*), choline (0.0012*) and hydroxybutyrate (0.0010*) as well as a decrease in systolic (0.0013*) and diastolic (0.0026*) blood pressure. The analysis of the metabolomic profile allows the identification of metabolites that have been associated in the literature with hypertension and excess weight (choline, hydroxybutyrate and amino acids) and with fiber intake (choline, hydroxybutyrate and amino acids) in addition to an increase in HDL-C. The increase in the detection of the described metabolites possibly occurs due to the presence of pathologies and the use of fiber in the intervention, which also contributes to elevated HDL-c and reduced blood pressure.

Keywords: dietary fiber; hypertension; obesity; metabolomics; nuclear magnetic resonance

1. Introduction

Despite the scientific advances in treatment, identification of pathophysiological mechanisms and public policies implemented in recent decades, hypertension remains a public health problem due to its high prevalence worldwide [1]. Considered one of the main causes of morbidity and mortality, it is an important risk factor for cardiovascular diseases (CVDs) and renal complications [2–5]. The etiology of hypertension is heterogeneous and multifactorial. This condition occurs as a consequence of genetic, environmental and lifestyle factors that trigger biological factors such as oxidative stress, endothelial dysfunction and the functionality of the renin-angiotensin-aldosterone system and the sympathetic nervous system [6,7].

Metabolomics has been used to evaluate metabolic disorders associated with hypertension. Studies comparing the metabolic profile of hypertensive patients to healthy ones point to changes in the metabolites involved in the metabolism of amino acids and lipids as the most prominent factor [8–11]. With advancements in technology, a large number of

metabolites can be measured in various body fluids, such as urine, feces, saliva and blood, providing more information on which metabolic pathways may be affected after exposure to several factors, especially food intake [9,12].

Considering the persistent increase in the prevalence of hypertension, the need for innovative prevention and control strategies has become important. The influence of fiber intake has been a topic discussed since the mid-1970s and this practice has already been associated with the prevention of cardiovascular disorders, with improvements in blood glucose, insulin resistance, weight loss and blood pressure (BP). However, there is still no consensus on its recommendation for the prevention and treatment of hypertension [13,14].

The increase in fiber intake by the general population seems to contribute to the prevention of hypertension, although there are controversies [15–17]. The decrease in BP values is due to soluble dietary fiber as well as to an increase in intestinal viscosity, delaying nutrient absorption and inhibiting the absorption of cholesterol and bile acids [18,19].

Current studies relating hypertension and metabolomic profiles are still in the initial stages. Therefore, more evidence and interpretation are needed, especially when related to cases with associated morbidities that are often found in the population, such as obesity and hypertension, as well as studies using interventions with specific nutrients. Thus, the present study aims to examine the metabolic profile of overweight and obese hypertensive women before and after intervention with mixed dietary fiber, a topic unreported in the literature.

2. Materials and Methods

2.1. Study Design and Sample Characteristics

We conducted a randomized intervention study in which 14 overweight/obese hypertensive women, aged 20 to 50 years, used fiber supplements (12 g/day of dietary fiber). Women with a medical diagnosis of hypertension and with high BP values were invited and recruited at the Blood Donor Centre in João Pessoa, Paraíba (PB), Brazil.

The initial number of participants was 20, however, there was sample loss due to the inclusion criteria (n = 5) and withdrawal due to the occurrence of adverse effects due to the use of fiber (colic and diarrhea n = 1).

This study was submitted to the Research Ethics Committee of the Healh Sciences Center, Federal University of Paraíba and was approved under CAAE number 64573917.4.0000. 5188 and registered in the Brazilian Registry of Clinical Trials under TRIAL: RBR-2PH4F9. The intervention with fiber was performed for a period of eight weeks; fiber was packed in sachets (12 g) and consisted of soluble fiber (7 g) and insoluble fiber. The fiber supplement was prepared in a pharmacy in João Pessoa, PB, Brazil. The composition of the supplement, which contained a nutraceutical based on mixed fibers, was soluble fiber (7 g), guar gum (4 g; lot ALL 0605354), NutraFlora (FOS; 1 g; lot Galena (CIQ): 1505006204), psyllium (2 g; scientific name *Plantago ovata*; manufacturer batch: 949/2013) and insoluble fiber (5 g; microcrystalline cellulose 101—5 g, batch 14116094A, manufacturing batch C1404014, formula C6nH10n + 205n = 1).

In this study, only women diagnosed with primary hypertension with a body mass index (BMI) of 25–35 m^2/kg and BP above 130/80 mmHg were included.

The exclusion criteria were diabetes, liver failure, congestive heart failure (grades 3 and 4), renal failure with creatinine values greater than 3.0 mg/dL and secondary hypertension or systolic BP values $\geq$ 180 mmHg or diastolic BP values $\geq$ 110 mmHg.

The women who agreed to participate in the study kept their eating and physical activity habits stable for at least four weeks before beginning the intervention and reported not having the intention to change those behaviors during the study.

2.2. Analysis of Biochemical Parameters

Lipid profile analyses were performed before and after the intervention of serum cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL-C), low-density lipoproteins (LDL-C), fasting glucose and high-sensitivity C reactive protein (hs-CRP). For bio-

chemical analyses, blood was collected by venipuncture in three different sterile tubes: tube 1 (with the anticoagulant K_3 EDTA-ethylenediamine tetra-acetic acid), tube 2 (with the anticoagulant sodium fluoride) and tube 3 (with the clot activator). The samples from tubes 2 and 3 were centrifuged immediately to obtain plasma and serum, respectively, and were subjected to analysis less than 2 h after collection.

The lipid fractions, TC and TG were determined by enzymatic assays [20,21]. TC and TG (enzymatic method—Trinder) were measured in serum aliquots in an automated analyzer using a Labtest kit.

2.3. Blood Pressure Assessment

Blood pressure was assessed using the criteria proposed by the Brazilian Society of Cardiology in its Hypertension Guideline of 2010. The procedure consisted of performing three BP measurements, using an OMRON HEM-742INT blood pressure monitor, with a 1-min interval between each measurement. The women were resting (for at least 5 min) in the sitting position with their feet on the ground and arms supported on the table.

2.4. Nutritional Evaluation

To calculate BMI, the body weight (kg) was divided by height (meters) squared, adopting the cut-off values recommended by the World Health Organization (WHO) for adults aged 20 to 59 years: <18.5 kg/m^2 (underweight), 18.5–24.9 kg/m^2 (normal weight), 25.0–29.9 kg/m^2 (overweight), 30.0–39.9 kg/m^2 (obese), and $\geq$40 kg/m^2 (extremely obese) [22]. Weight and height were measured in triplicate, taking the mean of the three values [23].

The usual intake of total calories, carbohydrates, proteins, lipids and fibers were analyzed using two recalls (24-h food recall), applied by professional nutritionists on the first day of the study and after eight weeks. Food consumption was analyzed using the Virtual Nutri Plus software and was subject to intra and interpersonal adjustment using the MSM software (multiple sources method).

The usual intake of total calories, carbohydrates, proteins, lipids and fibers was 2390.91 $\pm$ 1449.42 kcal, 267.82 $\pm$ 103.26 g, 68.29 $\pm$ 25.69 g, 61.06, $\pm$ 30.90 g and 15.57 $\pm$ 5.59 g, respectively, after analysis of two 24-h food recalls, applied on the first day of the study and at eight weeks. The usual intake of dietary fibers in the diet, before the intervention and after, was 15, 57 $\pm$ 5.59 g. That added to 12 g of fibers from the intervention with mixed dietary fibers, totaling 27, 57 g of mixed fiber consumption during the eight weeks of the study.

2.5. Metabolomic Profile

Metabolomic profile analyses were carried out using ^{1}H nuclear magnetic resonance (NMR) at the Multi-User Laboratory of Characterization and Analysis (LMCA, for its initials in Portuguese) of the Federal University of Paraíba (UFPB, for its initials in Portuguese) using a Bruker spectrometer at 400 MHz. The analysis of the women's serum was carried out pre- and postintervention. Initially, 5 mL of venous blood was collected in polypropylene tubes containing EDTA in the early morning under fasting conditions. After collection, the blood was centrifuged at 3000$\times$ g for 10 min at 4 °C to isolate the serum. The serum was transferred to a sterilized tube and stored at −80 °C; it was thawed for use, removing 300 µL of serum and adding 300 µL of phosphate buffer (TSP) as a reference standard, and homogenized by vortexing for 30 s, followed by centrifugation at 14,000$\times$ g for 10 min at 4 °C. After centrifugation, 60 µL of heavy water was added. This was followed by NMR analysis; the supernatant, in a 5 mm tube, was transferred to a 400 MHz NMR. The pulse sequence used to obtain data was CPMG-BRUKER and was performed in triplicate.

The obtained spectra were processed using MestreNova software (version 6.02, Mestre-Lab Research S.L.-Spain), and the chemical shifts were expressed in PPM. The metabolites were identified by comparing the obtained chemical shifts with chemical shifts of authen-

ticated samples available in the human metabolome database HMDB The spectra were referenced by tetramethylsilane (TSP), in a total range chosen for analysis of between 0.1 and 9 ppm, with *binnings* of 0.02 ppm, generating a data matrix of 96 samples and 225 variables that was exported in ASCII format to the statistical software "Unscrumbler" (version 9.7, CAMO Analytics AS, Norway). The data were not normalized by area, and the peaks for TMS, D_2O, methanol and acetone were discarded.

2.6. Statistical Analysis

To describe the general characteristics of the groups, data are expressed as the mean and standard deviation, proportion or N (%). Continuous variables were tested for normality and homogeneity of variance using the Shapiro-Wilk test. To evaluate possible pre- and postintervention differences in relation to the studied metabolites, paired t-tests or Wilcoxon tests were used based on the distribution of the variables. The Spearman correlation test was applied to determine the existence of a relationship between systolic and diastolic BP and the metabolites (choline, hydroxybutyrate, alpha-glucose and HDL). The statistical software R version 3.3.2 was used for analyses, and statistical significance was set as $p < 0.05$.

The NMR data were processed using MestreNova software (version 6.02, MestreLab Research S.L.). Principal component analysis (PCA) was used and the chemical shifts were expressed as PPM.

3. Results

The characteristics of the sample regarding demographic, epidemiological and lifestyle variables are presented in Table 1.

Table 1. Clinical and demographic characteristics of the sample of hypertensive women recruited from the Blood Donor Center of João Pessoa, PB, Brazil.

Variable	Mean/SD and %
Age (years)	44.28 ± 9.52
Weight (kg)	88.83 ± 14.25
Height (m)	1.60 ± 0.07
BMI (kg/m^2)	34.60 ± 5.90
Overweight	5 (35.7%)
Obese	9 (64.3%)
Use of medication	9 (64.3%)
Education level	
Less than 14 years	7 (38.89)
More than 14 years	7 (38.89)
Household income in US dollars	1243.04 ± 1035.94
Physical activity	5 (27.78%)
Frequency of physical activity/week	1.21 ± 1.89
Tobacco use	1 (0.06%)
Alcohol intake	2 (11.11%)

The lipid profiles and BP values were compared before and after the intervention, and there was a difference between the HDL cholesterol and BP values (Table 2). Observing the lipid profile of the 14 women who participated in the present study, 35.7% had HDL-C values below those recommended, and 42.9% of the participants had high TG levels.

The usual food consumption of total calories, carbohydrates, proteins, lipids and fibers was, respectively, 2390.91 ± 1449.42 kcal, 267.82 ± 103.26 g, 68.29 ± 25.69 g, 61.06 ± 30.90 g and 15.57 ± 5.59 g.

Figures 1 and 2 show, based on the figures, the most prominent metabolites before and after the intervention.

Table 2. Comparison of the blood pressures and lipid profiles of hypertensive women with excess weight, recruited from the Blood Donor Centre of João Pessoa, PB, Brazil, before and after intervention with mixed dietary fiber.

Variables	Before	After	*p* *
Total cholesterol (mg/dL)	180.71 ± 44.78	186.21 ± 39.02	0.5083
TG (mg/dL)	139.93 ± 58.62	134.29 ± 66.86	0.6546
HDL-C	50.5 ± 13.49	58.57 ± 11.76	0.0127 *
LDL-C	105.93 ± 36.89	104.07 ± 28.78	0.8013
VLDL-C	24.36 ± 7.18	23.57 ± 7.99	0.6480
SBP (mmHg)	145.93 ± 19.10	129.64 ± 17.17	0.0013 *
DBP (mmHg)	92.78 ± 12.01	86 ± 10.98	0.0026 *

* Paired *t*-test. VLDL: Very low density lipoprotein. SBP: Systolic blood pressure. DBP: diastolic blood lipoprotein.

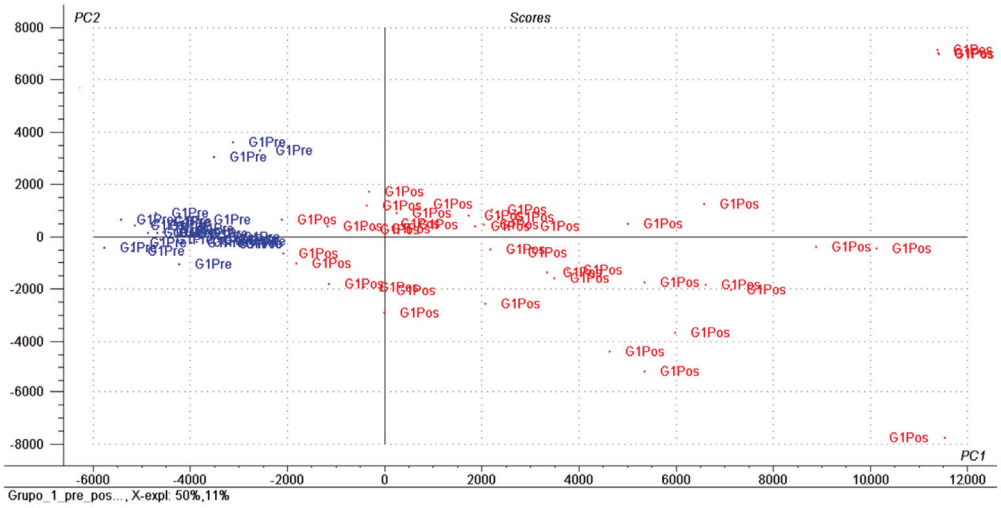

Figure 1. Graphical representation of the principal component analysis (PCA) comparing the metabolomic profiles of hypertensive women with excess weight before and after intervention with mixed dietary fiber (blue-preintervention, red-postintervention).

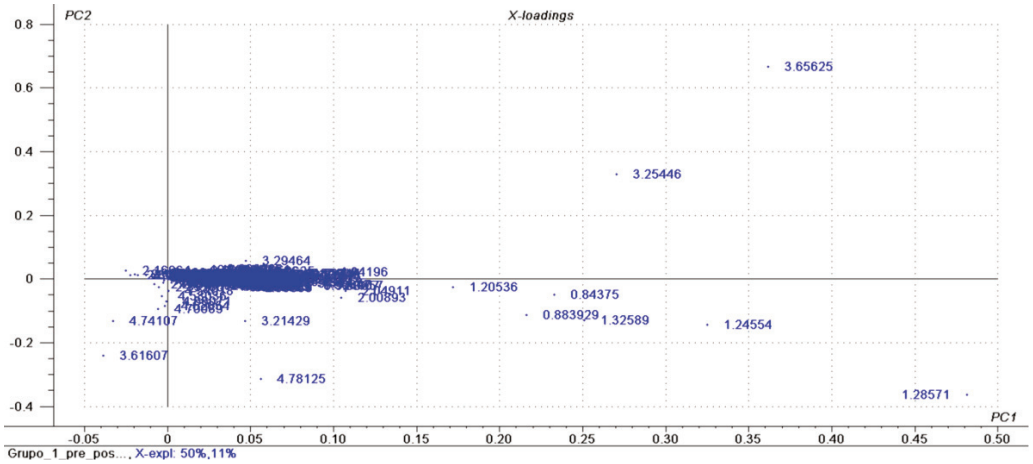

Figure 2. Metabolites identified and highlighted based on the principal component analysis (PCA), presented as a loading graph, comparing the metabolic profiles of hypertensive women with excess weight before and after intervention with mixed dietary fiber.

The following metabolites were notable based on the peak area (* = PPM—chemical shift): *0.88 (HDL); *0.84 (HDL); *1.32 (HDL); *1.28 (HDL); *3.25 (choline); *3.65 (choline); *1.24 (HDL); *1.20 (3-hydroxybutyrate); *4.78 (water); *4.90 (alpha-glucose); *4.94 (HDL).

The representative 1D Carr Purcell Meiboom Gill (CPMG) NMR spectrum of hypertensive patients after fiber supplementation is presented in Figure 3. It was possible to identify the following main metabolites: lipids, glucose, valine, leucine, isoleucine, alanine, choline, tyrosine, histidine and 3-hydroxybutyrate. Spectral area assignments were based on a 1H-NMR spectrum representative of serum. The assignment of NMR to metabolites was based mainly on literature research.

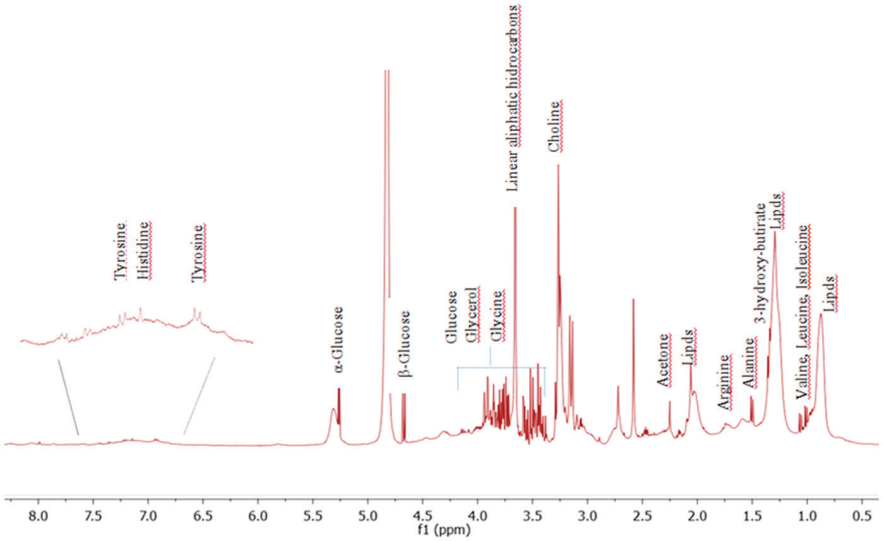

Figure 3. 1D Carr Purcell Meiboom Gill (CPMG) 1H NMR spectrum representative of serum metabolites.

The metabolites that were significant for HDL, choline, and hydroxybutyrate when comparing the groups, as presented in Table 3. Regarding the Spearman correlation test, there was no correlation between metabolite values and BP after the intervention with mixed dietary fibers (results not shown).

Table 3. Comparison between metabolites before and after intervention with mixed dietary fiber in women recruited from the Blood Donor Centre of João Pessoa, PB, Brazil.

Metabolites Signal/NMR PPM ** PPM **	Before	After	*p* *
HDL 1.24	492.79 ± 215. 06	1894.39 ± 0.12	0.0010 *
HDL 0.88	747.41 ± 323.87	2245.25 ± 792.05	0.0000 *
HDL 1.32	1048.69 ± 405.98	2669.57 ± 1131.73	0.0000 *
HDL 1.28	1411.59 ± 688.89	4776.78 ± 2082.98	0.0000 *
HDL 4.94	400.48 ± 258.96	959.79 ± 694.40	0.0258 *
Choline 3.25	992.72 ± 285.88	2668.72 ± 1547.87	0.0012 *
Choline 3.65	699.94 ± 877.23	2946.51 ± 2527.32	0.0035 *
Hydroxybutyrate 1.20	191.03 ± 59.24	1537.21 ± 571.08	0.0010 *
Alpha-glucose 4.90	1292.96 ± 949.88	1421.54 ± 958.67	0.9750

* Paired *t*-test or paired Wilcoxon test, based on the distribution of variables. $p < 0.05$. ** Chemical shift (ppm).

4. Discussion

In the present study, we identified an increment in HDL-C, choline and hydroxybutyrate metabolites and reduction in systolic and diastolic BP in hypertensive women with

excess weight, after dietary fiber supplementation. In the literature consulted, there were no studies related to the metabolomic profile of overweight and obese hypertensive women who consumed mixed dietary fiber.

Regarding metabolites studies conducted with women and intervention with fiber, an analysis was found of the serum of hypercholesterolemic postmenopausal women, who consumed a minimum of 20% of their daily energy intake as rye bread rich in insoluble fiber, with lower amounts of leucine and isoleucine and higher amounts of betaine [24,25].

Changes in these metabolites are often related to intestinal microbiota dysfunction. Branched-chain amino acids (valine, leucine and isoleucine) are associated with insulin resistance and obesity (a morbidity included in the sample of the present study), probably due to excess energy intake, as intestinal microbiota are an important modulator of branched-chain amino acid levels [26].

The study described above indirectly corroborates the increase in choline found in the present study because betaine is derived from choline, and this metabolite is also positively related to obesity, lipid metabolism and hypertension [25,27–31].

The differences in the results regarding the metabolites found are possibly due to the fiber composition, which in the present study was rich in soluble fiber and originated from various foods, such as guar gum, NutraFlora (FOS), psyllium seed bark and cellulose. The characteristics of the studied populations also differ, although both are composed of women; in the present study, obesity and hypertension were inclusion criteria.

A study conducted in premenopausal overweight/obese women with (n = 36) and without metabolic syndrome (n = 42) found that branched-chain amino acids (leucine, isoleucine and valine), aromatic amino acids (phenylalanine, tyrosine, tryptophan and methionine) and several types of fatty acids and phospholipids were associated with metabolic syndrome [32].

The results [32] partially corroborate those of the present study, which also identified the hydroxybutyrate metabolite and an increase in phosphatidylcholine (rich in choline), as well as branched-chain amino acids, histidine, tyrosine, arginine and alanine, although they were not significantly different after the intervention with dietary fiber.

Bagheri et al. [33] used a metabolomic approach to differentiate specific metabolites of healthy and obese individuals of both sexes (18 to 50 years of age) to identify profiles of metabolites associated with obesity.

Those authors observed that some amino acids and polar lipids, such as branched-chain amino acids and phosphatidylcholine, were higher in obese individuals. Thus, certain choline-based metabolites, such as phosphatidylcholine and lysophosphatidylcholine, are commonly altered in the presence of obesity, indirectly corroborating the present study regarding the significant increase in choline in the sample studied.

Choline at adequate levels is an essential nutrient for maintaining health, including adequate lipid metabolism [30], and is involved in the pathogenesis of metabolic syndrome and cardiovascular diseases [34,35]. Gao et al. [30] observed that adequate choline and betaine intake was associated with a lower percentage of body fat and higher lean mass, specifically in men, corroborating the present study, which did not find an association of these metabolites with body composition in women. However, the mechanisms by which choline and betaine improve body composition are still unclear.

Different mechanisms have been proposed to explain the effects of short-chain fatty acids, such as butyrate, on the control of obesity [34]. Butyrate attenuates diet-induced obesity and insulin resistance in mice and negatively regulates PPAR-γ expression and activity, thus promoting a change in lipogenesis toward lipid oxidation, thus stimulating oxidative metabolism in the liver and adipose tissue and increasing energy expenditure [36–39]. In their recent study, Wang et al. [34] found a positive association between BMI and short-chain fatty acids (butyrate and isobutyrate) measured by plasma metabolomics in Chinese adults aged 30–68 years. In addition, butyrate has anti-inflammatory effects that are presumed to be mediated by the inhibition of histone deacetylase (HDAC).

Regarding studies with metabolomic profiles of hypertensive women, no studies with this objective were found in the literature. A recent study with a sample that included both sexes developed by Goïta et al. [29] stands out; they evaluated plasma metabolic profiles using metabolomics by means of mass spectrometry, in 64 individuals aged between 34 and 60 years, 28 of whom were hypertensive (13 women and 15 men) and 36 nonhypertensive (18 women and 18 men).

Goïta et al. [29] identified a considerable increase in the levels of phosphatidylcholine in the blood of hypertensive patients of both sexes. In women, other metabolites increased, including the amino acids arginine, leucine, isoleucine, tryptophan, phenylalanine, tyrosine, lysine, glutamine, histidine, methionine and citrulline. In part, the results of the present study corroborate this author by observing a significant increase in choline and identifying leucine, which may indirectly have contributed to the significant increase in hydroxybutyrate. Moreover, the studies are coincident in other amino acids such as arginine, isoleucine, tyrosine, glycine, valine, alanine and histidine, although without differences in the comparison between pre- and post-intervention samples. Choline is recognized as an essential nutrient for maintaining human health, as it is involved in the donation of a methyl group and the synthesis of phospholipids, lipoproteins and betaine and is a precursor of acetylcholine [30,40–42].

Regarding studies on the lipid profile of obese and hypertensive individuals with or without fiber intake, Pasanta et al. [43] investigated the lipid profiles of serum metabolites using 1H NMR in 46 young obese and healthy adults of both sexes (18 to 25 years of age). The spectrum for the overweight group showed slightly higher lipids, α-glucose and β-glucose. Therefore, the TG, LDL, VLDL, HbA1c and blood glucose levels were significantly higher in the obese group, in addition to significantly lower HDL. In the present study, the mean HDL-C values were within the reference values, and after the intervention with fiber, these values increased significantly (Table 2), subsequently leading to a significant increase in HDL metabolites.

In a study by Pasanta et al. [43], there was a nonsignificant increase in choline in the obese group compared to the healthy group, suggesting a change in lipid metabolism, thus corroborating the present study in which choline was the metabolite that stood out the most in women after fiber supplementation.

Thus, scholars have supported an association between lipid metabolism and BP [11]. Dietary fiber intake seems to promote adequate serum lipid levels, and this benefit may be associated with improved endothelial vasodilation and oxidative stress, and consequently, decreased BP levels [44,45].

We observed a significant increase in butyrate in the metabolites derived from the serum of women after eight weeks of mixed fiber intake. This is a short-chain fatty acid that is produced in large quantities by bacterial fermentation of dietary fiber in the intestinal lumen. Butyrate can regulate the secretion of apoA-IV and, therefore, modulate reverse cholesterol transport, which may explain the increase in HDL-C in the present study [46,47].

In addition to stimulating the secretion of lipoprotein particles containing apoA-IV, favoring the removal of cholesterol from peripheral cells [47], butyrate also plays an important role in the modulation of immune and inflammatory responses and intestinal barrier function [48,49].

Thus, in agreement with our results presented here, other studies that employed metabolomics and NMR identified that pea fiber intake significantly increased the plasma levels of 3-hydroxybutyrate in rats [50]. Therefore, the results suggest that pea fiber can exert antioxidant activity, altering lipid metabolism and decreasing bile acid metabolism [25,50]. On the other hand, Dewulf et al. [51], in a study with obese women who consumed a mix of inulin and oligofructose for three months (16 g/day), reported that there was no significant impact on TC, HDL, LDL and TG compared to the control group, results that do not agree with those of the present study, in which an increase in HDL-C was observed, reinforcing the reduction in BP by cholesterol metabolism.

As limitations of the study, we point out the following aspects: (1) the absence of plasmatic dosages of choline and hydroxybutyrate, (2) that the study population was made up only of women, and (3) that it did not take into account possible genetic backgrounds that may have influenced the response to supplementation. The results found encourage further studies including both genders that seek to elucidate the mechanisms by applying molecular techniques.

5. Conclusions

In the present study, there was a significant increase in the concentrations of HDL, choline and hydroxybutyrate and a reduction in BP in hypertensive women with excess weight after intervention with dietary fiber. These significant increases in the different metabolites observed after the intervention were possibly a result of the presence of these metabolites in morbidities such as obesity and hypertension, in addition to those resulting from fiber intake, especially HDL. Therefore, based on the results found in the present study, the intake of mixed dietary fiber should be promoted to combat hypertension.

Author Contributions: Conceptualization—C.P.N.d.S.F., M.G.C.A.M., D.C.P., M.J.d.C.C. and C.S.O.d.S.; data curation—C.P.N.d.S.F., M.J.d.C.C., R.C.P.L., C.S.O.d.S., D.C.P. and M.G.C.A.M.; formal analysis—C.P.N.d.S.F., R.C.P.L., J.A.d.S. and A.L.S.; funding acquisition—C.P.N.d.S.F., S.G.d.S., D.C.P. and M.G.C.A.M.; methodology—C.P.N.d.S.F., C.S.O.d.S., D.C.P., J.F.T., M.G.C.A.M. and M.J.d.C.C.; project administration—C.P.N.d.S.F., C.S.O.d.S., D.C.P., J.F.T. and M.G.C.A.M.; resources—C.P.N.d.S.F., D.C.P., C.S.O.d.S., M.J.d.C.C. and A.L.S.; software—A.L.S. and J.F.T.; supervision—C.P.N.d.S.F. and M.J.d.C.C.; validation—C.P.N.d.S.F., M.G.C.A.M., J.F.T., A.L.S., R.C.P.L., C.S.O.d.S. and D.C.P.; visualization—C.P.N.d.S.F., D.C.P. and M.J.d.C.C.; writing—original draft—C.P.N.d.S.F., D.C.P. and M.J.d.C.C.; writing—review and editing—C.P.N.d.S.F., M.G.C.A.M., J.F.T., A.L.S., R.C.P.L., C.S.O.d.S., J.A.d.S., S.G.d.S., M.J.d.C.C. and D.C.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Ethics Committee of the Health Sciences Center, Federal University of Paraíba (protocol code CAAE 64573917.4.0000.5188 and date of approval in April, 2017).

Informed Consent Statement: All participants involved in the study signed an informed consent form.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors thank the women who participated in the study and the Laboratory of Characterization and Analysis of the Federal University of Paraíba (UFPB).

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

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nutrients

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Review

Diet and Nutrition in Gynecological Disorders: A Focus on Clinical Studies

Sadia Afrin [1,†], Abdelrahman AlAshqar [1,2,†], Malak El Sabeh [1], Mariko Miyashita-Ishiwata [1], Lauren Reschke [1], Joshua T. Brennan [1], Amanda Fader [1] and Mostafa A. Borahay [1,*]

[1] Department of Gynecology and Obstetrics, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA; safrin1@jhmi.edu (S.A.); abdashqer@gmail.com (A.A.); melsabe1@jhmi.edu (M.E.S.); mishiwa1@jhmi.edu (M.M.-I.); lreschk1@jhmi.edu (L.R.); jbrenn20@jhmi.edu (J.T.B.); afader1@jhmi.edu (A.F.)

[2] Department of Obstetrics and Gynecology, Kuwait University, Kuwait City 13110, Kuwait

* Correspondence: mboraha1@jhmi.edu; Tel.: +1-(410)-550-0337

† These authors contributed equally to this work.

Abstract: A healthy lifestyle and a balanced diet play a paramount role in promoting and maintaining homeostatic functions and preventing an array of chronic and debilitating diseases. Based upon observational and epidemiological investigations, it is clear that nutritional factors and dietary habits play a significant role in gynecological disease development, including uterine leiomyoma, endometriosis, polycystic ovary syndrome, and gynecological malignancies. Diets rich in fruits and vegetables, Mediterranean diets, green tea, vitamin D, and plant-derived natural compounds may have a long-term positive impact on gynecological diseases, while fats, red meat, alcohol, and coffee may contribute to their development. Data regarding the association between dietary habits and gynecological disorders are, at times, conflicting, with potential confounding factors, including food pollutants, reduced physical activity, ethnic background, and environmental factors limiting overall conclusions. This review provides a synopsis of the current clinical data and biological basis of the association between available dietary and nutritional data, along with their impact on the biology and pathophysiology of different gynecological disorders, as well as an outlook on future directions that will guide further investigational research.

Keywords: diet; nutrition; dietary habits; uterine leiomyoma; endometriosis; polycystic ovary syndrome; gynecological malignancies

Citation: Afrin, S.; AlAshqar, A.; El Sabeh, M.; Miyashita-Ishiwata, M.; Reschke, L.; Brennan, J.T.; Fader, A.; Borahay, M.A. Diet and Nutrition in Gynecological Disorders: A Focus on Clinical Studies. *Nutrients* **2021**, *13*, 1747. https://doi.org/10.3390/nu13061747

Academic Editors: Sílvia Rocha-Rodrigues, José Afonso and Monica Sousa

Received: 12 April 2021
Accepted: 19 May 2021
Published: 21 May 2021

1. Introduction

Worldwide, women are afflicted by a spectrum of gynecological disorders, ranging from benign entities, such as uterine leiomyoma, endometriosis, and polycystic ovary syndrome, to various gynecological malignancies. These disorders represent a significant source of morbidity by causing bothersome heavy menstruation, debilitating pelvic pain, chronic anovulation, hyperandrogenism, infertility, and even death. Although these conditions are discrepant in nature, they share a common feature: they lack a curative medical treatment that would allow the preservation of functioning reproductive organs. Most medications are only temporarily effective, have undesirable side effects, interfere with pregnancy, and carry a risk of disease recurrence upon discontinuation [1–3]. This stresses the importance of fully understanding the pathophysiology of these disorders and the potential risk factors associated with their development and maintenance to mitigate and modify their consequences on health whenever possible.

Diet and health have been among the most complex topics in public discourse and scientific circles. There have long been debates regarding the role that nutritional components and dietary habits play in modulating the risk of gynecological diseases, such as uterine leiomyoma [4–6], endometriosis [7,8], polycystic ovary syndrome (PCOS) [9,10], and different gynecological malignancies [11–14], with the majority of evidence retrieved from

epidemiological studies. Evidence suggests that a diet rich in fruit and vegetables, green tea, vitamins, and plant-derived compounds may help prevent gynecological disorders, compared to a diet deficient in vegetables and fruit and high in animal or dietary fats, red meat, and alcohol [6,7,12,14]. Understanding the role of diet in gynecology will change our perspective of how common gynecological diseases develop, progress, and lead to substantial adverse effects on women and will guide us toward pioneering novel diagnostic and therapeutic frameworks to reduce their burden. Most importantly, implementing preventive strategies aimed at changing certain dietary habits may ameliorate the occurrence of a wide array of gynecological diseases. Herein, we reviewed the latest research regarding the role of diet and nutrition in gynecological disorders, with an emphasis on clinical and epidemiological studies.

2. Search Criteria

This article provides a comprehensive review of the available literature discussing the role of diet and nutrition in the biological development of various gynecological disorders, emphasizing clinical and epidemiological data. A literature search was conducted using electronic databases, including PubMed of the National Library of Medicine, Google Scholar, Web of Science, and relevant clinical trials addressing diet and nutrition and gynecological disorders. The keywords "diet", "nutrition", "fruit", "vegetables", "vitamin", "fat", "meat", "fish", "alcohol", "coffee", "tea", "grain", "fiber", "dairy" and "natural compound" combined with "uterine leiomyoma", "uterine fibroid", "endometriosis", "polycystic ovary syndrome", and "gynecological malignancy" were used. All relevant reports were retrieved, and the corresponding reference lists were systematically searched to identify any additional studies that could be included. Only papers published as full-length articles in English were considered.

3. An Overview of Dietary Constituents

3.1. Sources of Dietary Elements

Dietary patterns differ in the type of nutritional constituent they contain. Whole grains, such as brown rice, barley, and wheat, are rich in minerals, vitamins, fibers, and phytochemicals, including vitamin E, carotenoids, inulin, and lignans, which play notable roles in modulating immune responses and oxidative stress [15]. Nuts, on the other hand, are rich in healthy (unsaturated) fats, which have been shown to confer a lower risk of obesity, diabetes, and cardiovascular disease. Fruit and vegetables are a known source of vitamins, minerals, and dietary fibers, many of which are notable antioxidants, such as β-carotene, vitamin C, and vitamin E; meat is high in protein and saturated (unhealthy) fatty acids and is a source of N-nitroso compounds, heterocyclic amines, and polycyclic aromatic hydrocarbons, which are known mutagens. Fish, by contrast, provides dietary protein, minerals, and omega-3 fatty acids, which are implicated in reducing the risk of cardiovascular disease [15]. Green tea has been extensively studied for its beneficial effects, and its component epigallocatechin-3-gallate (EGCG), a catechin, has received a great deal of attention for its antioxidant and anti-tumorigenic properties [16]. Later in this review, we will elaborate in depth how different food components and dietary constituents influence the occurrence of common disorders in gynecology.

3.2. Role in Health and Disease

Our daily diets consist of a myriad of nutritional constituents that have indispensable roles in maintaining physiological processes by participating in intricate homeostatic cascades. It has been well established that deficiencies in certain nutrients and excess of others can have detrimental consequences by means of disrupting physiology and promoting disease. Among all, micronutrients, consisting of minerals, vitamins, and other trace elements, seem to possess particularly vital roles on cellular grounds. For example, zinc and selenium participate as cofactors in modulating enzymatic activity in hundreds of pathways, whereas zinc has an additional role in gene transcription, depicting their

profound effects in regulating cellular functions [17]. Vitamins play an essential role in maintaining health and preventing disease. Tocopherols (vitamin E) and carotenoids (vitamin A) are remarkable in their potential to scavenge reactive oxygen species (ROS), which alongside other zinc- and selenium-containing enzymes, such as glutathione peroxidase and superoxide dismutase, are potent antioxidants. Furthermore, riboflavin and niacin participate in the electron transport chain and cellular energy production, whereas folic acid is involved in DNA synthesis and its deficiency has long been implicated in fetal neural tubal defects in pregnancy [18]. The actions of micronutrients extend to affect the immune system. With regard to the innate immune barrier, iron and zinc are responsible for sustaining epithelial integrity and repair, while calcitriol (vitamin D) promotes a healthier composition of the intestinal microbiota in the gut barrier. On the other hand, vitamins C, E, and B6 enhance T cell differentiation and proliferation and antibody production, emphasizing the prominent role of diet in combating infections [19].

Moving to macronutrients, dietary fats have been shown to have both beneficial and adverse effects on human physiology. On the one hand, trans-fatty acids accentuate inflammation and oxidative stress and influence autophagy and apoptosis. For example, it has been shown that trans-fatty acids potentiate the levels of inflammatory mediators, such as C-reactive protein (CRP) and interleukin (IL) 6, predisposing to atherosclerosis [20]. By contrast, omega-3 fatty acids dampen leukocyte chemotaxis and production of various proinflammatory cytokines, suggesting their use as possible therapeutic agents given their anti-inflammatory properties [21]. It has been found that high glucose levels can initiate several pathophysiologic cascades culminating in drastic outcomes, as already seen in patients with diabetes mellitus. While hyperglycemia mediates vasculopathy, neuropathy, and immunosuppression, it is also implicated in tumorigenesis as will be further discussed in this review. In brief, advanced glycation end products can induce DNA damage, which alongside hyperglycemia-induced ROS, epigenetic modulation, and oncoprotein upregulation, can initiate neoplastic transformation [22]. Similarly, obesity, by means of subclinical inflammation, creates an aberrant adipokine profile that promotes tumorigenesis of benign and malignant nature in women [23].

3.3. Role of Diet in Oxidative Stress and Inflammation

Knowing the deleterious effects of oxidative stress and inflammation in the development of various diseases, dietary constituents may have a prominent role in modulating their risk given the antioxidant activity of many. On the other hand, increased intake of certain macronutrients such as carbohydrates can in fact promote oxidative damage. For example, increased caloric intake from high-carbohydrate and high-fat diets dramatically activates the electron transport chain, potentiating superoxide production. Additionally, meat is rich in protein, which if fermented in excess in the gut can produce metabolites, such as ammonia and hydrogen sulfide, that are toxic to the mucosa [15]. When addressing these effects in the context of women's health, studies have reiterated the effects of diets on various obstetric and gynecological conditions. For instance, Rayman et al. have found that British women with low selenium levels have a higher risk of preeclampsia and pregnancy-induced hypertension. Selenium has been shown to influence eicosanoid synthesis and actions involved in inflammation, thrombosis, immune response, and blood pressure regulation [24]. In settings of oxidative stress, selenium-deficient endothelium loses some of its ability to produce prostacyclin, a vasodilatory eicosanoid, possibly increasing the risk of vascular dysfunction in pregnancy [25]. Similarly, a study has shown that lower copper levels in early pregnancy may increase the risk of pregnancy-induced hypertension, possibly by allowing oxidative stress to progress uninhibited [26]. The role of diet in oxidative stress will be further addressed below in relation to common gynecological diseases.

4. Diet and Nutrition in Uterine Leiomyoma

4.1. Uterine Leiomyoma

Uterine leiomyomas, also known as uterine fibroids or myomas, are the most common benign tumors of the female reproductive tract, affecting up to 70–80% of women of reproductive age [27], with symptoms ranging from heavy and prolonged menstruation and pelvic pain to subfertility. Although quite prevalent, the precise cellular and molecular culprits of uterine leiomyoma are still unclear [28]. With only few, mostly hormonal, medications that provide short-term relief, surgery (myomectomy or hysterectomy) is the definitive treatment method to date [29]. In fact, uterine leiomyoma is the leading indication for hysterectomy in the United States, with the annual overall leiomyoma-associated costs estimated at $5.9–$34.4 billion [1]. Research over the decades has suggested a number of risk factors for leiomyoma, including age, race, heredity, genetics, epigenetics, sex hormones, endocrine disruptions, obesity, lifestyle factors, and diet [4,5]. Epidemiological evidence suggests that specific dietary components and nutritional factors may be associated with various hormone-related diseases, including uterine leiomyomas (Figure 1) [4,5].

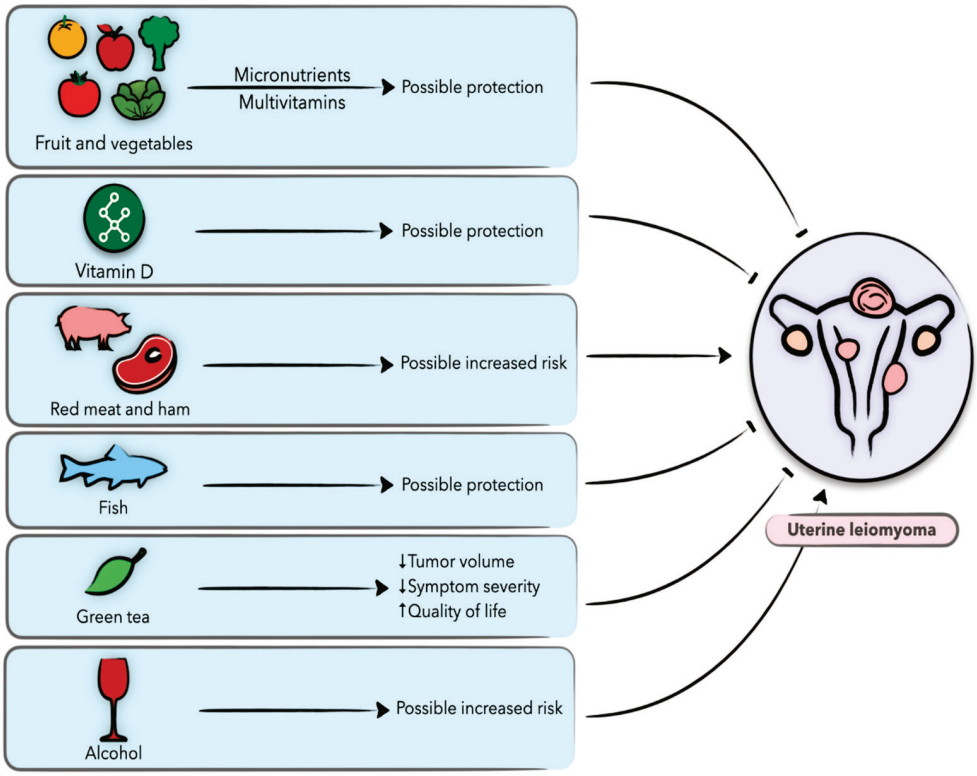

Figure 1. Schematic presentation of the role of diet and nutrition in uterine leiomyoma and the possible underlying biological mechanisms.

4.2. Vegetables and Fruit

Several studies have shown a protective effect of vegetable and fruit intake against uterine leiomyomas, most notably with citrus fruit, apples, cabbage, broccoli, and tomatoes [30–32]. In the US Black Women's Health Cohort Study, the frequency of food items has been determined on the premise of how many servings are consumed per day or month among women before menopause [30]. Four servings of fruit and vegetables each day reduced the risk of uterine leiomyoma in women, compared to just one serving per day. The association was more pronounced among those who consumed several servings of fruit each week, compared to one serving per month [30]. Similarly, a case–control study conducted in premenopausal Chinese women found a protective effect of fruit and vegetable intake against uterine leiomyoma development [33]. It has been suggested that

plant-based diets may decrease bioavailable estrogen levels and increase estrogen excretion, thus decreasing the risk of leiomyoma [34]. Another study examined the dietary habits of over 1000 Chinese women using a questionnaire; women with uterine leiomyoma consumed less vegetables compared to healthy women [31]. An Italian case–control study investigated the relationship between green leafy vegetables and fruit and the prevalence of uterine leiomyoma [32]. With weekly frequencies, the risk of surgically confirmed fibroids was inversely associated with intake of green vegetables and fruit, with the exception of carrots, which did not appear to alter this risk [32]. Phytonutrients present in vegetables and fruit may protect against leiomyoma by inhibiting proliferation, inflammation, and fibrosis, inducing apoptosis, and inactivating hormonal or growth factor-related pathways in laboratory studies [35], thus providing a plausible biological basis that may prompt future experiments. A negligible number of dietary nutrients have been examined to date, and more research is needed to clarify the associations in full.

4.3. Vitamins

Studies investigating the relationship between vitamins and leiomyoma risk are based on data collected in the Black Women's Health Study [30]. This study concluded that vitamin A appears to have an inverse association with uterine leiomyoma risk. The results may be attributed to intake of preformed vitamin A derived from animal sources, not from provitamin A derived from fruit and vegetables [30]. However, no correlation was found between leiomyoma and intake of carotenoids, including lycopene [30]. Similar results were obtained in the Nurses' Health Study II after 10 years of follow-up in a cohort of premenopausal women [36]. The same assessment also showed that intake of β-carotene slightly increased leiomyoma risk, but these effects were restricted to current smokers [36]. In the National Health and Nutrition Examination Survey (NHANES), after adjustments for age, race, education, BMI, and oral contraceptive use, a statistically significant dose–response relationship was identified between serum vitamin A concentrations and uterine leiomyoma, but not with β-carotene [37]. Modern studies reveal no significant association between intake of vitamins C and E and the incidence of leiomyoma [30,37].

In comparison with other vitamins, the value of vitamin D in leiomyoma prevention is significantly stronger. Several studies have examined serum vitamin D concentration in relation to uterine leiomyoma. An analysis of the National Health and Nutrition Examination Survey (NHANES) pointed out that the incidence of leiomyoma is inversely associated with serum vitamin D levels, although specifically among white women [38]. Conversely, a different study concluded that American women aged 35 to 49 with normal levels of vitamin D had an estimated 32% lower risk of leiomyoma occurrence than those who were deficient [39]. However, since the association between serum vitamin D and uterine leiomyoma in both of these studies may be confounded by non-sedentary lifestyles and hence increased sunlight exposure, even after adjusting for BMI, further studies should strongly consider adjusting for such potential confounders to fully elucidate the role of dietary vitamin D intake in leiomyoma risk. Additionally, a statistically significant negative correlation was found between serum vitamin D concentration and leiomyoma in African American women, but not in Caucasian women. This observed association is not limited to leiomyoma occurrence but also related to tumor volume [40]. Similarly, in Indian and Italian studies of women with leiomyoma, vitamin D3 levels were significantly lower in women with leiomyomas compared to those without [41,42]. Notably, a clinical trial in Italy demonstrated that vitamin D supplementation in vitamin D-deficient women with leiomyoma reduced the need for surgery and medical treatments [43]. A number of studies conducted on Turkish, Chinese, and Indian women provided similar results, confirming that vitamin D levels are highly correlated with uterine leiomyoma risk [44–46]. An additional study suggested that low 25(OH)D levels may be a risk factor for uterine leiomyoma in individuals with obesity, a positive leiomyoma family history, and a higher level of transforming growth factor 3 [47]. Among studies assessing vitamin D status with uterine leiomyomas, only one study found that vitamin D deficiency and low 25(OH)D

serum concentrations did not correlate with uterine leiomyomas in the studied population [38]. Vitamin D is extensively documented throughout the literature for its effects on uterine leiomyoma development and may serve as a potential pharmacological agent for the prevention and treatment of these tumors.

4.4. Dietary Fat, Meat, and Fish

There was no correlation between total dietary fat and trans fats and leiomyoma risk in a prospective cohort study of premenopausal African American women after a five-year follow-up [48]. On the other hand, marine omega-3 polyunsaturated fatty acids, namely docosahexaenoic acid, were positively associated with a 49% higher uterine leiomyoma incidence [48]. A Japanese cross-sectional study of premenopausal women found no significant association between dietary fat and uterine leiomyoma [49], which was also shown by Italian and Chinese case–control studies that examined the effect of butter, margarine, eggs, and oil [33]. While the association of dietary fat with uterine leiomyoma remains inconclusive, it is imperative to broadly investigate the role of various fats and fat-containing foods in eliciting a biologically plausible association. Interestingly, there is evidence suggesting that the anti-hyperlipidemic drug class statins may protect against uterine leiomyomas [28,50–52], proposing a possible association between leiomyomas and lipid intake and metabolism.

Data regarding the effect of meat and fish consumption on leiomyoma risk are conflicting. Results from an Italian case–control study revealed that significant consumption of meats, such as beef or ham, was associated with an increased risk of leiomyoma [32], but this association was rendered nonsignificant in Chinese populations [33]. Although an inverse association was similarly observed in an Italian case–control study addressing fish consumption and leiomyoma risk [32], other studies found no association between leiomyoma risk and total fish and seafood consumption [33].

4.5. Alcohol and Coffee

It is well established that alcohol and coffee intake increases the risk of certain diseases, but the available data regarding uterine leiomyoma risk remain controversial. Several lines of research have shown a link between alcohol consumption and higher leiomyoma risk [49,53]. The Black Women's Health Study has concluded that leiomyoma is associated with heavier drinking, which may be attributed to phytoestrogens found in beer, as compared to wine or liquor [54]. In the California Teachers Study (CTS), subjects who consumed at least 20 g of alcohol daily had a significantly increased risk of leiomyoma [55] while a case–control study in China did not find such an association [33]. There have been no definitive data regarding whether caffeine consumption increases the risk of uterine leiomyoma in women, except among younger women who consume a high amount of coffee or caffeine (500 mg/day) [54].

4.6. Epigallocatechin-3-Gallate (EGCG)

EGCG is a flavonoid found in green tea. A randomized, double-blinded study examined the efficacy of green tea extract in treating women with uterine leiomyoma and improving their quality of life compared to placebo. The findings demonstrated a significant decrease in leiomyoma volume and symptom severity and improvement in health-related quality of life scores [56]. A trial is currently recruiting patients to assess the pharmacokinetics and hepatic safety of EGCG in women with and without uterine fibroids (NCT04177693). A total of 36 women (age groups 18–29 and 30–40) are being given 800 mg EGCG daily alone, EGCG daily with clomiphene citrate, and EGCG daily with letrozole for up to 2 months. Changes in epigallocatechin gallate, epigallocatechin, epicatechin gallate, and 4'-O-methyl-epigallocatechin are listed as primary outcomes whereas changes in direct and total bilirubin, alanine aminotransferase/aspartate aminotransferase, alkaline phosphatase, beta human chorionic gonadotropin (β-hCG), follicle-stimulating hormone (FSH),

luteinizing hormone (LH), estrogen, progesterone, anti-mullerian hormone (AMH), and endometrial thickness are listed as secondary outcomes.

4.7. Miscellaneous

A number of studies looking at whole grain food intake in Chinese and Italian populations indicated no significant relationship between whole grain intake and leiomyoma prevalence [32,33]. Similarly, there was no significant link between high-fiber diets and leiomyoma development in the Black Women's Health Cohort study and a Japanese study [30,49].

According to the Italian study, there was no association between milk and cheese intake and uterine leiomyoma risk [32], even though an association was found in a Chinese prospective cohort study [57]. Among African American women, a study found a protective effect of frequent consumption of milk and milk products against leiomyoma occurrence and growth, with no association with butter, cheese, and ice cream, and a minor effect for yogurt consumption [58], warranting the need for further research.

5. Diet and Nutrition in Endometriosis

5.1. Endometriosis

Endometriosis is a chronic and recurrent disease affecting 6–10% of reproductive-age women and it is a leading cause of pelvic pain and infertility. The formation and growth of endometriotic lesions are dependent on various biological processes that result in the persistence of endometrial-like glandular epithelium and stroma outside the uterine cavity. Endometriosis is an estrogen-dependent disease, and conservative therapeutic strategies are limited to hormonal treatment (combined oral contraceptives, progestins, or gonadotropin-releasing hormone agonists) [59]. Although surgical removal of the endometriotic lesions is an alternate treatment modality, the recurrence rate is up to 50% within five years of surgery. An increasing number of studies have explored diet as a therapeutic strategy for endometriosis, especially in the light of current evidence suggesting an association between benign gynecological conditions, including endometriosis, and cardiometabolic risk and systemic inflammation [23,60]. In the following section, we review the current evidence regarding the association of nutrition and diet with endometriosis, focusing on clinical studies (Figure 2).

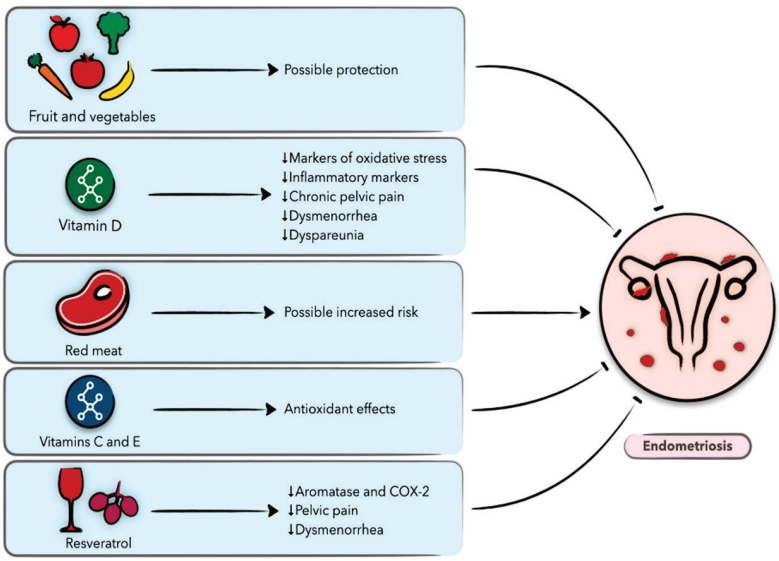

Figure 2. Schematic presentation of the role of diet and nutrition in endometriosis and the possible underlying biological mechanisms.

5.2. Vegetables and Fruit

The risk of endometriosis was found to be inversely related to vegetable and fruit consumption in an Italian population [61]. A significant risk reduction can be seen in women in the highest tertile of intake when compared to women in the lowest tertile, with findings persisting after adjusting for confounding factors [61]. Based on the number of food servings consumed throughout the day, Trabert et al. [62] assessed the role of diets rich in vegetables and fruit in endometriosis, similar to an Italian case–control study. On the contrary, fruit served twice a day was associated with increased risk of endometriosis, but no association was found with vegetables [62].

5.3. Vitamins

In a randomized, placebo-controlled clinical trial, 34 women with endometriosis received vitamins C and E or placebo for 6 months. Vitamin C and E supplementation was associated with a decrease in oxidative stress markers in women with endometriosis; however, no improvement in pregnancy rates was noted during or after the intervention [63]. Another study by the same group observed that women with endometriosis had lower intake of vitamins A, C, and E, zinc, and copper, and diminished peripheral oxidative stress markers, compared to women of higher intake [64]. Santanam et al. conducted a clinical trial involving 59 women with pelvic pain and endometriosis or infertility [65]. Patients who received vitamins C and E reported less pain, dysmenorrhea, and dyspareunia, compared to the placebo group. There was a significant decrease in peritoneal fluid inflammatory markers, including the chemokine CCL5/regulated on activation, normal T cell expressed and secreted (RANTES), IL-6, and monocyte chemotactic protein-1 (MCP-1), compared to patients not taking vitamins [65]. These effects may be mediated through the antioxidant properties of vitamins, potentially ameliorating the clinical manifestations of endometriosis.

Moreover, women with endometriosis were noted to have lower serum vitamin D levels compared to women with mild or no endometriosis [66]. There are several clinical trials examining the effect of vitamin D on pelvic pain in endometriosis patients. In randomized, double-blind, placebo-controlled trials, vitamin D supplementation significantly decreased pelvic pain in women with endometriosis [67,68], while no effects were observed by Almassinokiani et al. [69], wherein vitamin D treatment did not significantly reduce dysmenorrhea and/or pelvic pain. Additionally, vitamin D treatment also decreased the total/HDL cholesterol ratio, high-sensitivity CRP, and total antioxidant capacity levels but did not affect other clinical symptoms or metabolic profiles [67]. In another case–control study, women with stage III/IV endometriosis received vitamin D treatment weekly for 12 to 14 weeks, and the expression of β-catenin active form (an important molecule in the Wnt/β-catenin signaling pathway) was significantly reduced after treatment [70].

5.4. Dietary Fat, Meat, and Fish

In a large US cohort study, 12 years of prospectively collected data were used to assess dietary fat consumption and endometriosis risk [71]. The results showed that while total dietary fat consumption did not affect endometriosis risk, increased long-chain n-3 fatty acid consumption decreased the risk whereas trans-fat intake increased it [71]. Butter intake was marginally associated among Belgian women with an increased risk of peritoneal endometriosis [72]; however, no association was observed among Italian women [61].

A large Italian study showed that women reporting higher meat consumption had a greater endometriosis risk [61]. In contrast, a study conducted in Belgium showed no significant correlation between endometriosis and meat consumption [72]. Diets rich in red meat seem to correlate modestly with estradiol and estrone sulphate levels, contributing to human circulating steroid hormone concentrations, and thus disease maintenance [73]. However, the Italian and Belgian studies did not reveal any statistically significant association between fish consumption and endometriosis [61,72]. In a meta-analysis by Parazzini et al., endometriosis risk was notably higher among women in the highest tertile of beef

and other red meat consumption (OR = 2 [1.4–2.8]) compared to women in the lowest tertile of intake [61].

5.5. Whole Grain and Fiber

According to the study by Trabert et al., consumption of whole grains was not associated with endometriosis [62], similar to reports by an Italian case–control study [61]. Alternatively, a study by Savaris and Amaral found a higher fiber intake among women with endometriosis compared to controls, though the sample size was too small to draw any solid conclusions [74]. Despite a higher intake in women with the disease, fiber consumption was still inadequate in this group, which made the authors propose that suboptimal fiber intake might have led to more pronounced inflammation and oxidative stress. Nevertheless, a more biologically plausible mechanism that connects dietary fiber and endometriosis risk needs to be investigated.

5.6. Plant-Derived Compounds

5.6.1. Resveratrol

Resveratrol is a phytoalexin polyphenol produced naturally by several plants invaded by bacterial and fungal pathogens. High resveratrol levels are found in grapes, wine, berries, and nuts [75,76]. There are conflicting data concerning resveratrol's therapeutic effect on endometriosis. A clinical trial included 42 women with endometriosis, of which 16 received oral contraceptives alone and 26 received a combination of oral contraceptives and 30 mg resveratrol. Interestingly, aromatase and COX-2 expressions were reduced in the eutopic endometrium of patients treated with combined oral contraceptives and resveratrol therapy compared to the control group [77]. On the contrary, in another study, 40 mg/day resveratrol with monophasic contraceptives did not improve endometriosis-related symptoms when compared to placebo groups [78].

5.6.2. Epigallocatechin-3-Gallate (EGCG)

In vitro, several groups demonstrated the effect of EGCG on endometriosis [79,80]. The Chinese University of Hong Kong is currently recruiting patients for a randomized, clinical trial on prospective endometrioma patients to study the effects of high-purity EGCG (SUNPHENON EGCgVR; 400 mg, twice per day) versus placebo for 3 months prior to planned surgery. Change in endometriotic lesion size is listed as a primary outcome, whereas pain scores, quality of life, change in lesion growth (by biopsy), change in neovascularization, and monitoring of adverse effects are listed as secondary outcomes (NCT02832271).

5.6.3. Curcumin

Curcumin is a polyphenolic compound found in turmeric, which is derived from the rhizomes of the plant Curcuma longa Linn [81]. The anti-inflammatory and anti-proliferative effects of curcumin on endometriosis were reported previously in in vitro and animal studies [82–85]. The Vienna-based ENDOFLEX study is currently recruiting patients with endometriosis for an interventional clinical trial randomized for placebo versus the dietary supplement flexofytol, with planned administration of 42 mg of curcumin twice a day for a duration of 4 months. The study defines a possible change in the average pain score, from baseline to 4 months after the onset of treatment, as a primary outcome, and changes in the number of days with pain, alleviation of dyspareunia, dysuria, and dyschezia, as well as changes in quality of life and sexual function as secondary outcomes (NCT04150406).

6. Diet and Nutrition in Polycystic Ovary Syndrome

6.1. Polycystic Ovary Syndrome (PCOS)

Polycystic ovary syndrome (PCOS) is a very common endocrine disorder in reproductive-aged women, globally affecting 4–21% of this population depending upon the diagnostic

criteria applied [86]. This syndrome is characterized by chronic anovulation, hyperandrogenism, and polycystic ovarian morphology, and is associated with increased risks of reproductive disorders, cardiovascular disease, metabolic sequelae, and psychological morbidity [87]. The etiology and pathogenesis of this disease are widely unknown, but a combination of factors including genetic, epigenetic, and environmental influences are thought to play a significant role [88]. Women with PCOS are at increased risk of insulin resistance and associated conditions, including metabolic syndrome, nonalcoholic fatty liver disease, dyslipidemia, hypertension, and obesity-related disorders. Although not required for the diagnosis, the prevalence of insulin resistance has been described in up to 80% of women with PCOS [89], and further research has shown this to occur independently but in an additive manner to obesity [10,89]. While there is no cure for PCOS, first-line management includes conservative treatment through lifestyle interventions that emphasize weight loss and dietary modifications, with efforts to improve insulin sensitivity and prevent long-term health sequelae [9]. In the following section, we provide evidence on the effects of diet and nutrition on PCOS, focusing on clinical studies (Figure 3).

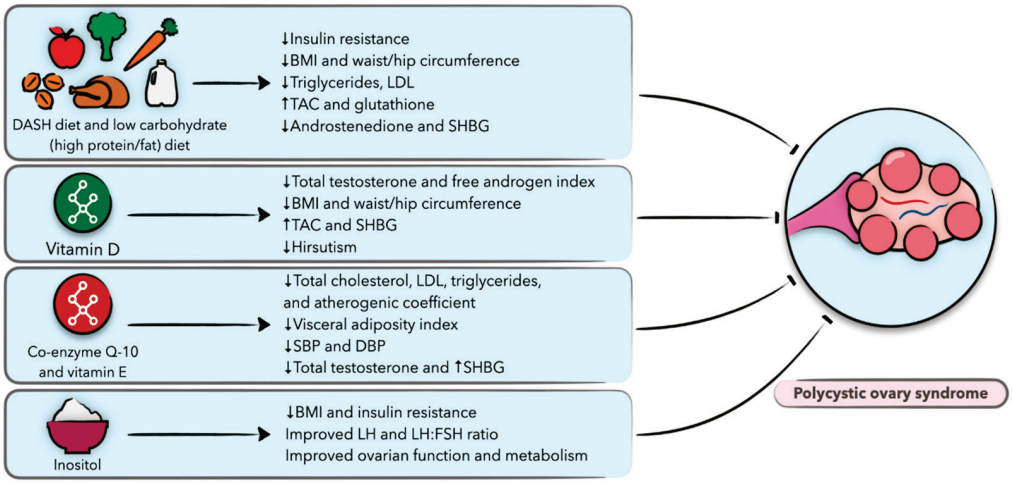

Figure 3. Schematic presentation of the role of diet and nutrition in polycystic ovary syndrome (PCOS) and the possible underlying biological mechanisms.

6.2. Weight Loss Diets

While no treatment is available for PCOS, research indicates that certain dietary and lifestyle changes can improve the overall metabolic health of women affected by this condition. Modest weight loss, which decreases circulating insulin and androgen concentrations, has been shown to improve the symptoms of PCOS, with the resumption of menstrual cyclicity, spontaneous ovulation and pregnancies, and improvement in overall quality of life [90–94]. However, there is no consensus on which diet is superior for patients with PCOS. Several clinical trials have been conducted to establish which diet is most suitable for PCOS patients, but given the relatively small sample sizes, heterogeneity in dietary interventions, and treatment duration, it is difficult to establish which diet is superior. For the purpose of this review, we discuss randomized controlled trials (RCT) that exclude the use of supplementary medications.

The pulse-based diet, a diet rich in beans, lentils, and chickpeas, was shown to be superior to the therapeutic lifestyle change (TLC) diet, a nutritionally balanced diet in a clinical trial of 95 women enrolled in a 16-week intervention [95]. The pulse-based diet resulted in greater insulin sensitivity, diastolic blood pressure, triglycerides, low-density lipoprotein (LDL) cholesterol, and total cholesterol [95]. Furthermore, in a trial that included 48 patients with PCOS, the dietary approaches to stop hypertension (DASH) diet, a diet rich in fruit, vegetables, and whole grains, and low in fats, cholesterol and sodium, resulted in a significant reduction in insulin resistance markers, waist and hip

circumference, total body weight, body mass index (BMI), serum triglycerides, and LDL levels [96]. The benefits of diet were additionally seen in a similar study that included 60 women where the diet reduced serum androstenedione levels, and increased sex hormone-binding globulin (SHBG), thus decreasing total androgens [97].

The effect of calorie distribution per meal has been studied, with the total daily caloric intake standardized to 1800 kcal, but with differences in caloric meal timing distribution: a breakfast diet and a dinner diet, with most of the daily calories consumed during the respective times [98]. The group that consumed most of the calories at breakfast had a significant reduction in glucose and insulin levels, a favorable decrease in testosterone, an increase in SHBG, and improved ovulatory function [98]. These effects were not achieved in the dinner group, suggesting that meal timing and caloric distribution may play a role in women with PCOS [98]. In efforts to analyze the effect of meal frequency on PCOS, a crossover RCT of 40 women with PCOS evaluated the consumption of six meals compared to three meals per day. Findings revealed that subjects with six meals per day resulted in an improved post-intervention oral glucose tolerance test with significant reductions in fasting insulin levels, compared to three meals per day; however, there were no significant differences in hemoglobin A1C and blood lipids [99].

Recent interest in high-protein diets among women with PCOS has been evaluated; however, there is little evidence suggesting this is superior to a standard diet. Moreover, potential concerns regarding aggravation of insulin resistance and impairment of glucose metabolism limit the use of these diets [100–105]. In efforts to compare the low-protein, high-carbohydrate diet (LPHC) to a high-protein, low-carbohydrate diet (HPLC), women with PCOS were randomized to either diet. While women in the HPLC diet had a significant reduction in depression and improvement in self-esteem, there was no difference in weight between the groups after adopting the diet for 4 months [106].

In regard to carbohydrate intake, two studies have shown that modest reductions in carbohydrate intake decrease serum insulin, total testosterone, and total cholesterol [107]. The implementation of an isocaloric low-glycemic index diet has also shown promising results, with improvements in insulin sensitivity in women with PCOS. Further epidemiological studies have additionally shown that a low-glycemic index diet reduces the risk of cardiovascular disease, type 2 diabetes, and metabolic syndrome in women; thus the type of carbohydrate intake may have a more significant role in promoting metabolic health than the total amount of carbohydrate intake [108–110].

Diets substituting carbohydrates with poly- and monounsaturated fats in obese women with PCOS have been shown to reduce hyperinsulinemia, but no benefit was observed in total cholesterol, triglyceride, and HDL levels [111]. The effects of polyunsaturated fatty acids (PUFA) have further been investigated in women with PCOS. While a PUFA-rich diet resulted in decreased fasting plasma free fatty acids, impaired insulin sensitivity was observed [112]. Additionally, supplementation with PUFA reduced plasma bioavailable testosterone levels; however, there was no significant change in total testosterone, androstenedione, dehydroepiandrosterone sulfate (DHEAS), luteinizing hormone (LH), estrogen, follicle-stimulating hormone (FSH), or SHBG concentrations [113].

Two small studies targeting adolescents with PCOS looked at dietary modifications. No difference between a low-glycemic load and a low-fat diet was observed [114], but both diets resulted in weight loss and improved menstrual regularity. Lastly, a low-glycemic vegan diet was assessed in one small RCT, in which obese women were randomized to either a low-glycemic vegan diet or a low-calorie diet. Though limited by sample size and high attrition rate, obese women randomized to the low-glycemic vegan diet were found to lose significantly more weight after 3 months, but no difference was observed following 6 months of intervention [115].

6.3. Vitamin D

A few small studies have looked at the beneficial effects of vitamin D supplementation on PCOS, with conflicting results. Ardabili et al. conducted a clinical trial that included

50 women randomized to receive either three oral treatments of 50,000 IU of vitamin D or three placebo pills over 2 months [116]. There was no difference in fasting serum insulin, glucose levels, or insulin sensitivity between the two groups [116]. Another group randomized women to either receive 4000 IU of vitamin D, 1000 IU of vitamin D, or placebo for 12 weeks [117]. High-dose vitamin D supplementation resulted in significant reductions in total testosterone, free androgen index, hirsutism, and C-reactive protein levels, as well as a significant increase in SHBG and total antioxidant capacity when compared to the low-dose supplementation and placebo groups [117]. The benefits of vitamin D and calcium supplementation were further investigated in two small studies among overweight and obese women with vitamin D deficiency and PCOS, with favorable effects on insulin levels, serum triglycerides, total testosterone, and blood pressure in those with elevated baseline blood pressure [118]. Finally, the effect of weight loss combined with vitamin D supplementation vs. placebo in women with PCOS was studied, with improved menstrual regularity in those subjected to supplementary vitamin D, but no significant difference in weight loss, BMI, or waist and hip circumference was observed [119]. Given the recent evidence that vitamin D plays a role in various metabolic pathways including insulin metabolism and resistance, further research regarding its role in PCOS is warranted [120].

6.4. Coenzyme Q10 and Vitamin E

The benefit of coenzyme Q10 (CoQ10), a natural antioxidant, with and without vitamin E has been explored, with supplementation resulting in a favorable cholesterol profile, blood pressure, and fasting blood sugar, reduced total testosterone levels, and an increased SHBG [121,122]. Additionally, in one small study, vitamin E with magnesium supplementation for 12 weeks was found to reduce hirsutism but not significantly alter testosterone or SHBG levels [123].

6.5. Inositol

Inositol-phosphoglycan (IPG) has stirred up interest as a potential treatment to improve cellular response to the metabolic secondary messenger pathways following insulin binding with its receptor. IPG has been associated with insulin action, and a deficiency of one of the stereoisomers, D-chiro-inositol, has been linked with insulin resistance [124]. As such, several groups have studied the potential benefit of inositol supplementation in women with PCOS. Forty-two obese patients with PCOS received 2 g of myo-inositol for 8 weeks [125]. Supplementation resulted in a reduction in body mass index and insulin resistance [125]. In patients that had an insulin level above 12 μU/mL, myo-inositol resulted in a significant decrease in fasting insulin levels and the area under the curve of insulin after oral glucose tolerance levels, indicating a greater benefit in obese patients with high fasting insulin levels [125]. The supplementation of a similar isomer, D-chiro-inositol, was studied in a group of overweight and obese women with PCOS patients. Supplementation resulted in an improvement in LH, LH/FSH ratio, insulin response to oral glucose tolerance test, and BMI, and these parameters were improved to a greater extent if the participant had a first-grade diabetic relative [126]. Another study compared the supplementation of myo-inositol to that of D-chiro-inositol for 6 months and found that both isoforms improved ovarian function and metabolism in PCOS patients. Myo-inositol was better at improving the participants' metabolic profile, while D-chiro-inositol reduced hyperandrogenism [127].

6.6. Alternative Therapies

Low selenium levels have been associated in women with PCOS [128]; thus a small RCT was done to test the effect of selenium supplementation in women with PCOS. Selenium supplementation resulted in a higher pregnancy rate, and a decrease in alopecia, hirsutism, and acne. There was no significant difference in testosterone, LH, FSH, nitric oxide, or glutathione (GSH) [129], and further studies are needed.

A study looking at the benefit of soy in women with PCOS showed that a soy diet resulted in a significant decrease in BMI, fasting plasma glucose, total testosterone, insulin resistance, triglycerides, and malondialdehyde. This diet also significantly increased nitric oxide and GSH [130], suggesting that a diet containing soy may offer therapeutic potential for patients with PCOS.

7. Diet and Nutrition in Gynecological Malignancies

7.1. Gynecological Malignancies

Gynecological malignancies, comprising uterine, ovarian, cervical, vaginal, and vulvar cancers, as well as breast cancer, represent the most common malignant entities in women. In the US alone, more than 90,000 new gynecological cancer cases and about 30,000 deaths were reported in 2017, in addition to 250,000 new cases of breast cancer and 42,000 deaths in the same year [131]. These statistics depict the substantial burden these malignancies impose on women and the economy, necessitating effective preventive measures. Despite their prevalence, the mechanistic aspects of their origins and progression remain largely obscure and are mainly reported as risk factors. A role for diet in modulating the risk of gynecological malignancies has long been described in the literature, with the majority of evidence derived from epidemiological studies. Various micro- and macronutrients, as well as disorders of metabolism and energy expenditure such as obesity, have documented potential roles in influencing these risks [11,12,132]. In the following section, we provide evidence on the effects of diet on women's cancers, including cervical, ovarian, endometrial, and breast cancers (Figure 4).

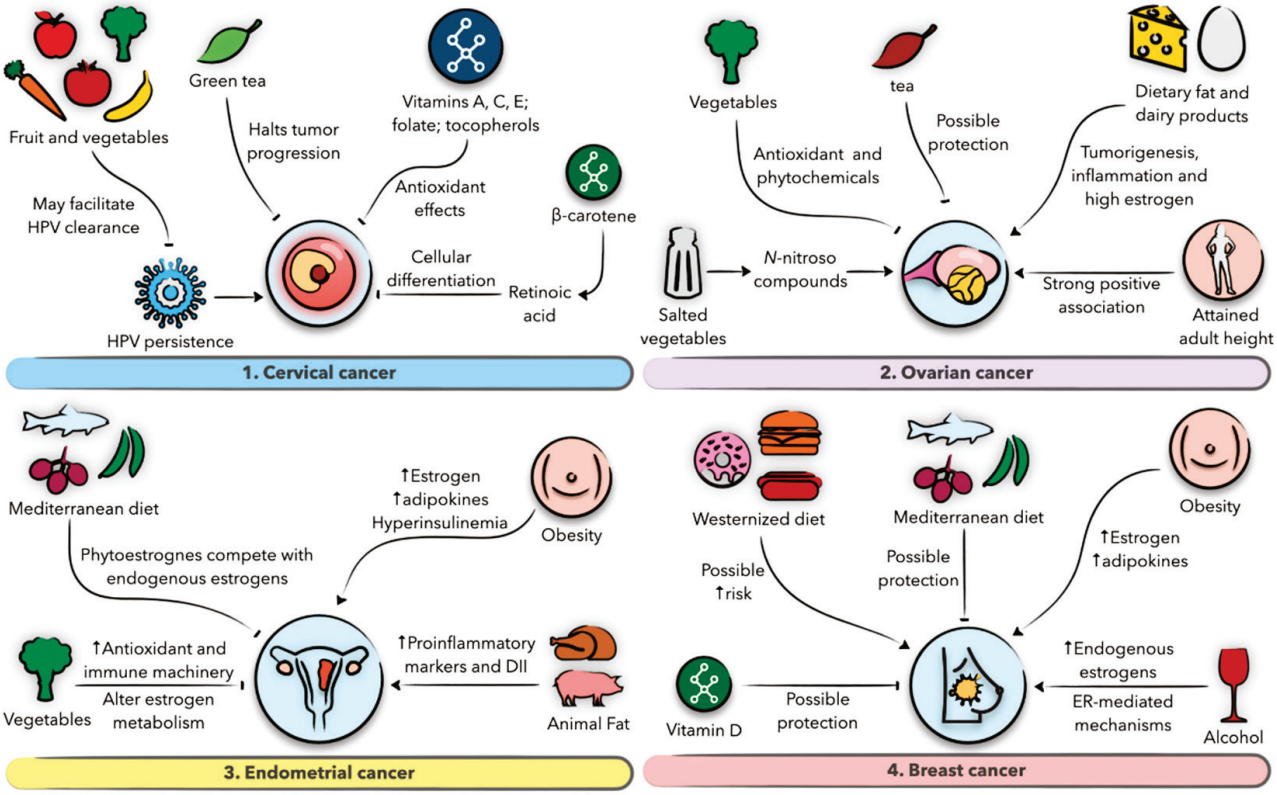

Figure 4. Schematic presentation of the role of diet and nutrition in gynecologic malignancies and the possible underlying biological mechanisms. (**1**) Role of diet in cervical cancer. (**2**) Role of diet in ovarian cancer. (**3**) Role of diet in endometrial cancer. (**4**) Role of diet in breast cancer.

7.2. Diet and Nutrition in Cervical Cancer

In cervical cancer, the interaction of dietary factors and carcinogenesis has been a matter of controversy, and most data have been conflicting, sparse, and limited by retrospective designs. Studies addressing this association have evaluated the effect of dietary habits on two main outcomes: human papillomavirus (HPV) persistence and squamous intraepithelial lesion (SIL)/invasive squamous carcinoma (ISC) risk [133]. A case–control study in China has concluded that fresh vegetable and green tea intake confers protection against cervical cancer and high SIL, whereas no association was reported for fruit, egg/milk/meat, or soybean intake [134]. This is in contrast to a European study that found a protective effect of fruit intake, but not vegetable intake [12]. The discrepancy in the results may be attributed to varied access of study participants to certain foods in different geographic areas and biases inherent to retrospective data ascertainment [134]. Lending support to these findings, green tea has indeed been shown to halt tumor progression and function synergistically with anti-cancer therapy, whereas fruit and vegetables may dampen cervical cancer risk through ameliorating HPV persistence [135–137].

In regard to micronutrient intake, several lines of research have concluded that cervical dysplasia and cancer risk may be significantly reduced in women with higher intake of vitamins C, E, and A, folate, β-carotene, and lycopene [138–140]. While experimental studies on the exact mechanisms by which these nutrients exert anti-neoplastic effects in cervical neoplasia are largely lacking, it is biologically plausible to consider their potent antioxidant properties in this context. Women deficient in folic acid, a vitamin necessary for DNA synthesis, are shown to manifest cervical dysplastic changes that are ameliorated upon folate supplementation, implying that folate deficiency may have a role in inducing cervical neoplasia, possibly through defective DNA synthesis and facilitating HPV persistence [141,142]. In addition to questionnaire-based studies, other studies found that low serum folate levels may interact with high-risk HPV to promote cervical intraepithelial neoplasia progression, which may prompt the experimentation of serum folate as a potential biomarker [143]. An excellent systematic review by García-Closas et al. has thoroughly evaluated the state of evidence on micronutrient intake and cervical carcinogenesis [133]. On the other hand, macronutrients, including carbohydrates, proteins, and fat, were not associated with cervical cancer risk [138,144]. When exploring the association of diet with cervical cancer, it is imperative to consider the complex, multifactorial etiology of this cancer as well as the reciprocal effect of latent disease on nutritional status to better elucidate a causal role of diet in this association.

7.3. Diet and Nutrition in Ovarian Cancer

Although most evidence on the association between diet and ovarian cancer remains largely inconclusive to date, some studies have investigated an etiological role for diet in this association. In a systematic review of the evidence for dietary intake and ovarian cancer risk, pooled studies indicated a higher risk with increased intake of dietary fat, dairy products, nitrites, and, to a lesser extent, fruit and vitamin C. By contrast, isoflavones, tea, and possibly vegetables may decrease that risk [13]. However, as data are inconsistent due to inherent biases or insufficient sample sizes, it proved difficult to make conclusive remarks about the role of specific dietary constituents compared to others, a notion that was reiterated by the Update Project Report on diet and ovarian cancer risk [145]. In another cross-sectional study conducted in China, animal fat and salted vegetable intake conferred a higher risk of ovarian cancer but vice versa with vegetable and fruit intake [146]. The latter dietary groups may mediate their protective effects through their antioxidant and phytochemical constituents [147,148], whereas salted vegetables may promote carcinogenesis as their nitrite substrates are converted to N-nitroso compounds, a mechanism similar to gastric and esophageal carcinogenesis [149,150].

Of all, most consistent evidence seems to come in favor of fat intake, particularly animal fat, which was shown by both epidemiological and mechanistic studies to increase cancer risk. The National Institutes of Health–American Association of Retired Persons

(NIH-AARP) cohort detected a 30% greater risk of ovarian cancer with animal fat intake [151], whereas Larsson et al. showed that dairy products, another source of animal fat, were linked to a 60% higher risk of invasive ovarian cancer [152]. At the cellular level, dietary fat appears to have tumorigenic, inflammatory, and estrogen-elevating properties, which collectively are plausible contributors to ovarian cancer [153–155]. Furthermore, the Women's Health Initiative (WHI) dietary modification trial inferred that low-fat diets followed for >4 years confer a lower risk of ovarian cancer among postmenopausal women [156].

Interestingly, the effect of diet on ovarian cancer appears to occasionally be subtype specific. For example, the Nurses' Health Study (NHS) and the AARP study found that lactose and total fat are associated with a greater risk of serous ovarian cancer [151,157], whereas a meta-analysis has detected a modest risk reduction for the endometrioid subtype with alcohol intake, as opposed to no association with the epithelial subtype [158]. Regarding obesity, the literature has reported a relatively weak association with ovarian cancer risk, possibly due to retrospective study designs and use of inaccurate measurements of excess body fat [159]. Intriguingly, convincing evidence now shows that increased adult attained height, reflective of genetic, environmental, hormonal, and nutritional factors affecting growth, confers a higher risk of ovarian cancer [145].

7.4. Diet and Nutrition in Endometrial Cancer

The association of dietary habits with endometrial cancer has been described in the context of their predisposition to chronic subclinical inflammation and hyperestrogenic states, culprits implicated in endometrial malignancy [160,161]. A recent case–control study done in Italy has concluded that vegetable intake, adherence to a Mediterranean diet, and low dietary inflammatory index confer protection against endometrial cancer [162], lending support to previously documented evidence [163,164]. From a mechanistic perspective, vegetables alter estrogen metabolism, induce antioxidant machinery, and activate the immune system [11], whereas phytoestrogens found in Mediterranean diets may compete with endogenous estrogens [165], possibly antagonizing their effects on the endometrium. On the contrary, animal products can lead to higher dietary inflammatory index and proinflammatory markers such as CRP that can be associated with endometrial cancer [161,166]. While it has been experimentally shown that monounsaturated fatty acid intake may decrease cancer risk through proapoptotic and anti-inflammatory mechanisms [167], clinical observations in this context should be carefully interpreted as they may be confounded by other factors, such as food source and total energy intake [168].

In contrast to ovarian cancer, the relationship between obesity and endometrial cancer is better established. The majority of observational studies, including prospective reports, have identified a significant positive association between body mass index (BMI) and central adiposity parameters, such as waist circumference and waist-to-hip ratio, and endometrial cancer risk [132]. In addition, it was shown that a weight gain of about 20 kg can increase endometrial cancer risk by twofold [132]. Interestingly, early adulthood obesity, and to a lesser extent childhood/adolescent obesity, were associated with a modest increase in endometrial cancer risk, but the results in this context should be interpreted with caution as inaccurate obesity parameters or recall bias might have affected the results [132,169,170]. The biological basis of the association between obesity and endometrial cancer can have multiple explanations. First, excess adiposity augments peripheral conversion of androgens to estrogens by harboring a larger aromatase reserve, especially in postmenopausal women [171], and decreases sex hormone-binding globulin, liberating more bioactive, unopposed estrogen [172]. Second, hyperinsulinemia, commonly co-existing with obesity, may activate mitogenic cascades in the endometrium, where insulin and insulin growth factor 1 (IGF-1) receptors are found to be overexpressed [173]. Third, obesity can create a systemic proinflammatory milieu by means of adipokine secretion, contributing to insulin resistance on the one hand, and to endometrial proliferation on other hand [174,175].

7.5. Diet and Nutrition in Breast Cancer

The role of diet in breast cancer has been investigated to a limited extent. Nevertheless, the most compelling evidence was reported for alcohol intake by a substantial number of studies [176,177]. Alcohol consumption is associated with a higher breast cancer risk, especially for hormone-dependent tumors, with a 12% increase in estrogen receptor tumor risk per 10 g ethanol per day [177]. Although unclear and still being investigated, alcohol may mediate this effect indirectly through induction of endogenous sex steroids [178] or directly through ER-dependent or ER-independent signaling pathways [179,180]. Regarding other nutrients, data are more controversial but were documented in the literature, nonetheless. The effect of total fat intake, for example, on breast cancer risk appears to be subtype specific [181]. On the one hand, omega-3 polyunsaturated fatty acids were found to confer a 14% less risk of breast cancer, perhaps through estrogen and adipokine modulating effects [182,183]. Additionally, trans-fatty acids and an increased monounsaturated to saturated fatty acids ratio may heighten a woman's risk of the tumor [184,185]. In regard to carbohydrates, the results are mixed and vary between pre- and postmenopausal women. Whereas the WHI found no association of dietary glucose load and glycemic index with breast cancer [186], a meta-analysis of observational studies reported a modest positive association between glycemic index and breast cancer risk [187], emphasizing the uncertain nature of this association. Theoretically, a high-glycemic index/glucose load diet can increase insulin release [188], which induces estrogen and IGF-1 and thus cellular proliferation [189,190], providing a plausible mechanism that may prompt future experimental work.

Epidemiological studies have recognized a potentially higher risk of breast cancer among vitamin D-deficient women. A meta-analysis has indeed detected an inverse association between serum vitamin D level and breast cancer risk [191], whereas several other studies failed to re-document it [192], warranting future investigation of this association. When addressing overall dietary patterns, Mediterranean diets comprising fish, vegetables, fruit, legumes, and vegetable oil are associated with a 46% lower risk of breast cancer in one study, whereas more Westernized dietary patterns that include processed meat, high-fat dairy products, sweets, and caloric drinks confer a higher risk of the tumor [14]. Excess adiposity may also signify a higher risk of breast cancer development through dysregulated estrogenic and adipokine profiles [193] and also a higher risk of recurrence and mortality among women with this cancer [194].

8. Conclusions and Future Directions

To date, the contribution of diet and nutrition to gynecological disorders remains a largely unexplored avenue that merits substantial future investigation. As most evidence is derived from epidemiological reports, experimental studies that consider potential confounding factors and accurately describe the independent effects of individual nutrients on the development and growth of gynecological diseases should be increasingly conducted. Observational studies addressing this association should shift toward randomized, prospective designs of sufficient power and follow-up periods to detect minor effects. As conservative therapeutic strategies for the prevention and risk reduction of some gynecological disorders are mainly limited to hormonal treatments, exploring the association between these disorders and diet will introduce women to novel therapeutic perspectives. With regard to gynecological malignancies, controlling for various confounding factors may prove important to delineate a precise etiological role for diet. Lastly, randomized controlled trials, wherein specific diets are assigned to different groups, should also be conducted on a wider population basis to better establish the protective or harmful effects of these diets on women's health.

Author Contributions: S.A. and M.A.B. conceived and designed the manuscript. S.A., A.A., M.E.S., M.M.-I., L.R. and J.T.B. searched the literature and wrote the manuscript. A.A. prepared the figures.

A.F. and M.A.B. edited the manuscript. All authors have read and approved the final version of the manuscript.

Funding: This work was supported, in part, by NIH grant 1R01HD094380.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

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

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Effects of 6 Months of Soy-Enriched High Protein Compared to Eucaloric Low Protein Snack Replacement on Appetite, Dietary Intake, and Body Composition in Normal-Weight Obese Women: A Randomized Controlled Trial

Neda Haghighat [1], Damoon Ashtary-Larky [2], Reza Bagheri [3], Alexei Wong [4,*], Neda Cheraghloo [5], Gholamreza Moradpour [1], Michael Nordvall [4], Omid Asbaghi [6], Nader Moeinvaziri [1], Masoud Amini [1,*], Zahra Sohrabi [7] and Frédéric Dutheil [8]

1 Laparoscopy Research Center, School of Medicine, Shiraz University of Medical Sciences, Shiraz 71348-14336, Iran; Neda.hag@gmail.com (N.H.); nedahaghighat@sums.ac.ir (G.M.); nmv1986@yahoo.com (N.M.)
2 Nutrition and Metabolic Diseases Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz 61357-15794, Iran; damoon_ashtary@yahoo.com
3 Department of Exercise Physiology, University of Isfahan, Isfahan 81746-73441, Iran; will.fivb@yahoo.com
4 Department of Health and Human Performance, Marymount University, Arlington, VA 22207, USA; mnordval@marymount.edu
5 Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran 1417613151, Iran; neda_cheraghloo721@yahoo.com
6 Cancer Research Center, Shahid Beheshti University of Medical Sciences, Tehran 1416753955, Iran; omid.asbaghi@gmail.com
7 Nutrition Research Center, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz 71348-14336, Iran; zahra_2043@yahoo.com
8 Université Clermont Auvergne, CNRS, LaPSCo, Physiological and Psychosocial Stress, CHU Clermont-Ferrand, University Hospital of Clermont-Ferrand, Preventive and Occupational Medicine, WittyFit, F-63000 Clermont-Ferrand, France; fred_dutheil@yahoo.fr
* Correspondence: awong@marymount.edu (A.W.); aminima@sums.ac.ir (M.A.)

Citation: Haghighat, N.; Ashtary-Larky, D.; Bagheri, R.; Wong, A.; Cheraghloo, N.; Moradpour, G.; Nordvall, M.; Asbaghi, O.; Moeinvaziri, N.; Amini, M.; et al. Effects of 6 Months of Soy-Enriched High Protein Compared to Eucaloric Low Protein Snack Replacement on Appetite, Dietary Intake, and Body Composition in Normal-Weight Obese Women: A Randomized Controlled Trial. *Nutrients* **2021**, *13*, 2266. https://doi.org/10.3390/nu13072266

Academic Editors: Sílvia Rocha-Rodrigues, José Afonso and Monica Sousa

Received: 20 May 2021
Accepted: 28 June 2021
Published: 30 June 2021

Abstract: (1) Background: The favorable effects of high protein snacks on body composition and appetite status in lean and athletic populations have been illustrated previously. However, the effects of soy-enriched high protein snacks have not been investigated in women with normal-weight obesity (NWO). Consequently, we aimed at comparing the effects of six months of soy-enriched high protein snack replacement on appetite, body composition, and dietary intake in women with NWO. (2) Methods: One hundred seven (107) women with NWO [(age: 24 ± 3 yrs, BMI: 22.7 ± 2.3 kg/m^2, body fat percentage (BFP): 38 ± 3.2%)] who were assigned to one of two groups; high protein snack (HP, n = 52) containing 50 g soybean or isocaloric low-protein snack (protein: 18.2 g, carbohydrate: 15 g, fat: 10 g, energy: 210 kcal) or isocaloric low protein snack (LP, n = 55) containing 3.5 servings of fruit (protein: <2 g, carbohydrate: ≈50 g, fat: <1 g, energy: ≈210 kcal) as part of their daily meals (as a snack at 10 a.m.), successfully completed the study interventions. Body mass (BM), body mass index (BMI), waist circumference (WC), BFP, skeletal muscle mass, dietary intake, and appetite levels were evaluated prior to and after the six-month intervention. (3) Results: Appetite (HP = −12 mm and LP = −0.6 mm), energy intake (HP = −166.2 kcal/day and LP = 91.3 kcal), carbohydrate intake (HP = −58.4 g/day and LP = 6.4 g/day), WC (HP = −4.3 cm and LP = −0.9 cm), and BFP (HP = −3.7% and LP = −0.9%) were significantly (p < 0.05) reduced, while skeletal muscle mass (HP = 1.2 kg and LP = 0.3 kg) significantly increased in the HP compared to the LP group, respectively. (4) Conclusions: Six months of a soy-enriched high protein snack replacement decreased appetite and improved body composition in women with NWO. Our findings suggest that soy-enriched high protein snacks are an efficacious strategy for body composition improvement.

Keywords: normal weight obesity; high-protein diet; high protein snack; body composition

1. Introduction

Normal-weight obesity (NWO) is recognized as a specific type of obesity and characterizes individuals as having normal body mass (BM) and body mass index (BMI) with an elevated fat mass (FM) (<25 (kg/m^2) and body fat percentage > 30%) and concurrent diminished lean mass [1]. It has been proposed that low dietary protein intake may be related to the lower lean mass observed in this population [2]. NWO is a widespread public health issue that may be prevalent in up to one-third of individuals of certain ethnicities [3]. For instance, a recent Iranian large population study involving 9704 individuals consisting of 2439 normal-weight adults showed that 38.5–46.2% of Iranian adults with normal BM met NWO criteria [4]. Further, the prevalence of NWO is drastically greater in women compared to men [5] suggesting that strategies for improving this condition be directed at specific cohorts.

Adipose tissue accumulation in obesity has been attributed to several factors, including increased snacking tendencies (defined as any eating occasion outside of mealtime), which may result in weight gain due to excess caloric intake [6,7]. Indeed, it has been previously reported that snacking accounts for as much as 30% of the daily energy intake [8] with women snacking more frequently than men [9] suggesting that hormonal differences may play a role in the development of NWO [10]. Because the nutrient and caloric density of snacks vary, altered body composition may be significantly influenced by macronutrient density, such as protein. Increased protein consumption is known to improve body composition, appetite control, and energy intake management [11,12]; suggesting that high protein snacks may offer useful approaches to achieve these adaptations. For instance, Astbury et al. demonstrated that high protein snacks significantly decreased dietary energy intake in lean men [13] and improved body composition in male athletes [14,15]. However, the effects of high protein snack replacement in women with NWO are currently unknown. Evidence suggests that improvements in body composition resulting from higher protein intake may result from elevated anorexigenic and reduced orexigenic hormones, leading to declines in adiposity [16,17]. Moreover, increased lean mass via muscle protein synthesis (MPS) may be enhanced by upregulating the mechanistic target of rapamycin (mTOR) signaling pathway activity [16]. It has been estimated that consumption of two to three meals a day, containing ~25–30 g of high quality protein is optimal for the stimulation of 24-h MPS in healthy adults [18]. Although prior studies have evaluated the acute effects of high protein snack replacement on satiety levels of both healthy and overweight women [19,20], there are no published investigations on long-term soy-enriched high protein snack replacement on body composition, appetite, and dietary intake. In other populations, such as Asians, vegetarians, and vegans, soy provides the main source of dietary protein [21], yet little is known about its applicability to the population in the present study. Therefore, we aimed to assess the effects of six months of soy-enriched high protein snack replacement on body composition, appetite control, and ad libitum caloric intake in women with NWO. We hypothesized that long-term soy-enriched high protein snack replacement would promote a primary outcome of improved skeletal muscle mass (SMM) and secondary outcome of appetite suppression.

2. Materials and Methods

2.1. Study Population

This parallel design (allocation ratio: 1:1) randomized clinical trial was conducted at Shiraz University of Medical Sciences (SUMS), Shiraz, Iran. Participants were recruited from the staff of companies supported by SUMS via posters and social media advertising. Participant inclusion criteria included healthy, pre-menopausal, BMI > 18.5 and <25 kg/m^2, body fat percentage (BFP) > 30% [1,22], and women between the ages of 20 and 40 years. Exclusion criteria included history or presence of bariatric surgery, any acute or chronic diseases, psychiatric disorders, soy allergy, alcohol consumption, smoking, medications use, engaging in any high-intensity physical activity, consumption of more than 300 mg of caffeine daily (described as caffeine users) [23], as well as being pregnant and/or lactating.

Further exclusion criteria were recent BM fluctuations, dietary changes, and the use of weight loss (green tea, caffeine, etc.) or other protein supplements (whey proteins, casein, etc.) within six months of the initiation of the study. Inclusion and exclusion criteria were assessed prior to the study following face-to-face meetings with each prospective participant. All study protocols were fully explained to participants who subsequently provided written consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and the Ethics Committee at SUMS approved the study protocol (IR.SUMS.REC.1398.776). The present study has been registered with the Iranian Registry of Clinical Trials {(IRCT), (IRCT20170412033393N3)}.

2.2. Study Protocol

Participants (N = 120) who met the inclusion criteria were randomly allocated into one of two groups; a high protein snack (HP; n = 60) or an isocaloric low protein snack (LP; n = 60) that was incorporated into their daily meals using a convenience allocation. The HP group received high protein content snacks {50 g of soybeans (protein: 18.2 g, carbohydrate: 15 g, fat: 10 g, energy: 210 kcal)} while the LP group received low protein content snacks (≈3.5 servings of fruit), as desired, from the exchange list of foods based on American Diabetes Association and American Dietetic Association guidelines [24,25] (Table 1). On a daily basis, participants in the HP group were instructed to weigh 50 g of soybeans on a digital scale whereas the LP group chose 3.5 servings of fruit based on the aforementioned guidelines. The snacks for both groups contained similar calories (≈210 kcal) [24,25] and all participants were instructed to consume their snacks daily at 10 a.m (~3 h before lunch). The exchange list of foods has been frequently utilized and validated in the Iranian population [26]. Throughout the intervention, participants consumed food and thus calories ad libitum and were instructed to maintain normal dietary habits and levels of physical activity while abstaining from supplement intake. Therefore, dietary macro-and micronutrient consumption remained a free-living condition of participants for the duration of the study [13]. Body composition testing, questionnaires for 24-h dietary recalls, physical activity, and appetite were completed at baseline and after six months of the intervention. Participants also visited the laboratory on two other occasions (at the end of months 2 and 4) to report snack compliance and physical activity levels, be reminded of consuming snack meals, as well as avoid lifestyle modification. Additional snack compliance reporting and reminders were performed once per week by phone or WhatsApp software.

Table 1. Characteristics of the soy enriched high and low protein snack meal. Abbreviations: HP, high protein; LP, low protein; g, gram.

	HP Snack (50 g Soybeans)	LP Snack (3.5 Servings Fruit)
Energy (g)	210	≈210
Protein (g)	18.2	<2
Carbohydrate (g)	15	≈50
Fat (g)	10	<1
Fiber (g)	4.6	4–6

2.3. Randomization and Blinding

After baseline assessments, randomization of participants was performed through a computer-generated random number table by an independent coordinator, not otherwise involved in the study, who created an allocation sequence that assigned participants to the HP or LP groups. A dietitian subsequently assigned participants to their respective groups (the allocation sequence was concealed by the independent coordinator until the moment of assignment), provided verbal (phone) and written (WhatsApp) instruction on their snack regimens, and facilitated one-on-one laboratory interventions but therefore, could not be

blinded. Therefore, study participants were aware of their treatment group for the duration of the study based on their snack allocation (HP or LP group). However, the dietitian did not participate in nor was their communication about data collection and/or analysis with any other researcher associated with this study. Further, the researchers associated with the study herein (except for the dietitian) were blinded to the treatment allocation until the database was unlocked for subsequent data analysis. At no time during the six-month intervention period did the researchers communicate with study participants.

2.4. Dietary Intake and Physical Activity Assessment

24-h dietary recalls were completed by all participants on three occasions over a week time period (one weekday and two weekend days) [27] prior to and at the conclusion of the six-month intervention [28]. The validity and reliability of this methodology for documenting caloric intake have been previously described [29–31]. Calorie and macronutrient combinations were assessed using the Nutritionist IV for Windows software program (The Hearst Corporation, San Bruno, CA). Physical activity level was recorded at baseline and six months using the international physical activity questionnaire (IPAQ) and presented as metabolic equivalent/wk (MET-min/wk) [32]. Metabolic equivalent values of 3.3, 4.0, and 8.0 were used to classify participants into low, moderate, or vigorous-intensity categories, respectively [28]. Within and between-group comparisons were made at baseline and six months for both dietary intake and physical activity levels.

2.5. Body Composition Assessment

Prior to arriving at the laboratory, participants fasted for 12 h (overnight and ideally following 8 h sleep), refrained from consuming alcohol and caffeinated or other diuretic beverages for 48 h before assessments [33]. Moreover, 30 min prior to assessment, participants were asked to urinate (void) completely and avoid consuming water according to standard procedures for bioelectrical impedance analysis (BIA) [34]. Height was measured using a wall-mounted stadiometer (SECA Model 222; Seca, Germany) having an accuracy of 0.1 cm. Body composition characteristics were measured by multi-frequency BIA (Inbody 270, Biospace, Korea). In advance of the BIA measurement, the participant's palms and soles were wiped with electrolyte tissue. Participant demographics were manually entered into the BIA display by a researcher. Participants were instructed to stand on the BIA device, placing the soles of their feet on the electrodes. To determine BFP, BMI, and SMM, participants grasped the handles of the BIA unit ensuring that the palm and fingers of each hand made direct contact with the electrodes while fully extending and abducting their arms at approximately 20° [35]. Multi-frequency BIA has been shown to be a valid and reliable method [36,37], which provides acceptable body composition estimates compared to dual x-ray absorptiometry (the gold standard) [35]. Indeed, the test-retest reliability of the BIA method was high (R = 0.95 to 0.99) and has been previously described [36]. Waist circumference (WC) measurement was obtained at the noticeable waist narrowing located approximately halfway between the costal border and the iliac crest [38]. Within and between group comparisons were made at baseline and six months for all body composition measurements.

2.6. Appetite Assessment

Each participant's appetite level was estimated by visual analog scales (VAS) [39] one day following body composition assessment via in-person questionnaires. VAS was completed 5 min prior to lunch (~3 h following a morning snack of the participant's choice with ad libitum water intake), which traditionally is the largest Iranian meal [40]. The VAS questionnaire contained three questions about appetite ("How hungry do you feel?", "How full do you feel?", "How much would you like to eat?") on separate 100 mm in length scales. To express their sensations of hunger and satiety, participants drew a vertical mark across the 100 mm scale with "no appetite" at one end (0 mm) and "uncontrollable appetite" (100 mm) at the other, with low, average, high, and very high demarcations in between [41].

After participants marked the questionnaires, subjective responses were converted to quantitative values according to the location of the mark ranging from 0–100 mm [41]. The numerical scores of between 0 and 100 mm were averaged from the three questions for each participant and presented as a single score which was subsequently analyzed for within and between-group differences at baseline and six months.

2.7. Statistical Analyses

A priori sample size calculation was conducted using the G*Power analysis software [42]. The rationale for sample size was based on our previous research, which documented significant improvements in lean mass after 12 weeks of a high protein diet in 24 women with NWO [43]. By utilizing the equation for effect size (ES) {(mean before-mean after the high protein diet)/the pooled standard deviation}, this study revealed an ES of 0.38 {(33.5–34.8)/3.45}. In the present study and based on $\alpha = 0.05$, a power (1- β) of 0.80, and an ES = 0.4 (highest approximate effect size), a total sample size of at least 88 participants ($n = 44$ per group) was needed for sufficient power to detect significant changes in the primary outcome of lean mass. To account for potential participant attrition during our six-month-long investigation, additional participants (25% above recommended sample size) were recruited, thus increasing the target sample size to 110 participants ($n = 55$ per group). Descriptive statistics were presented using mean ± standard deviation (SD). Independent t-tests were used to compare means between the HP and the LP groups for each variable, while paired t-tests were used to evaluate changes over time. An analysis of covariance (ANCOVA) was used to examine the impact of groups on variables in post-test controlling for the effects of pre-test values. All assumptions were assessed and when the homogeneity of variance was not met, the parameter was estimated with robust standard errors (based on the large sample robust estimator of the covariance matrix). Cohen's *d* effect size (ES) was calculated as post-training mean minus pre-training mean/pooled pre-training standard deviation means [44]. All analyzes were performed by SPSS (version 26) and *p*-values < 0.05 were considered as statistically significant. Figure 2 was produced using Graphpad Prism (8.4.3).

3. Results

3.1. Study Population and Compliance

Between October 2018 and April 2019, we screened 133 women with NWO. After exclusion criteria were applied, 120 qualified for baseline evaluation and were subsequently randomized to either the HP ($n = 60$) or the LP ($n = 60$) groups. After randomization, eight participants in the HP group dropped out for various personal reasons. In the LP group, five participants were excluded from continuing in the study due to new medication use and an inability to consume snacks. Data are shown for the 107 participants (age: 24 ± 3 yrs and height: 166 ± 7.4 cm), consisting of 52 and 55 participants in the HP and LP groups, respectively, that successfully completed the six-month intervention (Figure 1). Participants in both groups reported no ill side effects with the dietary snacks during the duration of the study. The overall participant compliance rate was 89.1% (86.6% in the HP group and 91.6% in the LP group).

3.2. Body Composition

There were no significant differences in body composition variables between LP and HP groups at baseline ($p > 0.05$). Participants in the HP group experienced significant declines in BM {−2.9 kg (95% CI = −2.4 to −3.4, $p < 0.001$, d = 0.4), (Figure 2A)} and BMI {−1 kg/m^2 (95% CI = −0.8 to −1.2, $p < 0.001$, d = 0.4), (Figure 2B)} from baseline to post-intervention. In contrast, those in the LP group experienced a significant increase in BM {0.8 kg (95% CI = 1.2 to 0.3, $p < 0.001$, d = 0.1)} and BMI [0.3 kg/m^2 (95% CI = 0.4 to 0.1, $p < 0.001$, d = 0.1)] during the same time. Additionally, both groups significantly decreased WC {HP = −4.3 cm (95% CI = −2.8 to −5.8, $p < 0.001$, d = 0.7) and LP = [−0.9 cm (95% CI = −0.6 to −1.2, $p < 0.001$, d = 0.1), (Figure 2C)] and BFP {HP = −3.7%

(95% CI = −3.3 to −4.1, $p < 0.001$, d = 1.1) and LP = −0.9% (95% CI = −0.5 to −1.2, $p < 0.001$, d = 0.3), (Figure 2D)} from baseline to post-intervention. Moreover, both groups significantly increased SMM {HP = 1.2 kg (95% CI = 1.5 to 1, $p < 0.001$, d = 0.9) and LP = 0.3 kg (95% CI = 0.7 to 0.02, $p = 0.035$, d = 0.2), (Figure 2E)} from baseline to post-intervention. ANCOVA results showed significant between-group differences for all body composition indices ($p < 0.001$, Table 2) with greater changes being noted in the HP group over time.

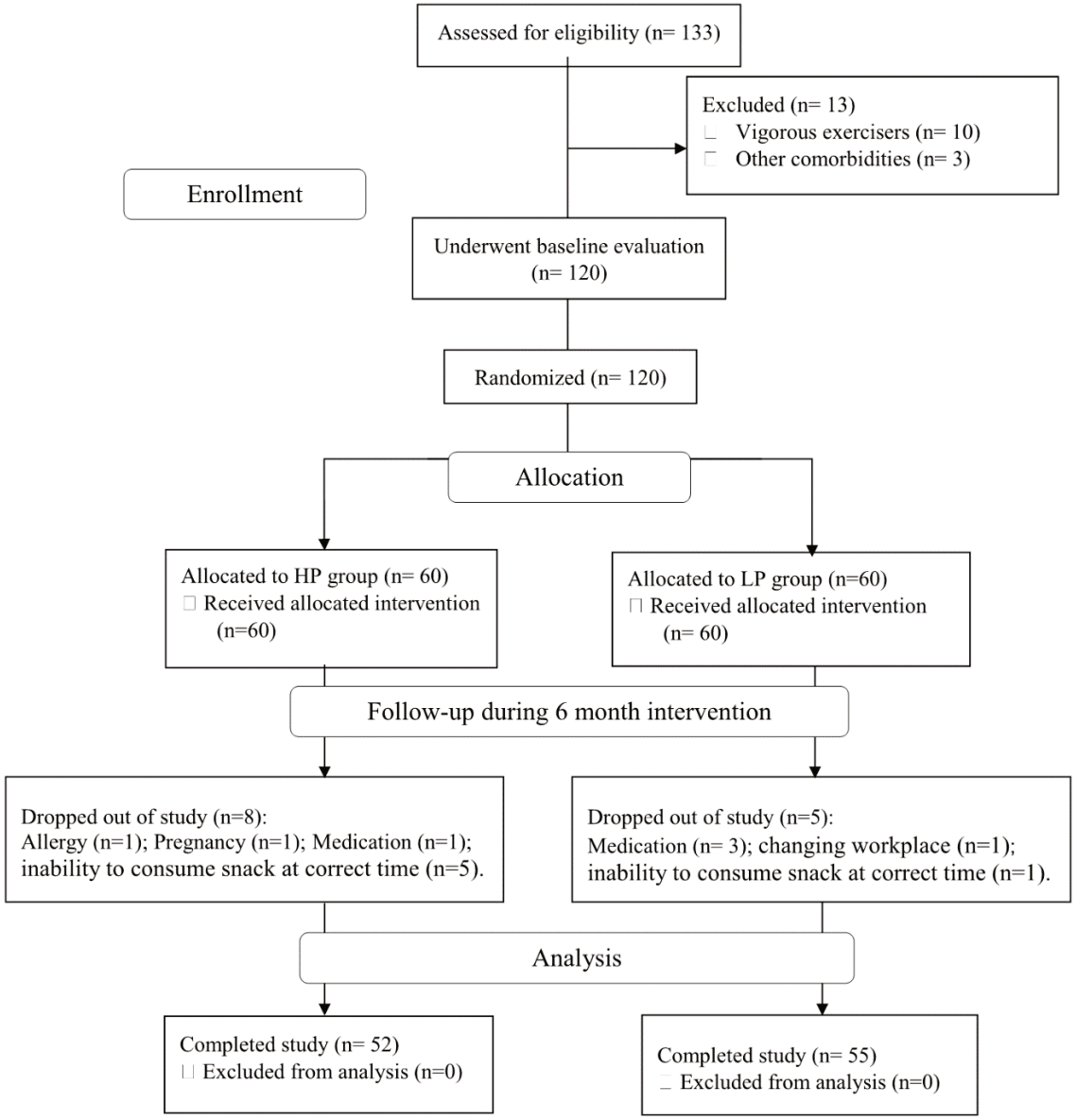

Figure 1. Participants flow diagram.

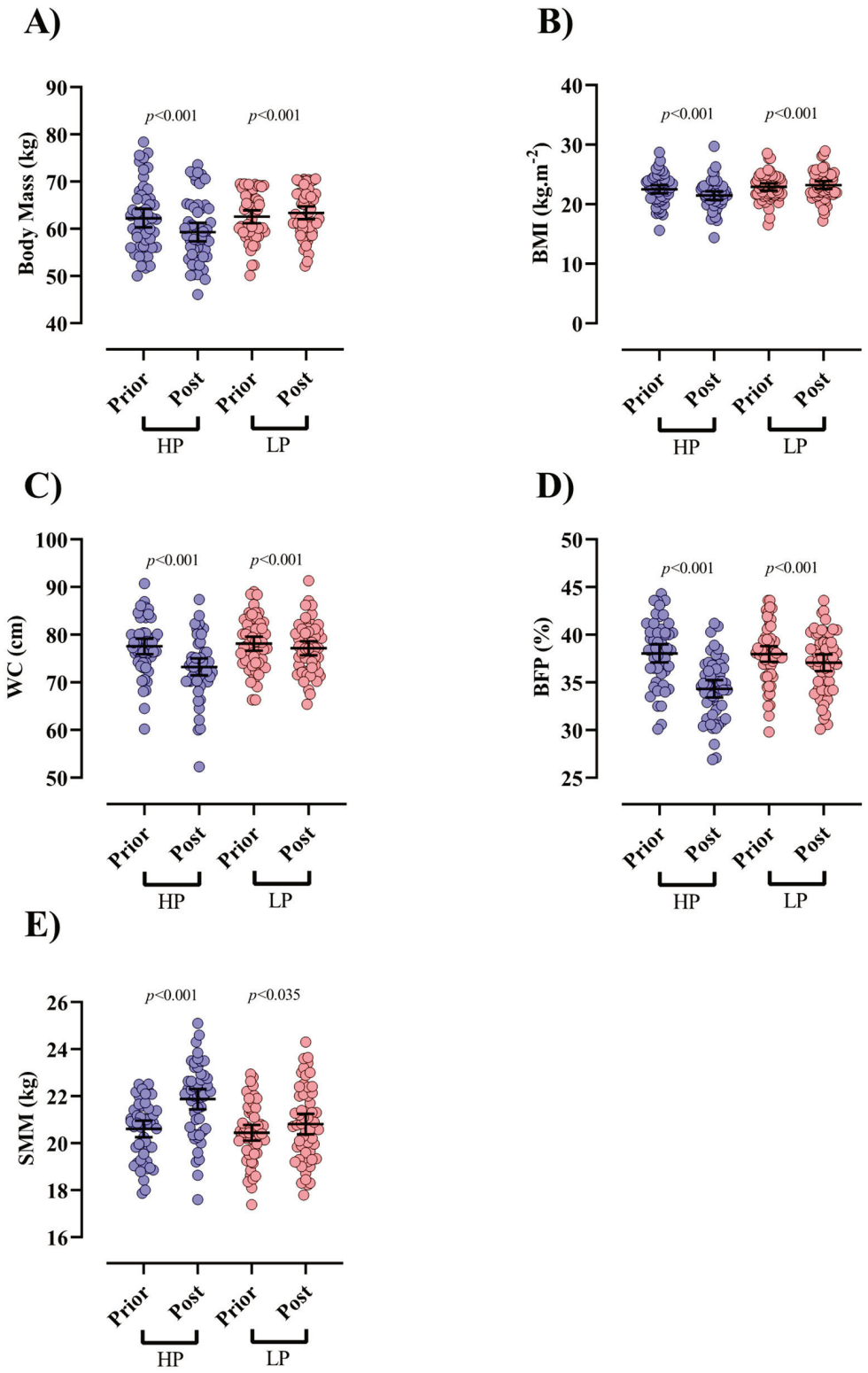

Figure 2. The effects of HP and LP interventions on body composition variables. (**A**) Body Mass, (**B**) BMI: body mass index, (**C**) WC: waist circumference, (**D**) BFP: body fat percentage, (**E**) SMM. Error bars indicate 95% CI. P-values of t-test are indicated above each group.

Table 2. The impact of HP vs. LP on variables.

Variables	Contrast	β (SE)	95% CI	*p*-Value
Body Mass (kg)	HP vs. LP	−3.7 (0.34)	−4.46 to −3.11	<0.001
BMI (kg.m^{-2})	HP vs. LP	−1.38 (0.13)	−1.63 to −1.13	<0.001
WC (cm) #	HP vs. LP	−3.46 (0.76)	−4.98 to −1.95	<0.001
BFP (%)	HP vs. LP	−2.80 (0.27)	−3.33 to −2.26	<0.001
SMM (kg)	HP vs. LP	0.91 (0.21)	0.5 to 1.3	<0.001
Appetite (mm) #	HP vs. LP	−11.44 (0.65)	−12.73 to −10.16	<0.001
Protein (g/day)	HP vs. LP	19.46 (1.23)	17.03 to 21.89	<0.001
Fat (g/day) #	HP vs. LP	−6.31 (1.67)	−9.62 to −2.99	<0.001
Fiber (g/day)	HP vs. LP	−0.59 (0.43)	−1.45 to 0.28	0.180
Carb (g/day) #	HP vs. LP	−62.43 (6.53)	−75.38 to −49.49	<0.001
Energy (kcal/day) #	HP vs. LP	−238.37 (30.93)	−299.70 to −177.03	<0.001
Relative protein (g/kg/day)	HP vs. LP	0.38 (0.03)	0.32 to 0.44	<0.001

Abbreviations. BMI, body mass index; WC, waist circumference; BFP, body fat percentage; SMM, skeletal muscle mass (kg); HP, high protein; LP, low protein; # Parameter estimates with robust standard errors based on the original asymptotic or large sample robust, empirical, or "sandwich" estimator of the covariance matrix of the parameter estimates.

3.3. Appetite and Physical Activity

There were no significant differences in these variables between LP and HP groups at baseline ($p > 0.05$). Appetite levels in the HP group {−12 mm (95% CI = −13.2 to −10.9, $p < 0.001$, d = 2.9)} significantly decreased whereas no change was observed in the LP group pre-to post intervention. Indeed, the change in appetite in the HP was significantly greater than the LP group ($p < 0.001$, Table 2) over time. No significant differences were observed for physical activity {(pre-HP, low: 19 (36%) and moderate: 33 (63%)} vs. {post-HP, low = 21 (40%) and moderate: 31 (60%)} and {pre-LP, low: 17 (31%) and moderate: 38 (69%)} vs. {post-LP, low: 20 (36%) and moderate: 35 (63%)} from baseline to post-intervention ($p > 0.05$).

3.4. Dietary Intake

Results of independent t-test showed that except fiber, the difference of markers between HP and LP was not significant at baseline. Following the intervention, energy {−166.2 kcal/day (95% CI = −109 to −223.5, $p < 0.001$, d = 0.9)} and fat intake {−2.98 g/day (95% CI = −0.2 to −5.7, $p = 0.035$, d = 0.4)} significantly decreased in the HP group; while significantly increasing in the LP group {energy = 91.3 kcal/day (95% CI = 127 to 55.6, $p < 0.001$, d = 0.3) and fat intake = 4.5 g/day (95% CI = 6.6 to 2.4, $p < 0.001$, d = 0.4)}. Absolute Protein {HP = 23.5 g/day (95% CI = 26 to 21.2, $p < 0.001$, d = 3.4) and LP = 6.2 g/day (95% CI = 8.6 to 3.8, $p < 0.001$, d = 0.9)} and relative protein intake {HP = 0.4 g/kg/day (95% CI = 0.5 to 0.4, $p < 0.001$, d = 2.6) and LP = 0.09 g/kg/day (95% CI = 0.1 to 0.04, $p < 0.001$, d = 0.7)} significantly increased in both groups. Furthermore, fiber intake significantly and similarly increased over time in both groups {HP = 1.1 g/day (95% CI = 1.7 to 0.4, $p = 0.001$, d = 0.4) and LP = 1 g/day (95% CI = 1.8 to 0.1, $p = 0.021$, d = 0.2)}; while carbohydrate intake {-58.4 g/day (95% CI = −45.8 to −70.9, $p < 0.001$, d = 1.2)} significantly decreased only in the HP group {(no change in the LP group); (all nutrients are shown in Table 3)}. ANCOVA results showed significant between-group differences with absolute protein (β = 19.46, $p < 0.001$), and relative protein (β = 0.38, $p < 0.001$) were significantly higher; while fat (β = −6.31, $p < 0.001$), carbohydrate (β = −62.43, $p < 0.001$) and energy (β = −238.37, $p < 0.001$) significantly lower in HP compared to LP group (Table 2).

Table 3. Energy and macronutrients (mean ± SD).

Nutrient	Group	Pre	Post	Post-Pre	*p*-Value of Paired *t*-Test
Protein (g/day)	HP	51.37 ± 7.36	74.94 ± 6.40 *	23.58 ± 8.35 *	<0.001
	LP	48.80 ± 7.21	55.02 ± 6.30	6.22 ± 8.84	<0.001
Fat (g/day)	HP	48.87 ± 5.42	45.88 ± 8.37 *	−2.98 ± 9.94 *	0.035
	LP	46.36 ± 7.97	50.91 ± 9.56	4.55 ± 7.87	<0.001
Fiber (g/day)	HP	11.65 ± 2.82 *	12.77 ± 2.29 *	1.12 ± 2.30	0.001
	LP	13.02 ± 3.70	14.02 ± 3.03	1.00 ± 3.12	0.021
Carbohydrate (g/day)	HP	253.48 ± 39.24	195.04 ± 33.47 *	−58.44 ± 44.96 *	<0.001
	LP	247.05 ± 57.55	253.45 ± 55.55	6.40 ± 32.29	0.147
Energy (kcal/day)	HP	1659.17 ± 172.29	1492.88 ± 175.82 *	−166.29 ± 205.64 *	<0.001
	LP	1600.69 ± 239.62	1692.07 ± 239.19	91.38 ± 132.04	<0.001
Relative protein (g/kg/day)	HP	0.84 ± 0.15	1.28 ± 0.20 *	0.45 ± 0.16 *	<0.001
	LP	0.79 ± 0.14	0.87 ± 0.12	0.09 ± 0.14	<0.001

Abbreviations. HP, high protein; LP, low protein; * $p < 0.05$ for independent t-test, significantly difference than LP.

4. Discussion

We examined the effects of a six-month soy-enriched high protein snack meal containing 50 g of soybean on appetite, body composition, and dietary intake in women with NWO. Accordingly, this intervention improved body composition by increasing SMM and reducing BM, BFP, and appetite levels in this population. Although it has been previously shown that a high protein macronutrient diet favorably impacts body composition and appetite [45–47], the present study demonstrated that long-term soy-enriched high protein snack consumption, in the absence of other dietary modifications, offers similar benefits.

Enhancing body fat loss while maintaining SMM is a primary outcome of obesity-related interventions and therefore, the amount of SMM loss relative to reduced BM presents a biomarker of clinical efficiency [48]. Increasing the proportion of dietary protein is critical in achieving the goal of minimizing SMM loss during BM reduction strategies [46]. To that effect, a meta-analysis of 87 short-term dietary studies found a critical protein intake level exists of greater than 1.05 g/kg/day of actual BM, which was associated with 0.6 kg additional lean mass gains compared to lesser or no lean mass gain with protein intake below that level [49]. Moreover, the authors reported that in studies conducted for >12 weeks, lean mass gains increased to 1.21 kg. In the present study, BM loss observed in the HP group occurred concurrently with increased lean mass. These results are consistent with prior work by our group where an HP diet increased lean mass in women with NWO [43]. With respect to the effects of high protein intervention studies on lean mass, a meta-analysis of 24 trials has shown that when compared to a standard protein (SP) diet, a prescribed eucaloric HP diet provides modest benefits of BM reduction and in mitigating fat-free mass (FFM) reductions [50]. With respect to high protein snack replacement, Treyzon et al. revealed that a concurrent higher protein meal replacement within an elevated protein diet resulted in similar BM reductions compared to a eucaloric lower protein meal replacement plan spanning 12 weeks [51]. Similar to our results here, they also reported a significantly greater fat loss in the high protein snack group while maintaining lean mass. The discrepancy in BM reduction results from the Treyzon et al. study and ours may be explained in several ways. First, all participants in their study received a dietary plan based on a 500-kcal deficit of estimated resting metabolic rate to achieve BM loss, whereas our participants followed an ad libidum strategy, which potentially addressed satiety levels (HP group). Second, baseline protein intake among participants in our study was relatively low and the intervention length was longer than the Treyzon et al. study (six vs. three months) suggesting body composition alterations were enhanced over time in our yet-to-be elucidated upon population. With regard to caloric intake and similar to our results, a double-blind, randomized, crossover design by Astbury et al. showed that

in an ad libidum condition, total daily energy intake was significantly lower when a high protein snack (whey protein and polydextrose) was consumed compared to a low protein snack (12.6 vs. 0.6 g of protein, respectively).

A potential mechanism for the observed SMM improvements following the HP intervention may be explained by dietary protein-induced alterations in protein turnover, particularly MPS [49]. It is widely acknowledged that the mechanistic target of rapamycin complex 1 (mTORC1) signaling regulates MPS in response to anabolic stimuli such as essential amino acid availability. In the present study, we used high biological value protein containing abundant essential amino acids, which may have led to an enhanced MPS [52]. Indeed, soybean is a high-quality protein that has a Protein Digestibility Corrected Amino Acid Score (PDCAAS) and Digestible Indispensable Amino Acid Score (DIAAS) [53] that are nutritionally equivalent to meat and eggs [54]. Moreover, soybean exhibits a higher amount of protein per equivalent volume of dry mass when compared to other common protein sources [55]; making soybean a relevant protein food choice to maximize muscle hypertrophy [56,57].

Dietary plans containing high protein snack replacements have been proposed as an efficient BM management approach through improved adherence in individuals with obesity [19,20,58]. To the best of our knowledge, this is the first randomized controlled trial that investigated the effects of soy-enriched high protein snack replacement in individuals with NWO. Our results suggested that compared to a low protein snack, six months of soy-enriched high protein snack replacement significantly decreases BM, BMI, and BFP in women with NWO. Similarly, it has been proposed that a high protein diet may serve as an efficient strategy to reduce FM and BFP compared to a standard macronutrient protein diet [45,46]. Indeed, Azadbakht et al. demonstrated that a combination of a high protein and low-fat diet reduced BM and WC compared to a standard high protein diet alone in overweight and obese women [59]. Moreover, we recently indicated that a eucaloric HP diet is more effective in reducing FM and BFP compared to an SP diet in women with NWO [43]. Such desirable impacts of high protein snack replacements in the present study may in part be due to a reduction in appetite and ad libitum dietary energy intake, as evidenced by our current findings. These results appear consistent with a study by Weigle et al. who observed increased dietary protein intake produces a sustained decrease in ad libitum caloric intake and theorized that the decline in caloric intake may be mediated by increased central nervous system leptin sensitivity resulting in significant BM loss [47]. It is important to note that altering a single snack meal may be more achievable and tolerable for the population in our study when compared to more drastic dietary modifications (e.g., high protein or low-fat diets) and may be considered a viable alternative for improved body composition goals.

Additionally, it has been shown that a soy-enriched meal replacement is effective in lowering BM and FM in individuals with obesity [60]. Several studies have reported that soy protein interventions in overweight and obese individuals decrease BM, WC, and BMI [61] and that FM reductions using soy products may be due to their protein, fiber, and isoflavones content [62,63]. With implications for future human trials, animal models have illustrated that adding protein to carbohydrates during dietary intake causes increased satiety and decreased food intake as fat becomes the predominant fuel source (as measured via decreased respiratory exchange ratio), which resulted in increased BM loss [64,65]. In the current study, six months of soy-enriched high protein snack replacement approximately doubled the protein to carbohydrate ratio from 0.2 to 0.38 in the HP group, indicating that other than increased satiety and decreased food intake (negative energy balance), enhanced fat metabolism may be considered among the mechanisms for reductions in BM, BMI, and BFP.

A growing body of evidence suggests that increasing dietary protein may be an effective strategy for decreasing appetite [66]. A primary methodology of several investigations included short-term feeding strategies utilizing subjective measures of appetite or analyzing the results of a single meal as the objective [11,47,67–69]. For example, a study by

Leidy et al. indicated that consuming a high protein soy-enriched snack for three days (26 g of protein and 6 g of fat per 27 g of carbohydrates) in the afternoon elicits greater appetite suppression compared to a high-fat snack (4 g of protein and 12 g of fat per 32 g of carbohydrates) [70]. Moreover, Weigle et al. found that by implementing a eucaloric yet higher protein ad libitum condition for two weeks (increasing dietary protein from 15% to 30%), satiety was markedly increased [47]. Potential mechanisms to consider for decreased food intake and increased satiety associated with high protein diets involve increased secretion of satiety hormones such as cholecystokinin (CCK), gastric inhibitory polypeptide (GIP), glucagon-like peptide (GLP-1), peptide YY (PYY), and reduced orexigenic hormone secretion such as ghrelin [70].

We are aware that our study has some limitations including that appetite analysis was based on a VAS containing a relatively new method not widely used in the nutrition/dietetics field [71] despite its proven validity and reliability [41,72]. In addition, the pre-VAS testing snack was not the same nor did we control energy intake prior to the snack. However, prior and similarly relevant studies did not control for these variables either [47,73–75]. Nevertheless, future investigations should aim for stricter control protocols prior to VAS testing. Moreover, subjective interpretations of biological mechanisms do not provide the full picture of appetite control and certainly, energy intake and other variables contribute to levels of satiety and satiation [76]. Beyond the self-perceived appetite level, there is a need to determine the strength of satiety during ad libitum intake [76]. Although the study duration (six months) is a strength of our study, the lack of mid-intervention analysis limits our conclusions. Urine sample collection and analysis, which would have been a useful tool to assess dietary adherence through 24-h urinary nitrogen [77] were not performed in the present study. Furthermore, aside from the inherent differences in protein content of the high protein and 'control' snacks, there were also differences in carbohydrate and fat content. Therefore, at least part of the significance of our results may be due to such differences in the snack macronutrient ratios. Additionally, the protein intake of our participants at baseline was relatively low (less than 1 g protein per kg of BM) [78] and thus, our results may not be generalizable to populations with normal protein intake. We performed a per-protocol analysis, which may have biased results due to the exclusion of participants that dropped out during the follow-up intervention period. Although this type of analysis better reflects intervention effects when performed optimally, the clinical applicability of this per-protocol approach is limited if participant compliance differs substantially from one cohort to another [79]. Lastly, multi-frequency bioelectrical impedance was used to measure body composition. This method is not as precise as dual-energy x-ray absorptiometry (the gold standard) and the clinical significance of small differences has not yet been determined [80]; however, previous studies have shown that it is a valid and reliable method [35–37].

5. Conclusions

In summary, long-term consumption of a soy-enriched high protein snack replacement significantly decreased appetite, ad libitum energy intake, and improved body composition by decreasing BFP, and increasing SMM in women with NWO. Our findings underline clinical applicability that consuming a soy-enriched high protein snack replacement may provide a practical approach in controlling caloric intake and improving body composition in cohorts with NWO.

Author Contributions: N.H., M.A. and G.M. conceived and designed research. N.H., Z.S. and N.M. conducted experiments. N.H. contributed new reagents or analytical tools. N.C. and R.B. analyzed data. D.A.-L. and R.B. wrote the manuscript. A.W., M.N., R.B., D.A.-L., O.A. and F.D. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by a grant (97-01-21-18694) from Shiraz University of Medical Sciences.

Acknowledgments: We would like to express our sincere appreciation to all of the study participants. Additionally, we would like to thank Morteza Zare from the Nutrition Research Center at Shiraz University of Medical Sciences for statistical assistance on this work.

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

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Dieting and Disinhibited Eating Patterns in Adult Women with Normal Body Weight: Does Rumination Matter?

Justyna Waliłko [1,†], Paulina Bronowicka [1,†], Jinbo He [2] and Anna Brytek-Matera [3,*]

[1] Institute of Psychology, University of Wroclaw, 50-527 Wroclaw, Poland; 291938@uwr.edu.pl (J.W.); 290259@uwr.edu.pl (P.B.)
[2] School of Humanities and Social Science, The Chinese University of Hong Kong, Shenzhen 518172, China; hejinbo@cuhk.edu.cn
[3] Faculty of Psychology in Katowice, SWPS University of Social Sciences and Humanities, 40-326 Katowice, Poland
* Correspondence: abrytek-matera@swps.edu.pl or anna.brytek-matera@uwr.edu.pl
† These authors contributed equally to this work.

Abstract: Dieting and disinhibited eating patterns are presented in both clinical and nonclinical samples. Repetitive negative thinking (i.e., rumination) may lead to maladaptive eating behaviors. While numerous studies have focused on dieting and disinhibited eating behaviors in clinical samples, less is known about these behaviors in nonclinical samples with normal body weight. Therefore, the present study aimed to explore how dieting, uncontrolled eating and emotional eating are related to rumination in adult women with normal body weight. One hundred eighty-eight women (M_{age} = 29.46 ± 8.94; M_{BMI} = 23.16 ± 4.04) were involved in the current study. The Eating Attitudes Test, the Three-Factor Eating Questionnaire-R18 and the Perseverative Thinking Questionnaire were administered to the participants. The results showed that repetitive negative thinking was a partial mediator in the relationship between dieting and uncontrolled eating, as well as in the relationship between dieting and emotional eating. Targeting repetitive negative thinking may be important for reducing disinhibited eating patterns in women with normal body weight.

Keywords: dieting; emotional eating; uncontrolled eating; rumination; weight; restraint theory

Citation: Waliłko, J.; Bronowicka, P.; He, J.; Brytek-Matera, A. Dieting and Disinhibited Eating Patterns in Adult Women with Normal Body Weight: Does Rumination Matter?. *Nutrients* **2021**, *13*, 2475. https://doi.org/10.3390/nu13072475

Academic Editors: Sílvia Rocha-Rodrigues, José Afonso and Monica Sousa

Received: 27 May 2021
Accepted: 17 July 2021
Published: 20 July 2021

1. Introduction

Dieting is defined as the intentional effort to control caloric intake by avoiding food consumption in order to maintain or lose weight [1]. There are three main categories of dietary strategies to promote weight loss: (1) diets based on the manipulation of macronutrient content (i.e., low-fat diet, low-carbohydrate diet); (2) diets based on the restriction of specific foods or food groups (i.e., gluten-free diet, plant-based diet); and (3) diets based on the manipulation of timing (i.e., fasting) [2]. Many women, especially adolescent girls and young women, diet because of body dissatisfaction and the desire to change both body size and shape [3].

1.1. Disinhibited Eating Patterns

Theoretical and empirical evidence suggests that dietary restraint may lead to an excessive intake of food [4–6], disinhibited eating and overconsumption [7]. Disinhibition is the tendency to overeat in response to different stimuli, and can occur in a variety of circumstances (e.g., exposure to palatable food cues, emotional distress) [8]. Dietary disinhibition was found to be associated with a loss of control, eating in response to emotional distress and overeating [7]. Uncontrolled eating (sometimes called external eating) is characterized by overconsumption, with the feeling of loss of control, of the amount of food intake products in response to external food cues (the sight and smell of attractive food) instead of internal cues such as the level of hunger and satiety [9], while

emotional eating means the tendency to overeat in response to negative emotions (e.g., anxiety) [10].

Uncontrolled eating and emotional eating can be conceptualized as a form of disinhibition, where individuals feel compelled to eat in response to external or internal (emotional) cues and lack control over inhibiting this behavior [11]. Both uncontrolled eating and emotional eating are behaviors mostly exhibited by women, including normal-weight, overweight, and underweight individuals [12,13]. Cross-sectional studies [14] and longitudinal data [15] have found that in women with normal body weight, the interaction between restrained and disinhibited eating (uncontrolled eating, emotional eating) predicts food intake, with dietary restraint moderating the impact of uncontrolled eating.

1.2. Repetitive Negative Thinking (Rumination)

The control of food intake and the preoccupation with eating may cause intrusive food-related thoughts. Repetitive negative thinking, commonly called rumination, is a cognitive process involving repetitive thoughts about one's problems or negative experiences and is characterized by repetitiveness, intrusiveness and difficulty in disengaging from this negative thinking [16]. Rumination has been implicated in mood and anxiety disorders [17,18], and there is a growing body of research on rumination in relation to eating disorder pathology [19–21]. Disorder-specific thoughts, focusing particularly on the control of eating, weight and shape [22,23], are presented in women with eating disorders and are likely to contribute to the increased severity in eating disorder symptomatology [24]. There are surprisingly few studies examining the process of rumination in normal-weight individuals.

Previous research has found effects of environmental food cues and food-related thoughts on eating behavior in healthy young women as well as women with eating disorders and overweight individuals [25,26]. Normal-weight individuals exhibit decreased susceptibility to food cue exposure, relative to overweight individuals [26]. They reported decreased desire for high-calorie food after exposure to tempting food words, whereas overweight individuals reported increased craving. In addition, normal-weight individuals respond to food-related cues with cognitions associated with eating less after a "thought–shape fusion" induction (imagining eating high-calorie food leads individuals to feel fatter, and to perceive weight gain and moral wrongdoing). In contrast, overweight individuals appear to respond to food-related cues with increased food desire with the absence of cognitions that may motivate reduced food consumption of high-calorie foods [26].

1.3. Objective of the Current Study

The evidence of negative repetitive thinking in the context of disinhibited eating patterns in normal-weight individuals is limited. Therefore, the present study aimed to examine the mediator effect of rumination on the relationship between dieting and both uncontrolled eating and emotional eating in a community sample of adult women.

Based on restraint theory [4] and taking into consideration that repetitive negative thinking is associated with disordered eating behaviors [21,27] and disinhibited eating patterns in normal-weight samples [28], the following hypotheses were proposed:

H1: A high level of concern about dieting is associated with more frequent uncontrolled eating and emotional eating, and higher levels of rumination.

H2: The relationship between a high level of concern about dieting and more frequent disinhibited eating patterns is mediated by higher levels of negative repetitive thinking among women with the body mass index (BMI) range from 18.5 to 24.99 kg/m^2 (normal weight) [29].

Dietary restraint, which assesses both concern with dieting and weight fluctuations, could in part explain failures in maintaining normalized eating [26]. Thus, a more detailed knowledge of variables related to dieting may point out targets for improved eating interventions to increase normalized eating behavior in normal-weight individuals.

2. Materials

2.1. Participants

Participants were 21- to 55-year-old normal-weight women (M_{age} = 29.46 ± 8.94; M_{BMI} = 23.16 ± 4.04). One hundred eighty-eight community adult women participated in the present study (response rate = 83%). The purposive sampling method was used in the present study. Female participants met the following inclusion/exclusion criteria: (1) age between 21 and 55 years; (2) non-vegetarian and tries to eat healthily on a regular basis, but also enjoys eating junk food and snacks; (3) not allergic to major groups of food (e.g., gluten allergy); (4) no current or past-year history of major depression (which can affect appetite and weight) or one of three major eating disorders (anorexia, bulimia, binge eating disorder); (5) no abuse of drugs or alcohol (which can affect appetite and weight); (6) and no lifetime history of psychosis, mania, hypomania, bipolar disorders, or suicidality, defined using the Mini International Neuropsychiatric Interview (MINI) [30]. The electronic version of the MINI was used through the Nview Health portal (portal.nviewhealth.com). The participants, with an identifying number, filled out the answers themselves. Detailed instructions on the online interview process were given. A PDF of generated participant data was automatically sent to the interviewer's email address (A.B.-M.). Only individuals with a low total score on the interview sections were invited to participate in the present study. The screening inclusion/exclusion criteria were made at the baseline of the study. The baseline study was conducted via an online platform (SurveyMonkey®, San Mateo, CA, USA) and consisted of three elements: first, confirming eligibility against inclusion and exclusion criteria; second, consent; and third, data collection.

The current study is part of the Harmonia 10 research project funded by the National Science Centre (Poland; grant no. 2018/30/M/HS6/00022), which focuses on the impact of negative affect on eating behavior in ecological and natural sittings. The first phase of the Harmonia 10 research project contained several self-administered questionnaires completed in a natural setting and assessed demographic variables and individual differences (eating behaviors, disordered eating behaviors, anxiety, negative affect, affect regulation, rumination).

2.2. Procedure

Collecting research data through traditional paper-and-pencil methods was not possible during the COVID-19 pandemic, so an online survey was used in the present study (SurveyMonkey®). Only community members who met study eligibility criteria participated in the present study. The participants were recruited through university centers, health clubs, fitness gyms (using flyers) and through social media such as Facebook. Participants received notice about the research with an announcement including all necessary information about the study. They received an email with the online link to the study. They were invited to visit a website that directed them to the consent form, information form and questionnaires. All participants offered their informed consent before starting the survey (by ticking a respective box at the first page of the online survey) and responded voluntarily to the survey. Women were informed about the anonymity of the study, and about the possibility of resignation at any stage of the study.

The research protocol of the Harmonia 10 research project was designed and conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Research Ethics Committee at the Institute of Psychology, University of Wroclaw, Poland (decision number IPE 0019).

2.3. Methods

2.3.1. The Eating Attitudes Test

The Eating Attitudes Test (EAT-26) [1,31] is one of the most widely used screening instruments to assess symptoms of eating disorders. It has also been used in nonclinical samples to detect characteristics and concerns related to eating disorders [32]. The EAT-26 contains 26 items in three subscales: (1) dieting (the pathological avoidance of fattening

foods and body shape preoccupation); (2) bulimia and food preoccupation (bulimic behaviors and thoughts about food); and (3) oral control (pertains to self-control regarding eating and perceived pressure from others to gain weight) [1]. A total score of 20 or more indicates a risk of eating disorders (e.g., anorexia nervosa, bulimia nervosa and binge eating disorder). We used the Polish version of the EAT-26 [31] ($\alpha_{\text{total score of the EAT-26}} = 0.80$). In the present study, internal reliabilities for the three subscales were as follows: dieting Cronbach's $\alpha = 0.811$, bulimia and food preoccupation Cronbach's $\alpha = 0.693$ and oral control Cronbach's $\alpha = 0.520$. The total EAT-26 Cronbach's alpha was 0.834. In the present study, we only used one subscale from the EAT-26, dieting, for assessing dietary restriction.

2.3.2. The Three-Factor Eating Questionnaire-R18

The Three-Factor Eating Questionnaire-R18 (TFEQ-R18) [33,34] is one of the most widely used measures to assess eating behaviors. Originally, the TFEQ was designed to measure the cognitive and behavioral components of eating in obese populations. It contained 51 items aggregated into three scales: cognitive restraint, disinhibition and hunger [15]. The TFEQ was modified by Karlsson et al. [33], who abbreviated it to 18 items (TFEQ-R18) and reconceptualized disinhibition as uncontrolled eating and hunger as emotional eating. The TFEQ-R18 has been found to be valid in the general female sample [13]. The TFEQ-R18 contains three subscales: (1) cognitive restraint (an individual's tendency to restrict dietary intake to control weight); (2) uncontrolled eating (an individual's tendency to eat in response to external food cues with episodes of loss of control and overeating); and (3) emotional eating (an individual's tendency to eat in response to negative emotion). We used the Polish version of the TFEQ-R18 [34]. The reliability estimates for each subscale were excellent: $\alpha_{\text{Uncontrolled Eating}} = 0.84$, $\alpha_{\text{Emotional Eating}} = 0.86$ and $\alpha_{\text{Cognitive Restraint}} = 0.78$ [34]. In the present study, internal reliabilities were as follows: uncontrolled eating Cronbach's $\alpha = 0.871$, emotional eating Cronbach's $\alpha = 0.735$ and cognitive restraint Cronbach's $\alpha = 0.800$. In the present study, we used two subscales of the TFEQ-R18, uncontrolled eating and emotional eating, for assessing disinhibited eating patterns.

2.3.3. The Perseverative Thinking Questionnaire

The Perseverative Thinking Questionnaire (PTQ) [35,36] measures repetitive negative thinking. It contains 15 items in three subscales: (1) the core characteristics of repetitive negative thinking: (a) its repetitive nature, (b) its intrusive nature and (c) the difficulty in disengaging; (2) the perceived unproductiveness of repetitive negative thinking; and (3) capturing mental resources. We used the Polish version of the PTQ [36]. The internal consistency of the PTQ was low but adequate ($\alpha = 0.64$–0.92) [36]. In the present study, internal reliabilities were as follows: the core characteristics of repetitive negative thinking Cronbach's $\alpha = 0.943$, unproductiveness of repetitive negative thinking Cronbach's $\alpha = 0.835$ and capturing mental resources Cronbach's $\alpha = 0.855$. In the present study, we used three subscales of the PTQ for assessing repetitive negative thinking (rumination): the core characteristics of repetitive negative thinking, the unproductiveness of repetitive negative thinking and capturing mental resources.

3. Results

3.1. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). We used the PROCESS version 3.5 macro by Hayes [37] to test the hypothesized mediation models between dieting, rumination and both emotional eating and uncontrolled eating. The results were reported as indirect and direct effects of measured variables. The indirect effect represents the relationship between dieting and both emotional and uncontrolled eating through rumination. A simple pathway analysis with 10,000 bootstrapping samples was conducted. To determine the significance of the mean indirect effects, 95% confidence intervals (CIs) were obtained. If the CI did not

contain zero, the indirect effect was considered statistically significant at the 0.05 level. The direct effect represents the relationship between dieting and both emotional eating and uncontrolled eating before adjustment for rumination. The regression analyses were performed for each path, regressing the predictor (dieting; one subscale of the EAT-26) on both the outcome (emotional eating and uncontrolled eating; two subscales of the TFEQ-R18) and the mediator (repetitive negative thinking; three subscales of the PTQ). We estimated the direct effects of the predictor on the mediator (path *a*), the mediator on the outcome (path *b*) and the predictor on the outcome (path *c′*), and the indirect effect (mediation) of the predictor on the outcome via the mediator (path $a \times b$) (Figure 1). Separate pathway analyses were conducted for each of the three subscales of the PTQ.

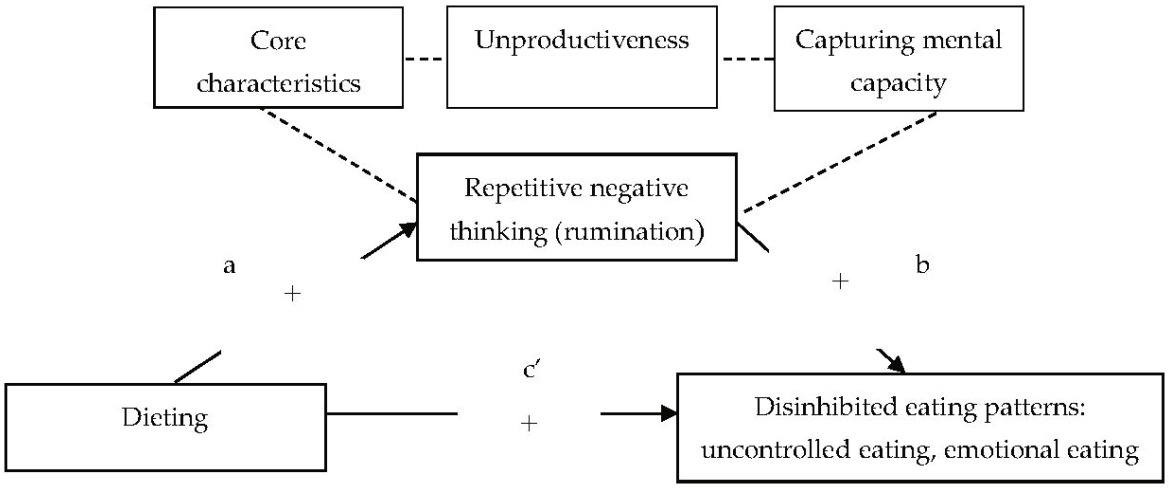

Figure 1. Hypothesized mediation model for relationship between dieting, rumination and both emotional eating and uncontrolled eating in women with normal body weight. Note: + positive prediction.

3.2. Correlations between Study Variables

Prior to the mediational analyses, Pearson correlation coefficients were calculated to examine associations of study variables (Table 1).

Table 1. Correlations of study variables.

Variable	1	2	3	4	5	6
1. Core characteristics of RNT	-					
2. Unproductiveness of RNT	0.75 ***	-				
3. Capturing mental capacity	0.79 ***	0.84 ***	-			
4. Dieting	0.18 *	0.21 **	0.27 ***	-		
5. Uncontrolled eating	0.30 ***	0.32 ***	0.32 ***	0.41 ***	-	
6. Emotional eating	0.31 ***	0.33 ***	0.37 ***	0.33 ***	0.61 ***	-

Note: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.005$; RNT: repetitive negative thinking.

3.3. Mediation Analysis for Relationship between Dieting, Rumination and Disinhibited Eating Patterns in Women with Normal Body Weight

In order to assess whether the relationship between dieting and disinhibited eating patterns is mediated by rumination, mediation analyses were used (Table 2). The results indicated that for:

Table 2. Mediation analysis for relationship between dieting, rumination and disinhibited eating patterns in women with normal body weight.

	Uncontrolled Eating		Emotional Eating	
	Direct Effect	**Indirect Effect**	**Direct Effect**	**Indirect Effect**
Core characteristics of RNT	Effect = 0.83	Effect = 0.09	Effect = 0.31	Effect = 0.05
	SE = 0.14	BootSE = 0.05	SE = 0.07	BootSE = 0.02
	95% CI [0.54, 1.13]	95% CI [0.01, 0.21]	95% CI [0.16, 0.45]	95% CI [0.00, 0.11]
Unproductiveness of RNT	Effect = 0.81	Effect = 0.12	Effect = 0.30	Effect = 0.06
	SE= 0.14	BootSE = 0.05	SE = 0.07	BootSE = 0.02
	95% CI [0.52, 1.10]	95% CI [0.02, 0.24]	95% CI [0.15, 0.44]	95% CI [0.01, 0.12]
Capturing mental capacity	Effect = 0.79	Effect = 0.02	Effect = 0.27	Effect = 0.08
	SE = 0.15	BootSE = 0.02	SE = 0.07	BootSE = 0.02
	95% CI [0.49, 1.09]	95% CI [0.05, 0.26]	95% CI [0.13, 0.42]	95% CI [0.03, 0.15]

Note: RNT: repetitive negative thinking; SE: standard error; CI: confidence interval.

1. Core characteristics of repetitive negative thinking: The path *a* model was significant, showing that greater dieting significantly predicted higher levels of core characteristics of repetitive negative thinking (β = 0.21, 95% CI [0.04, 0.37]). Path *b* was also significant, indicating that higher levels of core characteristics of repetitive negative thinking significantly predicted more frequent uncontrolled eating (β = 0.46, 95% CI [0.20, 0.72]) and emotional eating (β = 0.24, 95% CI [0.12, 0.32]). Path *c* was also significant, demonstrating that greater dieting significantly predicted more frequent uncontrolled eating (β = 0.83, 95% CI [0.54, 1.13]) and emotional eating (β = 0.31, 95% CI [0.16, 0.45]) when core characteristics of repetitive negative thinking were not included in the model.

2. Unproductiveness of repetitive negative thinking: The path *a* model was significant, showing that greater dieting significantly predicted higher levels of unproductiveness of repetitive negative thinking (β = 0.25, 95% CI [0.08, 0.42]). Path *b* was also significant, indicating that higher levels of unproductiveness of repetitive negative thinking significantly predicted more frequent uncontrolled eating (β = 0.47, 95% CI [0.23, 0.72]) and emotional eating (β = 0.25, 95% CI [0.12, 0.37]). Path *c* was also significant, demonstrating that greater dieting significantly predicted more frequent uncontrolled eating (β = 0.81, 95% CI [0.52, 1.10]) and emotional eating (β = 0.30, 95% CI [0.15, 0.44]) when unproductiveness of repetitive negative thinking was not included in the model.

3. Capturing mental capacity: The path *a* model was significant, showing that greater dieting significantly predicted higher levels of capturing mental capacity (β = 0.92, 95% CI [0.45, 1.38]). Path *b* was also significant, indicating that higher levels of capturing mental capacity significantly predicted more frequent uncontrolled eating (β = 0.15, 95% CI [0.06, 0.24]) and emotional eating (β = 0.09, 95% CI [0.05, 0.13]). Path *c* was also significant, demonstrating that greater dieting significantly predicted more frequent uncontrolled eating (β = 0.79, 95% CI [0.49, 1.09]) and emotional eating (β = 0.27, 95% CI [0.13, 0.42]) when capturing mental capacity was not included in the model.

4. Discussion

Consistent with our first hypothesis, we found that a high level of concern about dieting was related to more frequent uncontrolled eating and emotional eating, and higher levels of core characteristics of repetitive negative thinking, unproductiveness of repetitive negative thinking and capturing mental capacity in a community sample of adult women. Previous studies found that an increased conscious control of eating behavior (i.e., dietary restraint) was associated with obsessive thoughts about forbidden foods [38] and disinhibition [39]. Emotional eating, as a 'disinhibitor', requires prior inhibition (i.e., restraint) by definition [40]. Therefore, it can be supposed that individuals with disinhib-

ited eating might exert more dietary restraint at times in order to compensate for their greater disinhibition [41]. The fact that restrained eating is a cause of emotional eating has still not been resolved [42]. Some researchers [43] argue that the impact of overeating is limited by dietary restraint. We could suppose that adult women who diet may engage in emotional eating to cope with negative emotions and thoughts, which in the long term is a maladaptive emotion regulation strategy [44]. On the other hand, increased uncontrolled and emotional eating may be a consequence of women's dietary restriction, whereas the abstinence from food (dieting) may be the means of regulating the negative effect. This should be explored in future studies.

It is worth pointing out that although there is a strong relationship between uncontrolled eating and emotional eating and they can be conceptualized as different forms of disinhibited eating patterns, it has been empirically shown [45] that they refer to independent constructs. An essential difference between uncontrolled eating and emotional eating is that uncontrolled eating is attributed to a heightened sensitivity to external food cues, regardless of the internal state of hunger and satiety (externality theory), while emotional eating is attributed to a confusion of physiological states accompanying negative emotions with physiological correlates of hunger and satiety (psychosomatic theory) [45]. However, despite their differences, in both uncontrolled eating and emotional eating, individuals' misperception of their internal state prior to eating is considered to be a causal factor in overeating [45].

Consistent with our second hypothesis, the results showed that repetitive negative thinking (core characteristics of repetitive negative thinking, unproductiveness of repetitive negative thinking and capturing mental capacity) mediated the positive relationships between dieting and both uncontrolled eating and emotional eating in normal-weight women. Thus, rumination could be a cognitive mechanism between dieting and disinhibited eating patterns in these women. Cognitive distortions relevant to food intake may be implicated in difficulties with normalized eating [26]. It could be possible that beliefs about rumination act as an internal control mechanism that strengthens ruminative thinking [46], which may be one factor accounting for its persistence in normal-weight women. This hypothesis needs to be tested in future studies.

Our study may indicate that women with normal body weight do not suppress their negative repetitive thoughts, and that this consequently leads to disinhibited eating patterns. Rumination leads to unhealthy eating behavior [47]. Perhaps, the more time women spend dieting, the more likely they are to avoid food-related thoughts over time (an additive effect of food thought suppression) [48].

Our results are in line with the restraint theory [4], showing that both uncontrolled eating and emotional eating are caused by dieting, rather than preceding dieting (this is in contrast with the psychosomatic theory and externality theory).

In future research, it would be interesting to explore the impact of trying to suppress eating-related thoughts on subsequent eating-related thoughts in women with normal body weight. Findings have shown that individuals practicing dietary restraint and presenting disinhibited eating patterns used thought suppression (the conscious attempt to not think about something) [49]. Moreover, individuals practicing dietary restraint who tend to overeat try to suppress thoughts about food more often, but if they do, they think about food more often afterwards (a rebound effect following a thought suppression task about food). Some studies [50] have shown that food thought suppression increased food-related thoughts, notwithstanding weight status [50], and increased food intake among undergraduate women [51]. Future studies should take the rebound effect following thought suppression for both subsequent thoughts and eating behavior in normal-weight women into consideration. In addition, future studies should include other potential mediators (e.g., weight regulation, weight change, food choices) of relationships between dieting and disinhibited eating patterns.

In conclusion, increasing our understanding of the link between dieting and rumination could potentially inform future prevention programs intended to reduce disinhibited eating

patterns in women with normal body weight. In addition, psychological intervention should match with individuals' eating patterns. Furthermore, the type of disinhibited eating patterns ("habitual" disinhibition—the susceptibility to overeat in response to daily life circumstances, "emotional" disinhibition—the tendency to overeat in response to emotional states and "situational" disinhibition—the susceptibility to overeat in response to specific environmental cues [8]) would be worth investigating further to plan for future intervention.

Some limitations of the current study should be noted. Firstly, the cross-sectional nature of the study means that causal inferences cannot be made. To better examine causality, longitudinal or experimental designs should be used. Therefore, future studies should utilize longitudinal or laboratory-based paradigms to further examine the associations among dieting, rumination and maladaptive eating behaviors. Secondly, the widely used TFEQ-R18 also has restrictions; for example, the use of three items to measure emotional eating might not be sufficiently reliable or valid to reflect emotional eating. In a future study, other measures of emotional eating (e.g., the Emotional Eating Scale [52], the Dutch Eating Behavior Questionnaire [53]) should be used to confirm the findings of the current study. Finally, self-report measures may be affected by the common methods bias.

5. Conclusions

Our findings showed that dieting and rumination (core characteristics of repetitive negative thinking, unproductiveness of repetitive negative thinking and capturing mental capacity) were positively associated with each other and both predicted higher levels of uncontrolled eating and emotional eating. The effect of dieting on both uncontrolled eating and emotional eating was mediated by repetitive negative thinking (its core characteristics, its unproductiveness and capturing mental capacity). Some research has explored the impact of trying to suppress eating-related thoughts on subsequent thoughts about eating. Our results suggest that rumination should be taken into consideration in psychological intervention for decreasing disinhibited eating patterns in women with normal body weight.

Author Contributions: Conceptualization—A.B.-M.; project administration—A.B.-M.; formal analysis—A.B.-M.; investigation—J.W. and P.B.; writing—original draft—A.B.-M., J.W. and P.B.; writing—review and editing—J.H. and A.B.-M.; resources—A.B.-M.; funding acquisition—A.B.-M.; methodology—A.B.-M.; supervision—A.B.-M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Science Centre (NCN), Poland, under the Harmonia 10 research project (no. 2018/30/M/HS6/00022) awarded to A.B.-M.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Research Ethics Committee at the Institute of Psychology, University of Wroclaw, Poland (no. IPE 0019).

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

Data Availability Statement: The dataset used during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript or in the decision to publish the results.

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Type of Physical Training and Selected Aspects of Psychological Functioning of Women with Obesity: A Randomised Trial

Monika Bąk-Sosnowska [1], Magdalena Gruszczyńska [1,†], Damian Skrypnik [2,*,†], Sławomir Grzegorczyn [3], Joanna Karolkiewicz [4], Marzena Ratajczak [5], Edyta Mądry [6], Jarosław Walkowiak [7] and Paweł Bogdański [2]

1 Department of Psychology, Chair of Social Sciences and Humanities, School of Health Sciences in Katowice, Medical University of Silesia in Katowice, 40-055 Katowice, Poland; monika.bak-sosnowska@sum.edu.pl (M.B.-S.); mgruszczynska@sum.edu.pl (M.G.)
2 Department of Treatment of Obesity, Metabolic Disorders and Clinical Dietetics, Poznan University of Medical Sciences, 61-701 Poznań, Poland; pbogdanski@ump.edu.pl
3 Department of Biophysics, School of Medicine in Zabrze, Medical University of Silesia in Katowice, 40-055 Katowice, Poland; grzegorczyn@sum.edu.pl
4 Department of Food and Nutrition, Poznan University of Physical Education, 61-701 Poznań, Poland; karolkiewicz@awf.poznan.pl
5 Department of Anatomy, Poznan University of Physical Education, 61-701 Poznań, Poland; mratajczak@awf.poznan.pl
6 Department of Physiology, Poznan University of Medical Sciences, 61-701 Poznań, Poland; edytamadry@gmail.com
7 Department of Paediatric Gastroenterology and Metabolic Diseases, Poznan University of Medical Sciences, 61-701 Poznań, Poland; jarwalk@ump.edu.pl
* Correspondence: damian.skrypnik@gmail.com
† Magdalena Gruszczyńska and Damian Skrypnik equally contributed to the paper and are joint second authors.

Citation: Bąk-Sosnowska, M.; Gruszczyńska, M.; Skrypnik, D.; Grzegorczyn, S.; Karolkiewicz, J.; Ratajczak, M.; Mądry, E.; Walkowiak, J.; Bogdański, P. Type of Physical Training and Selected Aspects of Psychological Functioning of Women with Obesity: A Randomised Trial. *Nutrients* **2021**, *13*, 2555. https://doi.org/10.3390/nu13082555

Academic Editors: Sílvia Rocha-Rodrigues, José Afonso and Monica Sousa

Received: 27 May 2021
Accepted: 22 July 2021
Published: 26 July 2021

Abstract: Objective: We conducted a prospective randomised trial to assess whether a specific type of regular physical training performed by women with obesity is related to obtaining specific psychological benefits. Methods: Forty-four women qualified for the study and were divided into two groups. The applied intervention consisted of regular three-month physical exercises in the form of endurance training (group A) or endurance strength training (group B). Initially, and after the completed intervention, we examined anthropometric measurements and the level of: stress (PSS-10), general self-esteem (SES), body self-report (BSQ–34, FRS), and behaviours associated with diet (TFEQ-18). Results: As a result of the intervention, both groups had significantly lower anthropometric parameters and FRS scores with regard to the current figure (gr. A:δ FRS CS -0.90 ± 0.83, $p < 0.001$; gr. B:δ FRS CS -0.41 ± 0.50, $p = 0.01$) and BSQ –34 results (gr. A:δ BSQ–34 -14.90 ± 13.5, $p = 0.001$; gr. B:δ BSQ–34 $- 18.64 \pm 25.4$, $p = 0.01$). Additionally, an increase in cognitive restraint (δ TFEQ–18 CR1.65 $\pm$ 2.06, $p = 0.01$) and a decrease in emotional eating (δ TFEQ–18 EE -0.82 ± 1.28, $p = 0.01$) were observed in group B. There were no between-group differences in terms of the magnitude of changes achieved due to the intervention, except for asignificant improvement in the perception of their current figure (FRS) (δ FRSCS -0.90 ± 0.83, $p = 0.03$) in group A. Conclusions: Regular physical activity over a three-month period by women with obesity promotes the perception of their own body as slimmer and lowers body shape concerns. The change in body shape perception was more pronounced under the influence of endurance training than endurance strength training. Trial registration: ClinicalTrials.gov ID NCT04793451.

Keywords: obesity; physical activity; psychological aspects; body image

1. Introduction

Physical activity is recommended as one of the key methods of reducing excess body weight, in addition to caloric restriction. It helps to improve control of type 2 diabetes

(T2D) [1] and cardiovascular diseases (CVD) [2], reduces serum glucose levels and blood pressure, improves overall quality of life [3], reduces insulin resistance [4], serum levels of proinflammatory cytokines [5] and the risk of cancer [6], and helps to maintain physical fitness [7]. The benefits of physical activity are observed in people with obesity even when they do not significantly reduce their body weight, as maintaining a high level of cardiorespiratory fitness reduces the risk of obesity-related diseases [8]. Additionally, it helps older patients to maintain the appropriate telomere length as an indicator of cellular biological ageing [9]. However, this phenomenon applies to regular patterns of high physical activity but not occasional physical activity, which is more strongly associated with a number of anthropometric and mortality outcomes [10]. Some studies show that, although physical activity brings measurable health benefits regardless of gender, positive health outcomes can be observed in women even for low (<3 MET) or moderate (3–6 MET) intensity exercise performed for 15 min a day [11].

In the case of overweight people (BMI 25–29.9 kg/m^2), 45 to 60 min of moderate physical activity most days a week is recommended, while for people with obesity (BMI $\geq$ 30 kg/m^2), the recommended amount is 60 to 90 min [12]. It is estimated that regular aerobic physical activity for less than 150 min a week may only prevent further weight gain, but slight weight loss (2–3 kg) requires 150–225 min of physical activity a week. Weight reduction at the level of 5–7.5 kg may be achieved by regular physical activity for more than 225 min (to 420 min) weekly. In order to maintain the achieved results, moderate physical activity for 200–300 min a week is recommended [13].

Endurance training is recommended for people with obesity; however, it brings satisfactory results (at the level of a 5–15% reduction ininitial body weight) only in combination with caloric restriction [14]. Even if it does not bring about significant weight loss, it improves glucose and lipid metabolism in people who previously led a sedentary lifestyle [15]. In turn, strength training is considered ineffective in reducing excess body weight. However, it should be noted that it brings measurable health benefits, such as a reduction inadipose tissue, increased muscle mass and an improvement in the lipid profile [16]. In women with abdominal obesity who performed endurance training or mixed endurance strength training three times a week for three months, some beneficial effects were observed in terms of anthropometric parameters, body composition, physical fitness and cardiovascular function [17], liver [18] and kidney parameters [19], as well as mineral balance [20], functional skills and back pain [21].

Physical activity is beneficial both for somatic functions and mental health [22], as well asforthe efficiency of psychological mechanisms, even in terms of motivation, self-efficacy or self-regulation [9]. This is especially important in people with obesity, as the disease is often accompanied by depression, decreased quality of life and self-esteem, appearance-related embarrassment, withdrawal from social relationships and other psychological problems [23]. The relationship between the perception of one's own appearance and self-esteem, overall mental wellbeing and eating behaviour is important, especially in women [24–26]. Scientific reports confirm the psychological benefits of endurance training in people with excess body weight, which can lead to lower levels of depression, improved moods and overall self-esteem [27]. Similar effects can be brought about by less intensive Pilates training [28] or yoga [29]. On the other hand, a meta-analysis comparing the results of 17 studies on the effect of strength training on the mental status of people with obesity did not show any convincing evidence of such an effect. A small effect, however, was observed with regard to aspects such as self-esteem, inhibition, anxiety or depression [30].

A comparison of psychological benefits resulting from both types of physical training in a group of people with obesity has barely been studied to date. The available results are mainly focusedon the level of depression, anxiety and quality of life and indicate no significant differences between groups performing endurance and endurance strength training [31,32]. Therefore, the aim of our study was to compare the influence of endurance and endurance strength training on stress, self-esteem, body-esteem and eating behaviour in women with obesity. The innovative nature of the study is related to the use of acompar-

ative intervention model (endurance vs. endurance strength) and analysis of the impact of these two training models on the psychological state of women with obesity.

2. Materials and Methods

2.1. Trial Information

The study was a part of an interdisciplinary research project for which consent from the Bioethics Committee of the Medical University in Poznań was obtained (No. 1077/12 with supplement No. 753/13). The study took place between December 2012 and December 2017.

This work was supported by the National Science Centre, Poland, Grant No. 2014/13/B/NZ7/02209 and the Faculty of Medicine I, Poznan University of Medical Sciences, Poland, Grant for Young Scientists [grant number 502-14-01119172-41123].

The study has been registered as a clinical trial on ClinicalTrials.gov under the ID NCT04793451. The trial protocol can be accessed at https://clinicaltrials.gov/ct2/show/NCT04793451 (accessed on: 22 July 2021).

2.2. Participants

The screening was conducted among 163 people registered in the outpatient clinic of the Department of Internal Medicine, Metabolic Disorders, and Hypertension, University of Medical Sciences, Poznan, Poland. Inclusion criteria relevant to the issues presented in this study were as follows: (1) informed and written consent to participate in the study, (2) age between 18 and 65, (3) diagnosed obesity (BMI $\geq$ 30 kg/m^2), (4) stable body weight for a month before the study (acceptable deviation $\pm$ 1 kg). Exclusion criteria relevant for this paper were as follows: (1) intellectual disability, (2) mental illness, either currently or in medical records, (3) pregnancy, childbirth and lactation. The full list of criteria is presented in a publication by Skrypniket et al. [17]. A total of 44 people qualified for the study.

2.3. Study Design

The study was a prospective randomised trial. The study group was divided into two subgroups (group A and group B) using a randomisation list. Patient enrolment was undertaken by the physician. Patients were assigned to the study intervention by the physician using the subject's unique code according to the random allocation sequence generated by a computer. The groups did not differ in age, body mass, BMI, waist and hip circumference, or WHR [17]. Both groups participated in parallel physical training for three months. Group A (n = 22) took part in endurance training, while group B (n = 22) took part in endurance strength training. The subjects were informed of the need to keep their diet unchanged. Based on a dietary interview before and after the intervention and with the use of the specialised software Diet 2.0 for product consumption analysis, it was found that nutrient intake, total calories and caffeine intake during the study in both groups were at a constant, comparable level.

Initially and after three months of physical training, measurements of body weight, height, waist and hips were conducted [17]. The following psychological parameters were also assessed: stress level, overall self-esteem, body self-esteem and eating behaviour. The study was conducted by means of adiagnostic survey method using standardised questionnaires.

The Perceived Stress Scale (PSS-10) by Cohen, Kamarcki and Mermelstein, adapted in Polish by Juczyński and Ogińska-Bulik [33] examines general stress levels. It contains 10 questions concerning various subjective feelings associated with problems and personal experiences, behaviours and ways of dealing with them. The respondent marks the answers on a 4-point scale (0, never; 1, hardly ever; 2, sometimes; 3, quite often; 4, very often). The total score is the sum of all points, and the higher the score, the higher the examined person's stress intensity. The total score, after conversion in accordance with the guidelines of the authors into standardised units, is interpreted as low (1–4 sten), average (5–6 sten)

or high (7–10 sten). The Cronbach's alpha in our study was 0.81 before and 0.84 after the intervention.

The Rosenberg Self-Esteem Scale (SES) by Rosenberg, adapted in Polish by Dzwonkowska, Lachowicz-Tabaczek and Łaguna [34], examines levels of overall self-esteem. It is composed of 10 diagnostic statements to which respondents refer on a 4-point scale (1, I definitely agree; 2, I agree; 3, I have no opinion; 4, I do not agree; 5, I definitely do not agree). The final score is the sum of all points, and the higher the score, the higher the level of self-esteem of the examined person. The total score can be converted into stenunits and interpreted analogously in accordance with the guidelines of the authors, as described above. The Cronbach's alpha in our study was 0.82 before and 0.80 after the intervention.

The Body Shape Questionnaire (BSQ-34) by Cooper, Taylor, Cooper and Fairburn [35] examines the level of one's preoccupation with their own body shape. It is composed of 34 questions. Each question has a 6-point scale of answers thatreflects the frequency of experiencing the described situations by the subject in the last 30 days (1, never; 2, rarely; 3, sometimes; 4, often; 5, very often; 6, always). The sum of points gives the total score, making it possible to classify the examined person, in accordance with the authors' guidelines, in one of four categories describing the level of concern with their body shape: none, mild, moderate and considerable. The Cronbach's alpha in our study was0.94 before and 0.95 after the intervention.

The Figure Rating Scale (FRS) by Stunkart et al. [36] examines the perception of the size and shape of one's own body. It presents nine schematic silhouettes of men and women, from extremely thin to extremely obese. Subjects are asked to choose the silhouette that best reflects their current silhouette (CS) and ideal silhouette (IS) physical appearance. In the presented study, only female silhouettes were used due to the specificity of the study group.

The Three-Factor Eating Questionnaire-18 (TFEQ-18) by Karlsson and Persson, adapted in Polish by Brytek-Matera, Rogoza and Czepczor-Bernat [37] is used for measuring eating behaviour. The subject responds to the questionnaire using a 4-grade scale (0, definitely not; 1, rather not; 2, rather yes; 3, definitely yes). The scores are calculated separately for three sub-scales: cognitive restraint of eating (CR), uncontrolled eating (UE) and emotional eating (EE). The higher the score achieved in the given sub-scale, the greater the intensity of the tested behaviour is manifested. The average Cronbach's alpha for all sub-scales in our study was 0.71 before and 0.76 after the intervention.

The occurrence of any exclusion criteria (mentioned above) during the trial resulted in immediate cessation of participation in the study. There were no important changes to the methodology after the commencement of the trial. There were no changes to trial outcomes after the trial commenced.

2.4. Intervention

The intervention was planned for three months. During this time, the women participated in physical training three times a week. Each group had 36 training sessions. Training took place at a professional sports club, Sport Club City Zen in Poznań, and was conducted by a qualified and certified fitness instructor. Both groups were also under medical supervision. Training for both groups included the following exercises.

Group A: Endurance training on bicycle ergometers (Schwinn Evolution, Schwinn Bicycle Company, Boulder, CO, USA). Training sessions consisted of five minutes of warm-up, including stretching exercises of low intensity (50–60% of maximum heart rate), 45 min of training with an intensity of between 50 and 80% of maximum heart rate, five minutes of riding a cycloergometer without any additional load and five minutes of low-intensity stretching and breathing exercises.

Group B: Endurancestrength training that consisted of a five-minute warm-up (stretching exercises) of low intensity (50–60% of maximum heart rate), 20 min of strength exercises, 25 min of endurance exercises (50–80% of maximum heart rate), five minutes of cycling without any additional load and five minutes of stretching and breathing exercises.

As part of the strength exercises, the participants undertook some variable, repeated weekly exercises with a barbell and exercises with a gym ball. Endurance exercises were performedon a cycloergometer (Schwinn Evolution, Schwinn Bicycle Company, Boulder, CO, USA). Participants'heart rate during training was monitored using a Suunto Fitness Solution® (Suunto, Vantaa, Finland) device. Both types of training were comparable in exercise volume and varied only in the modality of the effort. The intervention ended when the last participant completed the training. The exact description of the intervention is presented in the publication by Skrypniket et al. [17].

2.5. Statistical Analysis

The Statistica13.1 statistical package was used. The normality of distributions was tested using a Shapiro–Wilk test. Statistical methods were used to compare groups for primary and secondary outcomes (in the area of analysed variables, e.g., the level of stress and self-esteem, body perception and eating behaviour), as well as for subgroup analysis (e.g., the difference between the initial and end value of variables, correlations between variables). The difference between the initial and end value (delta-δ) was calculated for each analysed variable. In addition, differences between the groups that implemented different forms of physical training were examined with regard to the size of change (δ) in the analysed variables. The relationships between changes of the studied parameters (delta results) were investigated using the correlation coefficient.

Parametric tests (t-Student tests for dependent and independent variables) were used for the analysis of variables with normal distribution, while nonparametric tests (a Wilcoxon signed-rank test for dependent variables and a Mann–Whitney U test for independent variables) were used in the case of a lack of normality in variables distribution. Differences in categorical variables were compared with Fisher's exact test. The strength of the relationship between variables was calculated using d-Cohen's effect size (for dependent variables) and g-Hedges' effect size (for independent variables). The adopted level of significance was $\alpha < 0.05$.

3. Results

During the intervention, six subjects, one from group A and five from group B, were withdrawn from the trial due to poor compliance, defined as attendance at fewer than 60% of physical training sessions. In the endurance training group, the average attendance at classes was 81.97%, while in the endurance and strength training group, it was 87.37%. There was no statistically significant difference between the groups of women in terms of attendance at classes ($p = 0.1652$). Thirty-eight patients (group A, $n = 21$; group B, $n = 17$) completed the study and underwent statistical analysis (Figure 1). The analysis was performed by the original assigned groups. No important harm or unintended effects in each group occurred.

Comparison of studied parameters between groups A and B before and after the intervention was performed. The results of the comparison are presented in Table 1.

In addition, in terms of nutrient, calorie and caffeine intake, there was no significant difference between groups A and B before and after the intervention (Table S1).

Before the intervention, groups A and B did not differ significantly in terms of any analysed variable. After the intervention, the only significant difference was emotional eating. In the endurance strength training group, as well as before the intervention, the level of this variable was higher than in the endurance training group. In the endurance training group, the average attendance at classes was 81.97%, while in the endurance and strength group, it was 87.37%. There was no statistically significant difference between the groups in terms of attendance at classes. The Mann–Whitney U test was $p = 0.1652$.

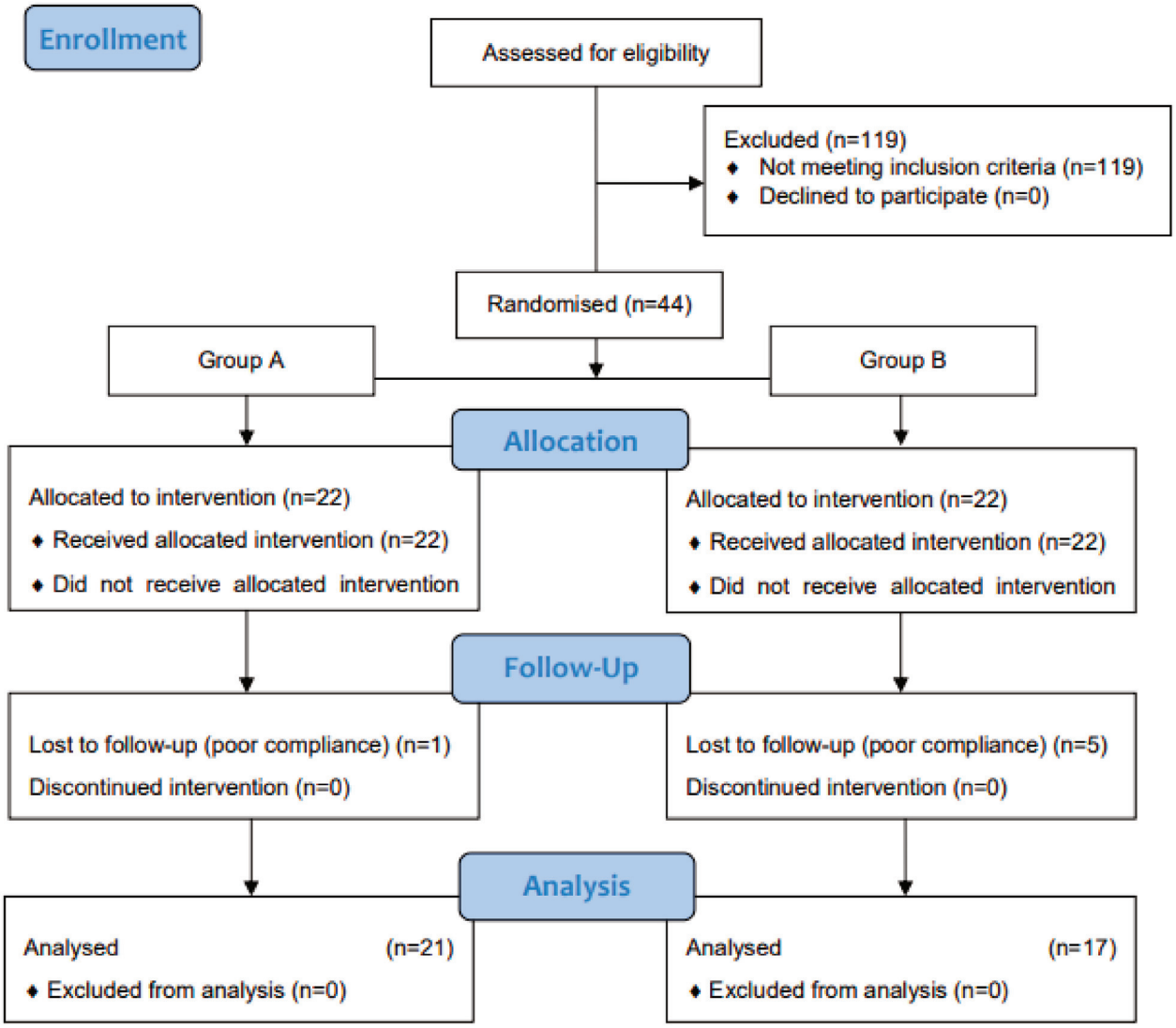


Figure 1. Flow diagram of study groups.

As a result of the intervention used, significant changes in anthropometric parameters were observed in both groups, which have already been presented in the paper by Skrypnik et al. [17]. With regard to psychological parameters after the intervention, there was a perception of the current figure as significantly slimmer and a significant reduction in level body shape concerns in both groups. Additionally, eating behaviour improved in group B, namely, the level of cognitive restraint increased, and emotional eating level decreased. Details are presented in the table below (Table 2).

The number of people experiencing lowstress levels increased, and the number of people experiencing average stress levels decreased in both groups. Moreover, the number of people experiencing highstress levels decreased in group B (Figure 2A,B). However, the distribution of results did not change significantly.

Table 1. Comparison of studied parameters between groups A and B before and after the intervention (anthropometric parameters have already been presented in the paper by Skrypnik et al., 2015 [17]).

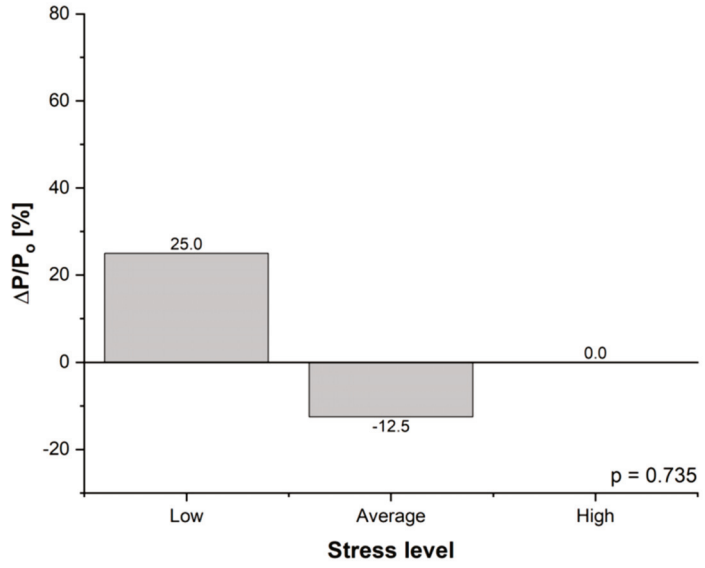
Variables		Group A before Intervention (n = 21)	Group B before Intervention (n = 17)	p	g	Group A after Intervention (n = 21)	Group B after Intervention (n = 17)	p	g
Body mass [kg]		91.7 ± 11.8	94.5 ± 13.4	0.325	0.2234	89.5 ± 11.8	91.8 ± 13.7	0.628	0.1814
BMI [kg/m^2]		35.2 ± 3.9	34.9 ± 3.8	0.747	0.0778	34.3 ± 3.9	33.9 ± 4.1	0.725	0.1002
Waist circumference [cm]		110.8 ± 10.2	111.6 ± 11.3	0.883	0.0747	105.5 ± 11.1	104.0 ± 10.5	0.538	0.1384
Hip circumference [cm]		115.0 ± 8.0	115.8 ± 9.4	0.577	0.0924	111.7 ± 8.5	112.4 ± 9.7	0.837	0.0773
WHR		0.96 ± 0.06	0.96 ± 0.07	0.770	0.00	0.94 ± 0.07	0.92 ± 0.07	0.445	0.2857
PSS-10		18.00 ± 7.3	18.8 ± 7.4	0.714	0.1089	17.1 ± 7.2	17.2 ± 7.3	0.973	0.0138
SES		29.1 ± 4.6	27.2 ± 4.1	0.183 *	0.4333	29.4 ± 4.09	28.3 ± 4.3	0.414	0.2629
BSQ-34		97.4 ± 23.07	99.2 ± 30.2	0.836	0.0680	82.5 ± 24.4	80.9 ± 25.8	0.814	0.0639
FRS	CS	6.62 ± 0.92	6.70 ± 1.16	0.978 *	0.0774	5.71 ± 0.78	6.29 ± 1.31	0.230 *	0.5528
	IS	4.04 ± 0.59	4.12 ± 0.60	0.726 *	0.1346	4.00 ± 0.63	4.00 ± 0.71	0.987 *	0.00
TFEQ-18	CR	14.5 ± 3.1	13.8 ± 2.8	0.483 *	0.2357	14.7 ± 2.5	15.5 ± 2.6	0.371	0.3144
	UE	20.4 ± 3.8	20.5 ± 4.6	0.948	0.0239	19.3 ± 3.7	19.2 ± 4.4	0.906	0.0248
	EE	6.8 ± 2.5	8.4 ± 2.09	0.0835 *	06877	6.4 ± 1.4	7.6 ± 2.001	**0.037**	0.7087

BMI, body mass index; CS, current silhouette; IS, ideal silhouette; CR, cognitive restraint; UE, uncontrolled eating; EE, emotional eating; p, level of statistical significance; g, Hedges' effect size; * Mann–Whitney U test. Significant p value is pointed bold.

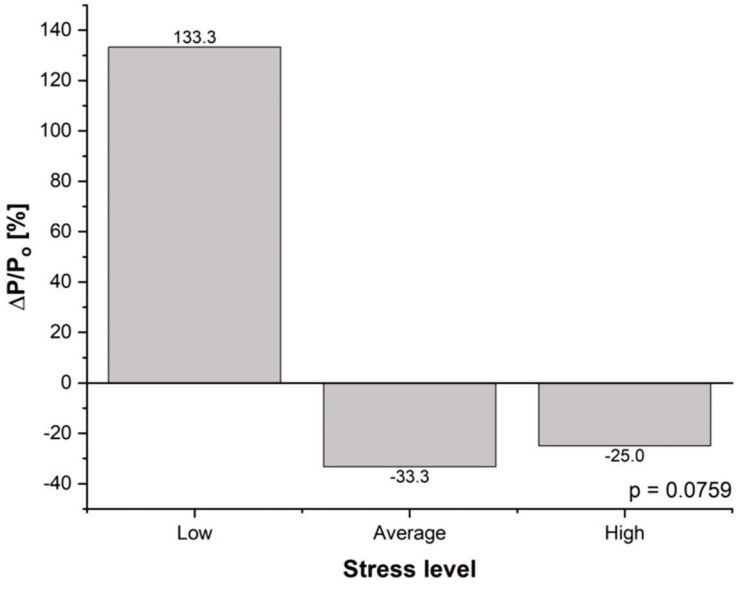
Table 2. Comparison of studied parameters before and after the intervention in groups A and B (anthropometric parameters have already been presented in the paper by Skrypnik et al., 2015 [17]).

Variables		Group A before Intervention (n = 21)	Group A after Intervention (n = 21)	p	d	Group B before Intervention (n = 17)	Group B after Intervention (n = 17)	p	d
Body mass [kg]		91.7 ± 11.8	89.5 ± 11.8	**<0.001**	0.181	94.5 ± 13.4	91.8 ± 13.7	**0.003**	0.193
BMI [kg/m^2]		35.2 ± 3.9	34.3 ± 3.9	**<0.001**	0.225	34.9 ± 3.8	33.9 ± 4.1	**<0.001**	0.245
Waist circumference [cm]		110.8 ± 10.2	105.5 ± 11.1	**<0.001**	0.485	111.6 ± 11.3	104.0 ± 10.5	**<0.001**	0.675
Hip circumference [cm]		115.0 ± 8.0	111.7 ± 8.5	**<0.001**	0.390	115.8 ± 9.4	112.4 ± 9.7	**0.001**	0.345
WHR		0.96 ± 0.06	0.94 ± 0.07	**0.010**	0.299	0.96 ± 0.07	0.92 ± 0.07	**0.005**	0.554
PSS-10		18.00 ± 7.3	17.1 ± 7.2	0.471	0.121	18.8 ± 7.4	17.2 ± 7.3	0.151	0.211
SES		29.1 ± 4.6	29.4 ± 4.09	0.298	0.067	27.2 ± 4.1	28.3 ± 4.3	0.106 *	0.254
BSQ-34		97.4 ± 23.07	82.5 ± 24.4	**<0.001**	0.612	99.2 ± 30.2	80.9 ± 25.8	**0.008**	0.632
FRS	CS	6.62 ± 0.92	5.71 ± 0.78	**0.001** *	1.041	6.70 ± 1.16	6.29 ± 1.31	**0.017**	0.321
	IS	4.04 ± 0.59	4.00 ± 0.63	0.767 *	0.063	4.12 ± 0.60	4.00 ± 0.71	0.361 *	0.177
TFEQ-18	CR	14.5 ± 3.1	14.7 ± 2.5	0.802 *	0.069	13.8 ± 2.8	15.5 ± 2.6	**0.004**	0.610
	UE	20.4 ± 3.8	19.3 ± 3.7	0.233	0.286	20.5 ± 4.6	19.2 ± 4.4	0.135	0.280
	EE	6.8 ± 2.5	6.4 ± 1.4	0.394 *	0.192	8.4 ± 2.09	7.6 ± 2.001	**0.017**	0.379

BMI, body mass index; CS, current silhouette; IS, ideal silhouette; CR, cognitive restraint; UE, uncontrolled eating; EE, emotional eating; p, level of statistical significance; d, Cohen's effect size; * Wilcoxon sign test. Significant p value is pointed bold.



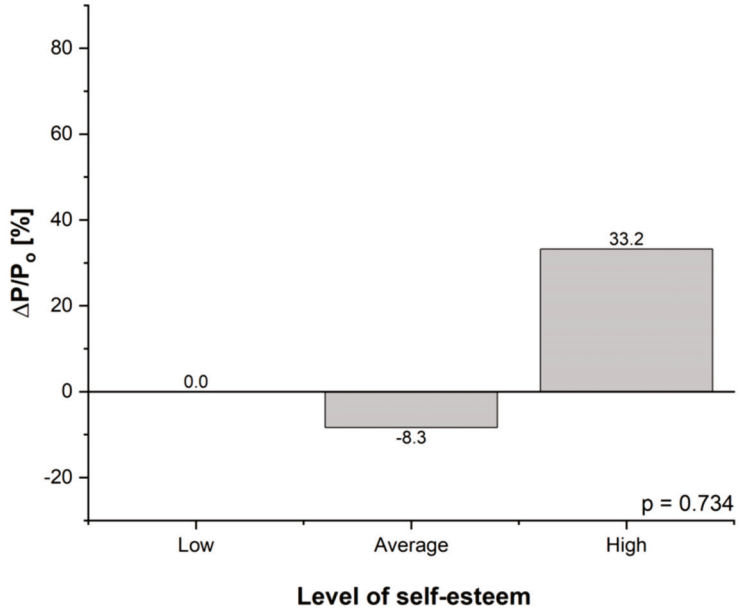
(**A**)



(**B**)

Figure 2. (**A**) Difference in stress level initially and after three months of intervention in group A: P, percentage of the group; $\Delta P = P_{After} - P_{before}$; $P_o = P_{before}$; $\Delta P/P_o$ relative change of percentage of the group (during experiment). (**B**) Difference in stress level initially and after three months of intervention in group B: P, percentage of the group; $\Delta P = P_{After} - P_{before}$; $P_o = P_{before}$; $\Delta P/P_o$ relative change of percentage of the group (during experiment).

In the case of general self-esteem, the number of people with high self-esteem increased in both groups. In the categories of low and average self-esteem, changes were clearer and potentially beneficial in group B (Figure 3A,B). However, the distribution of results did not change significantly.



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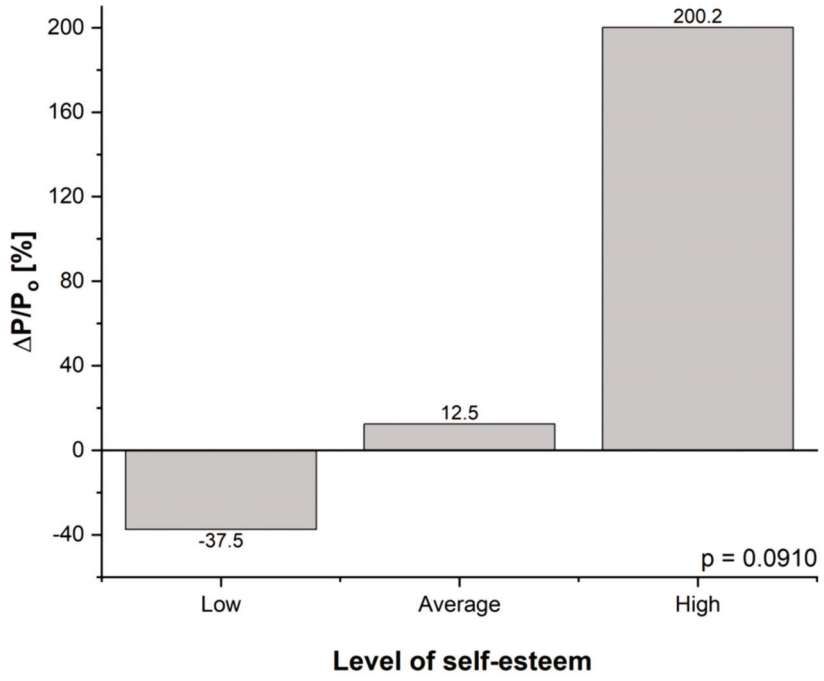

(**B**)

Figure 3. (**A**) Difference in self-esteem level initially and after three months of intervention in group A: P, percentage of the group; $\Delta P = P_{After} - P_{before}$; $P_o = P_{before}$; $\Delta P/P_o$ relative change of percentage of the group (during experiment). (**B**) Difference in self-esteem level initially and after three months of intervention in group B: P, percentage of the group; $\Delta P = P_{After} - P_{before}$; $P_o = P_{before}$; $\Delta P/P_o$ relative change of percentage of the group (during experiment).

As a result of the intervention, it was observed that as the level of self-esteem (δ SES) increased among the subjects, there were larger reductions in the following: BMI ($r = -0.438$), waist circumference ($r = -0.349$), EE ($r = -0.330$) and BSQ–24 ($r = -0.377$). Moreover, as the level of body shape concerns (δ BSQ–24) decreased, waist circumference ($r = 0.393$), WHR ($r = 0.379$), UE ($r = 0.446$) and CS ($r = 0.362$) also decreased. Addition-

ally, the greater the difference in perception of the current silhouette of subjects (δ CS), the greater was the change in BMI (r = 0.375).

In addition, analysis of the variables before and after the intervention showed that only the size of the change in the perception of the current silhouette differentiated both groups in favour of group A. Details concerning all the analysed variables are presented in Table 3.

Table 3. Comparison of change in the parameters studied from the baseline to the three-month point of the intervention in groups A and B (anthropometric parameters have already been presented in the paper by Skrypnik et al., 2015 [17]).

Variables		Group A (n = 21)	Group B (n = 17)	p	g
δ Body mass [kg]		−2.20 ± 2.12	−2.71 ± 2.25	0.371	0.234
δ BMI [kg/m^2]		−0.84 ± 0.80	−0.99 ± 0.80	0.348	0.187
δ Waist circumference [cm]		−5.26 ± 4.45	−7.65 ± 4.56	0.142 *	0.531
δ Hip circumference [cm]		−3.33 ± 2.83	−3.41 ± 3.58	0.547	0.025
δ WHR		−0.02 ± 0.03	−0.04 ± 0.05	0.207	0.498
δ PSS-10		−0.90 ± 5.64	−1.71 ± 4.66	0.642	0.154
δ SES		0.33 ± 2.43	1.12 ± 2.61	0.447 *	0.314
δ BSQ–34		−14.90 ± 13.5	−18.64 ± 25.4	0.565	0.189
δ FRS	CS	−0.90 ± 0.83	−0.41 ± 0.50	**0.035** *	0.697
	IS	−0.05 ± 0.67	−0.12 ± 0.48	0.766 *	0.118
δ TFEQ–18	CR	0.24 ± 2.96	1.65 ± 2.06	0.106	0.542
	UE	−1.05 ± 3.90	−1.29 ± 3.38	0.538 *	0.065
	EE	−0.43 ± 2.09	−0.82 ± 1.28	0.941 *	0.219

BMI, body mass index; CS, current silhouette; IS, ideal silhouette; CR, cognitive restraint; UE, uncontrolled eating; EE, emotional eating; p, level of statistical significance; g, Hedges' effect size; * Mann–Whitney U test. Significant p value is pointed bold.

4. Discussion

After three months of regular physical training, all subjects, regardless of the type of activity, showed significant changes in the objective parameters. A decrease in body weight, waist and hips circumference, as well as BMI and WHR, was observed. Certainly, this can be a reason for a better perception of the current figure and lowering of the level of concern about one's body shape. The importance of BMI and WHR for the mental well-being of women is due to the fact that both parameters are related to physical health [38]. The researchers did not agree on which of them is more important for the assessment of the attractiveness of the female figure. For instance, Furnham et al. [39] studied different body models of women with different body weights, WHRs and breast sizes. They assessed the influence of certain patterns on the assessment of women's attractiveness, femininity, health and fertility. They observed the strongest effect for WHR (0.34–0.52) but not for body weight (0.14–0.31). In the case of breast size, no major effect was shown [39]. In line with this observation, Płatek and Sighn [40] confirmed the importance of optimal (approx. 0.7) WHR in women's attractiveness. By contrast, Holliday et al. [41] showed that BMI, not WHR, modulates reward mechanisms in the brain in both men and women.

Despite these differences, it was clearly confirmed that both body shape improvement (expressed as a decrease in WHR) and weight loss (corresponding to BMI reduction) in women with obesity have a positive impact on the level of satisfaction with their own body and reduce concerns about their appearance [42,43]. Even though we did not prove a significant improvement in the self-esteem of the subjects in our study, we did observe a

tendency for its improvement, especially in the group performing endurance and strength training. The positive effect of excess weight reduction on self-esteem was also observed by the other authors [44]. Interestingly, most women show dissatisfaction with their own body regardless of their actual body weight, and the level of dissatisfaction is higher in women with overweight and obesity [45]. In women, dissatisfaction with one's appearance and worrying about one's figure has a strong negative effect on their overall sense of self-respect and on behaviour, as well as on nutrition [46]. From a psychological point of view, certain manifestations of eating behaviour may indicate risk or the occurrence of eating disorders. Common eating behaviours include, for example, cognitive food abstention, such as intentionally abstaining from eating or limiting food intake in order to control both body weight and body image. In turn, uncontrolled eating manifests itself in a tendency to overeat due to unrestrained hunger. Emotional eating is characterised by overeating due to depressed mood and anxiety. In our study, we confirmed the link between the severity of body shape concerns and uncontrolled eating. The more the level of body shape concerns decreased, the more the level of uncontrolled eating decreased.

Additionally, we have shown that the eating behaviour of women with excess body weight may be influenced by participation in a specific type of physical training. Namely, after three months' intervention, the endurance and strength training group had significantly higher levels of cognitive restraint and significantly lower levels of emotional eating compared with the endurance training group. The observed differences may be the result of the relationship between eating behaviours and the level of stress, which in the group with endurance and strength training decreased more significantly as a result of the intervention; however, the difference between groups was not statistically significant. Lower levels of stress improve cognitive functioning, including cognitive behavioural control and goal achievement [47]. It also promotes the effective regulation of emotions, which reduces emotional eating and unfavourable food choices [48]. Although physical training generally helps reduce stress, the fact that the specificity of training and the presence of such elements as breathing and stretching exercises, which takes place in endurance and strength training, are also important and cannot be ruled out. We observed this tendency in our study comparing endurance training versus endurance and strength training, but this hypothesis needs to be tested on a larger population.

Although changes in the intensity of stress experienced by women participating in the intervention turned out to be statistically insignificant, we observed a clear trend related to the increase in the number of people experiencing low levels of stress and a tendency towards more favourable changes in this regard in women performing endurance and strength training. The relationship between obesity and stress at both the cellular level (oxidative stress) and psychological and social levels have been already confirmed in other studies [49,50]. The lack of clear changes in stress intensity during the intervention may be related to the assessment of the results obtained as lower than expected by the subjects. However, the effort put into regular physical activity resulted in are duction of objective somatic parameters, but in the case of high initial body weight, the changes may be less noticeable both for the examined subject and for her social environment. Another reason may be the presence of a mediating factor between the change in appearance and appearance-related stress. The potential level of self-esteem, the level of self-compassion or the level of depression can be such a factor. However, these hypotheses need to be confirmed in further studies.

The main purpose of our study was to specify whether the size of changes in selected psychological variables differs depending on the form of physical training. Endurance training develops features such as agility, speed, flexibility and nimbleness. It involves all muscle groups and improves the functioning of the heart and entire circulatory system [51]. Endurance and strength training is additionally completed with resistance exercises aimed at increasing both muscle and bone strength and improving metabolism [52]. We did not observe any significant differences in the size of psychological changes, except for the assessment of one's current body shape, among the subjects who followed the abovemen-

tioned forms of training for three months. Although self-esteem related to the current figure changed with training towards a slimmer silhouette in both groups, this change was significant only in women who undertook endurance training.

4.1. Strengths of the Study

No similar studies have been found in the scientific literature that could be used as a comparison. It is only known that both types of training bring similar improvements in depression and anxiety as well as quality of life in people with excess body weight [29,30].

In our study, we used an innovative, comparative model of an endurance and endurance strength training intervention to analyse the impact of certain forms of physical activity on the psychological functioning of women with obesity. We applied strict inclusion and exclusion criteria, which resulted in a homogeneous group of participants. We have proven that both forms of training improve body image parameters, such as body perception and body concerns. In addition, we found that endurance strength training significantly improved two psychological parameters influencing eating behaviour: emotional eating and cognitive restraint, and that this form of physical activity promoted better stress management. Our findings could play a potential role in creating recommendations for future obesity treatment, as they proved that endurance and strength training brings some psychological benefits in addition to measurable physiological changes.

4.2. Study Limitations

Our results should be considered preliminary and requiring confirmation in a study intentionally designed to assess the changes in body image and eating behaviour under the influence of a specific type of physical activity. Further studies will also bean opportunity to correct the shortcomings that we could not avoid in this article. The basic limitation of the study is the relatively small number of participants and the significant age range.

5. Conclusions

Three months of regular physical activity in women with obesity promotes the perception of their own body as thinner, and it reduces concerns about their body shape. The change in body shape perception was more pronounced with endurance training compared with endurance strength training.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/nu13082555/s1, Supplementary Table S1: dietary intake of calorie, protein, carbohydrates, fat and caffeine in both the study groups before and after the intervention.

Author Contributions: Conceptualisation, M.B.-S.; data curation, M.B.-S., D.S., S.G., J.K., E.M. and J.W.; formal analysis, M.B.-S., D.S. and S.G.; investigation, M.B.-S., D.S. and M.R.; methodology, M.B.-S. and D.S.; project administration, M.B.-S.; resources, D.S.; supervision, J.W. and P.B.; writing—original draft, M.B.-S., M.G. and D.S.; writing—review and editing, J.W. and P.B. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the National Science Centre, Poland, Grant No. 2014/13/B/NZ7/02209 and the Faculty of Medicine I, Poznan University of Medical Sciences, Poland, Grant for Young Scientists [grant number 502-14-01119172-41123]. The source of funding had no role in the design of this study and had no role during its execution, analyses, interpretation of the data or in the decision to submit results.

Institutional Review Board Statement: All procedures performed in studies involving human participants were in accordance with the ethical standards of the Bioethics Committee of the Medical University in Poznań and with the 1964 Helsinki Declaration and its later amendments.

Informed Consent Statement: Informed written consent was obtained from all individual participants included in the study.

Data Availability Statement: The data are stored on a secured research server of Statistics Poland and are accessible only to authorised researchers based on collaborative agreements. Please contact Monika Bąk-Sosnowska (monika.bak-sosnowska@sum.edu.pl) for further details.

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

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Can Assessing Physical Activity Liking Identify Opportunities to Promote Physical Activity Engagement and Healthy Dietary Behaviors?

Patrice A. Hubert, Megan Mahoney, Tania B. Huedo-Medina, Tricia M. Leahey and Valerie B. Duffy *

Department of Allied Health Sciences, University of CT, Storrs, CT 06269-1101, USA; Patrice.hubert@uconn.edu (P.A.H.); megan.m.mahoney@uconn.edu (M.M.); tania.huedo-medina@uconn.edu (T.B.H.-M.); tricia.leahey@uconn.edu (T.M.L.)
* Correspondence: valerie.duffy@uconn.edu; Tel.: +1-860-486-1997

Abstract: Improving our understanding of what physical activities are enjoyed and the factors that are associated with physical activity liking can promote participation in regular physical activity. We aimed to study physical activity (PA) liking in college women by modelling interactions between body size perception and dietary behaviors on PA liking, and by examining discrepancies between PA liking versus engagement on body size perception and dietary behaviors. Women (n = 251; 74% white) utilized an online survey to report their level of liking for PA types (scored into a PA liking index) and frequency of PA participation. They also reported their perceived body size, level of dietary restraint, and frequency of consuming foods (scored into a diet quality index). In multivariate analyses, a greater perceived body size was directly associated with lower PA liking and indirectly through greater dietary restraint but lower diet quality. Healthiest dietary behaviors were reported by women who both liked and engaged in PA. Women who reported high PA liking but low PA participation reported a higher dietary restraint and lower diet quality. These findings support the empowerment of women across all body sizes to identify physical activities that they enjoy. Health promotion efforts should encourage women to couple physical activity liking and engagement with a healthy level of dietary restraint and consumption of a healthy diet.

Keywords: physical activity; preference; college women; body size; dietary restraint; dietary behaviors; diet quality; physical exercise; diet; online survey

Citation: Hubert, P.A.; Mahoney, M.; Huedo-Medina, T.B.; Leahey, T.M.; Duffy, V.B. Can Assessing Physical Activity Liking Identify Opportunities to Promote Physical Activity Engagement and Healthy Dietary Behaviors? *Nutrients* **2021**, *13*, 3366. https://doi.org/10.3390/nu13103366

Academic Editors: Sílvia Rocha-Rodrigues and Antoni Sureda

Received: 28 July 2021
Accepted: 22 September 2021
Published: 25 September 2021

1. Introduction

Physical activity contributes to a healthy body weight as women age into early adulthood [1]. The Physical Activity Guidelines for Americans recommend that adults engage in at least 150 to 300 min a week of moderate intensity or 75 to 150 min a week of vigorous intensity aerobic physical activity and muscle-strengthening activities 2 or more days a week for health benefits [2]. Physical activity also supports academic achievement [3] and psychological well-being [4,5]. However, according to the Spring and Fall 2019 American College Health Association (ACHA) surveys, most college women (56–64%) do not meet the physical activity recommendations [6,7]. Pre-COVID-19, lack of physical activity coupled with living on a college campus with ready access to palatable but less healthy foods presented a challenge to maintaining a healthy body weight in college students [8]. COVID-19 restrictions, such as gym closures, mandated mask wearing, and social distancing, present a further challenge to physical activity and well-being, particularly in women [9,10]. Understanding ways to encourage physical activity in young women continues to be an essential task and remains an objective of ACHA's Healthy Campus 2020 [11].

Attention to preferred physical activities encourages participation in physical activity [12–15], with the general belief that, overtime, liked physical activities will become ones that are sustainable. The present paper focuses on how body size perception and dietary

behaviors influence physical activity liking. Previous research has supported the notion that physical activity participation is associated with body size perception but not in a consistent way [16–18]. Less is known about the relationship between body size perception and physical activity liking. Greater body size perception has motivated [19,20] and dissuaded [21,22] women from physical activity participation. Regular physical activity participation can promote positive body image perception [23,24]. This raises the question of whether greater liking of physical activity can support more frequent physical activity participation even if women perceive their body size as large.

The relationship between body size perception and physical activity is also influenced by dietary behaviors, including cognitive control of eating (dietary restraint) and the healthiness of the diet (diet quality). While excessive dietary restraint has been of concern for disordered eating, appropriate levels of dietary restraint are associated with greater diet quality [25], successful body weight management, and health promotion [26,27]. College women, who may not be comfortable with public participation in physical activities, are more apt to control their dietary behaviors (dietary restraint/calorie restriction) when aiming to lose weight, without changing levels of physical activity [28]. However, in a longitudinal study, college women who presented weight concerns coupled with feelings of loss of control of eating and high hedonic value from food reported greater participation in physical activity over time [29]. This suggests that young women can use physical activity as a compensatory behavior for a poor or suboptimal dietary quality [30,31], or as a tradeoff when engaging in other unhealthy behaviors [32]. Additionally, physical activity has been seen to moderate dietary restraint and body weight changes. That is, appropriate levels of dietary restraint and adequate levels of physical activity support healthy weight maintenance [33]. Thus, assessing dietary behaviors can be key in understanding how to support sustainable physical activity behaviors. Participation in enjoyable physical activity should be encouraged among college women, with attention paid to the roles of body size perception and dietary behaviors on liking and frequency of physical activity.

In an online study conducted prior to the COVID-19 pandemic, we aimed to study physical activity liking among college women, including variability in physical activity liking related to body size and dietary behaviors as well as its relationship with frequency of engaging in physical activity. Survey assessment of liking can be a feasible way to identify motivators and barriers to exercise [34] and to promote preferred exercise patterns [35]. Research from our laboratory has shown that survey assessment of diet and physical activity likes and dislikes serves as a simple proxy of usual behaviors. Liking is part of a broader taxonomy used to describe complex behaviors, such as that described for dietary behaviors [36]. That is, reported liking of foods and beverages reflects usual food and beverage consumption as evidenced by associations with biomarkers of dietary intake [37], including in women [38] and young adults [39]. Therefore, we propose that using liking to measure physical activity can help to identify physical activity behaviors that are sustainable, as liking reinforces motivation, increasing adherence [40].

Our first aim was to model the liking of physical activity from body size perception and dietary behaviors (dietary restraint and diet quality). Few studies have specifically examined the interaction between body size perception and liking of physical activity [41], supporting a need for further examination. We hypothesize that greater body weight perception would be associated with lower liking of physical activity. Although there are mixed findings on the relationships between body size perception and frequency of physical activity [16,18–22], some studies report that greater body size perception may cause women to feel uncomfortable with physical activity [42–44], which could fuel lower liking of physical activity. Regarding dietary behaviors, we hypothesize that there are competing influences on the relationships between body weight perception and physical activity liking. That is, young women may be more willing to change dietary behaviors than physical activity in the presence of greater body size perception or concerns [26], whereas adolescent women without weight concerns report less healthy diet behaviors [45]. The greater focus on dieting may compel less interest and engagement in physical activity.

Our second aim extends the examination physical activity liking by comparing liking and reported frequency of engaging in physical activity. Previously, we demonstrated the greater ability to explain differences in dietary restraint and health outcomes in women who report that food and beverage liking and consumption are in agreement (e.g., high liking and high-frequency consumption) versus disagreement (e.g., high liking, low-frequency consumption) [38,46]. In regards to physical activity, we hypothesized that women who reported agreement between high liking and frequency of physical activities also would report the healthiest dietary behaviors and perceive the lowest or healthiest body size. Furthermore, we hypothesized that women who reported disagreement between liking and frequency of physical activities would report the least healthy behaviors. Findings from this study can help to identify how understanding liking of physical activity can be used to inform tailored interventions aimed at promoting physical activity in college women.

2. Materials and Methods

2.1. Participants

This was an observational, cross-sectional study using a convenience sample of 251 female students recruited through student newspaper postings to complete an online survey between February and March, 2018. The survey was open for all students; however, only 41 men participated. We found this number to be insufficient to reflect female vs. male differences in survey responses. There are significant gender effects on body size perception [47], dietary behaviors, and physical activity [48]. As a result, we only included responses from women in the analysis. The study was approved by the University Internal Review Board. The first page provided information about the study followed by yes/no consent to participate. There were no incentives or compensation provided to participants for survey completion.

2.2. Procedures

The online survey was programmed into Qualtrics (Provo, UT, USA) through the University and consisted of reported liking/disliking of physical activity, food and beverages, non-food-health-related behaviors; reported frequency of health behaviors (physical activity and diet); body size perception; dietary restraint; other health behaviors (perceived stress, and sleep); demographics; and additional student information.

2.3. Liking of Physical/Sedentary Activities and Foods/Beverages

Participants were oriented to the scale by reporting level of liking of activities that are generally liked (winning the lottery, succeeding, fun parks), neutral (doing a routine chore), and disliked (running out of money, paper cut, waiting in traffic). These examples and the survey items were represented with both pictures and a circle indicator, which participants could move anywhere on the scale containing seven faces (Figure 1) labeled as "love it", "really like it", "like it", "it's okay", "dislike it", "really dislike", and "hate it". The Qualtrics program reported the distance measured from the scale center (0: "it's okay") to the ends of the bar (±100 "love/hate it)", with intermediate values of "really dislike" (−75), "dislike it" (−50), "like it" (35), "really like it" (60), and "love it" (100). The participants could also mark "never tried/done" for any item.

For the survey items, participants reported liking of 12 exercises under 4 domains of physical activity: aerobic (power walking, running at a slow/steady pace, running at a moderate pace, and sprinting), functional training (free weights, circuit training, and cable exercises), flexibility (yoga, stretching, and stability training), and resistance training (squats, deadlifts, bench press, and leg press). The physical activity items were content validated by experts in student health and physical activity assessment and pilot tested among university students [49]. The pilot study showed good internal reliability and variability among participants [49]. Three exercises were removed (participation in intramural sports, cable exercises, and deadlifts) due to low response rates. Participants also reported liking of 5 behavioral inclinations (working out alone/with a partner, taking

the stairs, going to the gym, group fitness classes, and working up a sweat) and 4 sedentary activities (watching TV, taking the bus around campus, playing video games, and watching videos or movies on YouTube). These individual physical activity groups achieved or neared acceptable internal reliability (Cronbach's α's > 0.6).

Figure 1. Sample question from the online survey with the hedonic facial scale, picture of the item, moveable scale, and ability to report "never tried or done".

Participants reported liking of several foods and beverages (protein powder, sports drinks, pre-packaged coffee drinks, energy drinks, milk, fruits, and vegetables) [49]. Items were chosen to capture food groups and sweetened beverages consumed by college students as seen in previous pilot testing [49] and our findings with young adults [39].

The Physical Activity Liking Index (PAI) is comprised of the liking ratings for individual physical activities. These ratings are averaged into groups and theoretically weighted (group average × weight), and the weighted groups are then averaged into the PAI. The multiplier weights follow the American College of Sports Medicine guidelines: aerobic exercise (+3), functional training (+1.5), flexibility training (+2), resistance exercises (+2.5), sedentary activities (−3), and behavioral inclinations (+3). Higher weights represent increased cardiometabolic health benefits. Through content validation, aerobic exercise was deemed to be the most influential factor on health and provided the highest weighting [49]. This physical activity liking index score (PAI) was tested for reliability and validity following the framework used to evaluate the Healthy Eating Index [50,51]. This framework outlines criteria to test the validity and reliability of survey-generated indexes for health behaviors. For validity, the index should give maximum and minimum scores to behaviors, score variation among individuals, and distinctions between groups with known differences in behavior. For reliability, internal consistency is assessed by examining relationships among the index components and identifying which components have the most influence on the total score. This PAI score met the requirements for validity (outlined further in the Results Section) and neared sufficient internal reliability (Cronbach's α = 0.65).

2.4. Frequency of Physical/Sedentary Activities and Foods/Beverages

For physical activity engagement, the participants responded to questions about their level, frequency, and intensity of physical activity. For physical activity level, responses were either sedentary, lightly active, moderately active, or extremely active. For frequency and intensity, the participants identified the number of days per week that they worked out, the number of exercise repetitions in a set, and the length of workout (minutes). These frequency and intensity responses were multiplied together to create a physical activity exposure score, with good reliability (Cronbach's α = 0.801).

For food and beverage intake as well as measures of diet quality, the participants reported the frequency of consuming fruits, vegetables, fast food, sweets or salty snacks,

and sweetened beverages. Responses were either never, couple times/month, weekly, daily, or more than one time/day. Each response was recoded into a value from 0 to 5 and then theoretically weighted based on the 2015 Dietary Guidelines [52] by multiplying the corresponding category: eating fruits (+5); eating vegetables (+7); eating fast food (−3); eating sweets or salty snacks (−5); and drinking sugary, sweetened beverages (−7). This method of weighting has been validated in similar studies using liking surveys to create diet quality index scores among college students, with higher weights reflecting a healthier diet and reduced cardiometabolic risk factors [39,53]. The resulting values were then added together to create a dietary index score (Cronbach's $\alpha = 0.54$). Scores of >4 aligned with healthier diet quality and score ≤ -7 aligned with less healthy diet quality. Participants also reported their diet quality as poor, fair, good, very good, or excellent for comparisons with the calculated diet quality index [54].

2.5. Body Size Perception

Participants were questioned about their body size perception in two ways: via use of the Figure Rating Scale [55] and a single question regarding perception of being underweight, normal weight, overweight, or obese. The Figure Rating Scale asks participants to select the figure that best represents their current body size from 9 male or female body figures, which increase in size from underweight to obese (1–2 = underweight, 3–4 = normal weight, 5–6 = overweight, and 7–9 = obese). This scale has been validated as an easy tool used to measure body size perception and body dissatisfaction in college women [56–58], with good test–retest reliability [56]. For the present study and following the figures in the Figure Rating Scale [55], women were categorized as underweight/normal weight (body size Figures 1–4) and overweight/obese (body size Figures 5–9).

2.6. Dietary Restraint

Dietary restraint was assessed using three items from the Dutch Restrained Eating Scale [59]. These items were used in pre/post-survey assessment of The Body Project, a dissonance and healthy weight eating disorder program aimed at improving body image in young women [60] delivered at our university. The 3 chosen questions primarily regarded diet behaviors influenced by weight with high item-test correlation coefficients among past students who participated in the program. Different from the original Likert response format, items were responded to on a five-point frequency scale (0–4) ranging from never to daily, and participants responded to the following questions: (1) (Eat Less) If you put on weight, did you eat less than you normally would? (2) (Weight Decide Eating) Did you take into account your weight in deciding what to eat? (3) (Avoid Eating) How often did you try not to eat between meals because you were watching your weight? Restraint scores could range from 0 to 12. Responses were summed to obtain the dietary restraint score with good reliability (Cronbach's $\alpha = 0.788$).

2.7. Statistical Analysis

Data were analyzed using SPSS statistical software for Mac (version 24, Chicago, IL, USA) with Process v3.4 (2019); significance was set at $p < 0.05$. Power analysis variables undergoing parametric testing (e.g., PAI) were assessed for normality. Descriptive analyses were performed with and without outliers to determine effect. Results with outliers removed (PAI scores greater than −100; $n = 5$) are presented, as these responses also had abnormal values for the pleasant/unpleasant items used for scale orientation. Testing of the reliability and validity of PAI was adapted from the methods used to evaluate the Healthy Eating Index [50,51]. Paired sample t-tests examined the differences in liking of physical activity groups among participants. Reliability was tested with Cronbach's alpha (α) and correlational statistics. Concurrent criterion validity of the PAI was tested by comparing values to self-reported physical activity (category and exposure score), diet quality score, perceived body size (categorical and Figure Rating Scale), and dietary restraint score.

Linear regression was used to conduct a path analysis of three variables (body size perception, dietary restraint, and dietary index scores) on physical activity liking (PAI). Based on the regression-based approach proposed by Hayes [61], analysis of variance (ANOVA) was used to test for differences in the concordant and discordant groups in reported physical activity and liking. Variables tested included perceived body size, dietary quality, and dietary restraint. PAI scores and reported physical activity levels were split at the median to form concordant (low liking/low reported and high liking/high reported) and discordant (low liking/high reported and high liking/low reported) groups. These groups allowed for the identification of individuals who were health promoting (high liking/high reported), health seeking, or trying to change behaviors (low liking/low reported), and individuals who may need behavior change intervention (high liking/low reported and low liking/low reported). The assumptions of ANOVA were tested, including evaluating normality and outliers of dependent variables at each level of the independent variable. Levene's test was used for equality of variances at each level of the independent variable.

3. Results

3.1. Descriptive Findings

The completion time for the survey ranged from 5 to 10 min. Table 1 displays the characteristics of the study sample. Most of the women were between 19–22 years of age, identified as white, and reported light-to-moderate physical activity.

Table 1. Characteristics of college women (n = 251).

Age Group	
17–18 years	18.3%
19–20 years	42.6%
21–22 years	31.5%
23+ years	7.6%
Race	
White	74.1%
Black	4.0%
Hispanic	8.4%
Other	13.5%
Reported Physical Activity Level	
Sedentary	8.0%
Lightly Active	35.9%
Moderately Active	46.2%
Extremely Active	10.0%
Body Size Perception	
Categorical	
Underweight	3.6%
Normal Weight	72.1%
Overweight/Obese	24.3%
Figure Scale Rating †	
Normal	71.3%
Overweight/Obese	28.7%
Diet Quality (Self-Reported ††)	
Poor	5.6%
Fair	28.3%
Good	36.3%
Very Good	27.1%
Excellent	2.8%

Table 1. *Cont.*

Dietary Restraint questions †††	
Eat Less	
Never	44.6%
Couple times/month	24.0%
Weekly	7.4%
Couple times/week	13.2%
Daily	10.7%
Weight Decide Eating	
Never	18.4%
Couple times/month	21.6%
Weekly	8.8%
Couple times/week	22.0%
Daily	29.2%
Avoid Eating	
Never	35.2%
Couple times/month	15.2%
Weekly	6.4%
Couple times/week	20.4%
Daily	22.8%

† Based on [55]; †† based on [54]; ††† three items from the Dutch Restrained Eating Scale: (1) Eat Less—"If you put on weight, did you eat less than you normally would?" (2) Weight Decide Eating—"Did you take into account your weight in deciding what to eat?" (3) Avoid Eating—"How often did you try not to eat between meals because you were watching your weight?" [59].

Of the total sample who completed the survey, 23.4% reported they were in a health-related major, while others reported a variety of majors in science, engineering, business, and liberal arts fields. Just over 25% reported as being overweight/obese based on the two measurements of body size perception, which showed a moderate correlation (rho = 0.61 ($p < 0.001$). There was good variability in the questions concerning dietary restraint, from infrequent behaviors to weekly and daily behaviors. Dietary index scores ranged from −13 to 8.4, with an average score of 0.26. The correlation between the categorical rating of dietary quality and dietary index scores was significant (r = 0.582, $p < 0.0001$), with women who reported poor diet quality averaging between −4 and −5 for the dietary index and women who reported very good/excellent diet quality averaging between 3 and 4. Thus, most of the women reported healthy diet quality but with room for improvement, as only 19% reported the consumption of multiple servings of fruits and vegetables per day.

3.2. Physical Activity Liking and the Physical Activity Liking Index (PAI)

Overall, physical activity was generally liked by the study sample (Figure 2). The average liking of physical activity groups (aerobic, functional, flexibility, resistance, and behavioral inclinations) was higher than average liking of sedentary behaviors (mean(s): 28.1 ± 1.4 SE vs. 19.6 ± 1.8 SE; t(225) = 3.86; $p < 0.001$). Reported liking of individual physical activity groups averaged between a neutral rating ("it's okay") and liking rating, demonstrating a positive preference for physical activity in this sample, ranging from the lowest average liking of aerobic activities (nearing "it's okay") to flexibility exercises and behavioral inclination categories averaging at "like it." Liking of aerobic exercises was significantly different from that of all other exercise groups (functional training t(232) = −7.70, $p < 0.001$; flexibility: t(250) = 10.88, $p < 0.001$; resistance: t(238) = −6.11, $p < 0.001$; general Exercise t(250) = −14.47, $p < 0.001$).

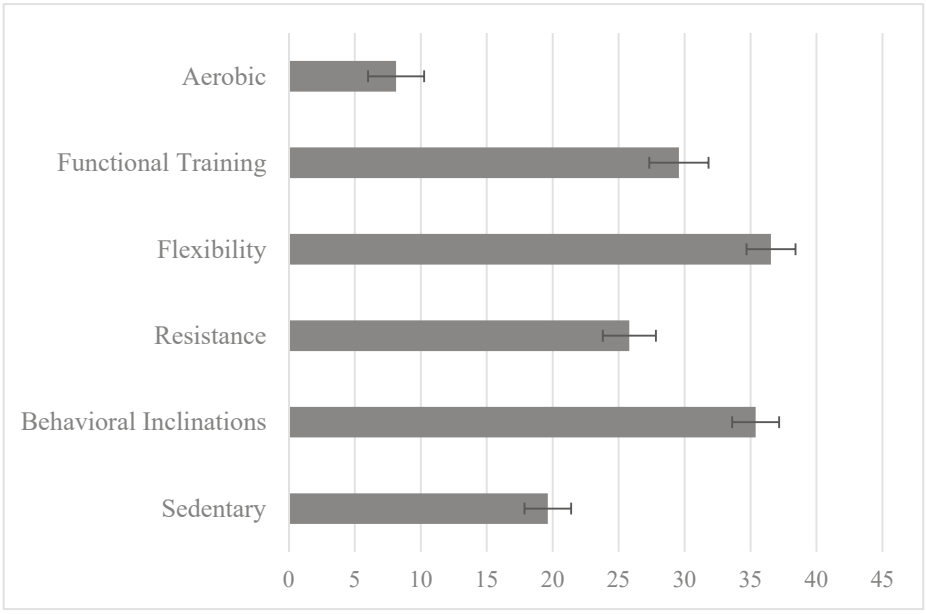

Figure 2. Average liking of groups of physical activities in the physical activity liking index, where participants rated the activities on a bidirectional hedonic scale (0 = "it's okay" to ± 100 "love/hate it") and intermediate values of "really dislike" (−75), "dislike it" (−50), "like it" (35), "really like it" (60), and "love it" (100).

Each physical activity liking group showed significant correlations ($p < 0.001$) with PAI. Sedentary and flexibility exercises (rho = −0.335 and 0.376, respectively) had the least influence on overall PAI score, while general exercise behaviors had the most influence (rho = 0.818). The PAI showed significant variation and a normal distribution (W(251) = 0.91), with scores ranging from −84 to 168, (mean = 41.23 ± 2.91 SE). Exploratory factor analysis indicated two dimensions within the PAI score (active and less active) that accounted for >60% of variability.

The PAI scores were higher in those who self-reported to be moderately and extremely active as a categorical rating (F(3, 251) = 38.29, $p < 0.0001$). There also was discordance between those who reported low or high liking and frequency of activity categories, suggesting other motivations or intent for engaging in physical activity. The PAI was also positively correlated with reported physical activity behavior as a continuous composite variable (physical activity exposure score) (r = 0.611, $p < 0.001$).

The PAI scores were lower in participants who perceived themselves as heavier, significant for perceived categorical ratings (normal/underweight vs. overweight obese; F(1251) = 3.81) but not reaching significance for those perceiving themselves as overweight/obese on the Figure Rating Scale (F(1251) = 2.59, $p = 0.11$). Similarly, for associations with diet, PAI scores were higher in participants who reported higher diet qualities, significant for both categorical response (F(4, 251) = 9.38, $p < 0.0001$) and for the continuous dietary index score (r = 0.339, $p < 0.0001$). However, unlike body size perception and diet quality, dietary restraint was not significantly correlated with PAI scores (r = 0.086, $p = 0.173$).

3.3. Multivariate Modeling of Physical Activity Liking

The simultaneous effect of body size perception, dietary restraint, diet quality (measured by dietary index scores), and PAI scores was modeled based on individual associations (Figure 3). The total effect (c = −7.809, SE = 2.11, t = −3.70, $p < 0.001$) of body size perception on PAI scores was significant, indicating greater body size perception associated with lower PAI scores. Body size perception had a positive direct effect on dietary restraint (b = 0.199, SE = 0.049, t = 4.025, $p < 0.001$) and a negative direct effect on diet quality (b = −0.413, SE = 0.195, t = −2.115, $p < 0.05$), implying that greater body size perception

is associated with greater dietary restraint and a lower diet quality. The direct effect of dietary restraint as the first mediating variable on the second mediating variable of diet quality (b = 0.603, SE = 0.243, t = 2.484, $p < 0.05$) was also significant, suggesting that diet quality increased alongside dietary restraint. A review of the direct effects of mediating variables on PAI scores showed that the effects of dietary restraint (b = 5.56, SE = 2.578, t = 2.157, $p < 0.05$) and diet quality (b = 3.322, SE = 0.666, t = 4.99, $p < 0.001$) were significant. A greater level of dietary restraint was associated with higher PAI scores, but the reverse relationship was seen with diet quality, where low diet quality indicated higher PAI scores. This could suggest compensatory behaviors in this study sample.

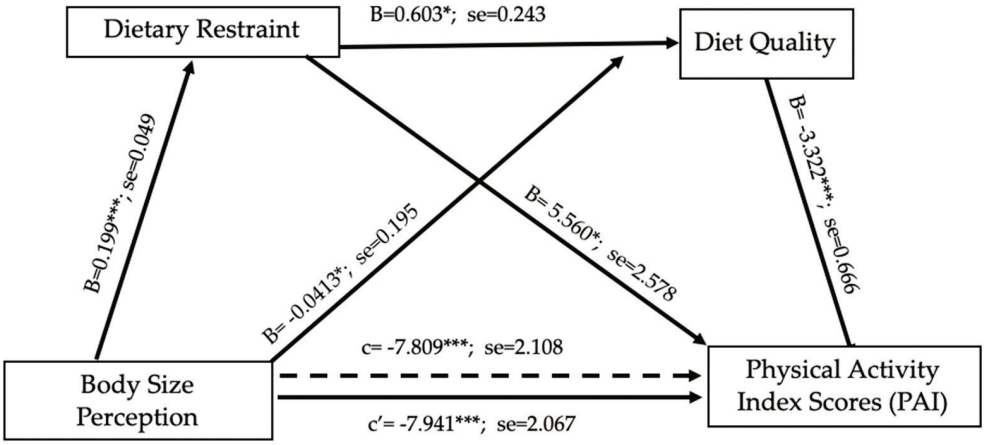

Figure 3. Serial multiple path analysis of dietary restraint and dietary quality in the relationship between perceived body size and physical activity index scores with non-standardized beta values. * $p < 0.05$, *** $p < 0.001$. Greater body size perception associated with decrease in liking of physical activity (PAI), greater dietary restraint, and lower diet quality. Higher dietary restraint associated with higher diet quality and higher liking of physical activity. Higher dietary quality associated with lower liking of physical activity. Simultaneously dietary restraint and diet quality indirectly influenced liking of physical activity in relation to body size perception.

When body size perception and the two mediating variables were entered simultaneously into the model, the direct effect of body size perception on physical activity liking index scores was found to be significant (c′ = −7.941, SE = 2.067, t = −3.842, $p < 0.001$) but slightly lessened, demonstrating evidence of serial mediation. This suggested that the combined effect of dietary restraint and diet quality indirectly influenced liking of physical activity related to body size. Some of the associations between greater body size perception and lower liking of physical activity were explained by a greater level of dietary restraint but lower diet quality.

3.4. Relationships between Physical Activity, Body Perception, and Dietary Behaviors

While liking and frequency of physical activity showed a significant correlation, there were women who were concordant (33.1% low in both; 39.8% high in both) and discordant (16.3% low liking/high frequency; 10.8% high liking/low frequency) in these measures of physical activity behaviors. Differences in body size perception, diet quality (dietary index scores), and dietary restraint were tested with ANOVA among participants who were concordant and discordant in liking (PAI) versus frequency of physical activity. Body size perceptions were significantly different among the concordant/discordant groups ($F(3251) = 5.12$, $p < 0.005$) (Figure 4). The lowest average body size was perceived by those who had both higher liking and frequency of physical activity, significantly lower than those who reported either both low liking and frequency of physical activity or low liking and high frequency of physical activity (all $p < 0.05$).

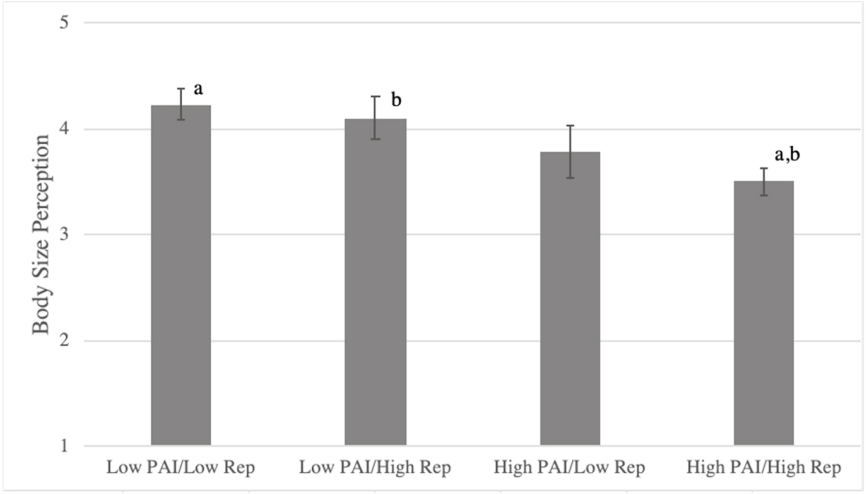

Figure 4. Body size perception among concordant/discordant groups in PAI (physical activity liking index) versus reported frequency of physical activity in college women; according to the Figure Rating Scale [55] 1–2 = underweight, 3–4 = normal weight, 5–6 = overweight, and 7–9 = obese. Matching letters denote significant difference ($p < 0.05$).

Similarly, the highest diet quality (dietary index scores) was observed among participants who reported high physical activity liking and frequency of physical activity ($F(3, 251) = 8.901$, $p < 0.001$; Figure 5), and the dietary index scores of such participants were significantly greater than those in the two groups with low frequency of physical activity ($p < 0.005$).

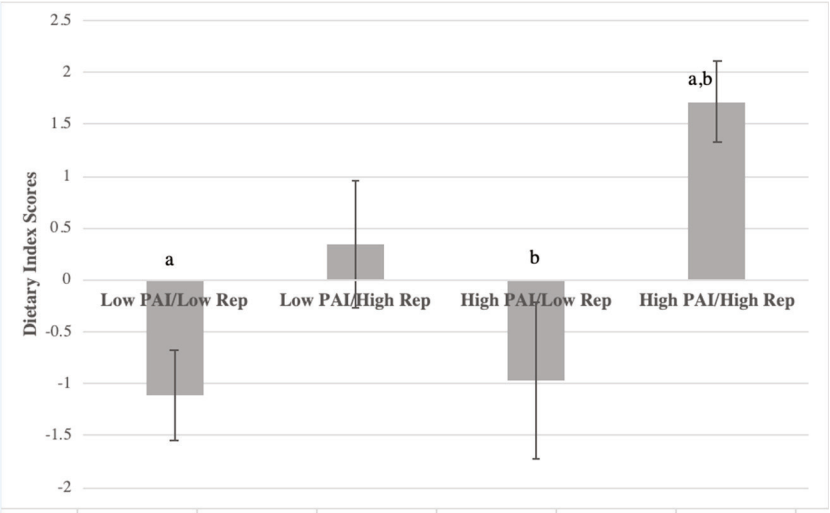

Figure 5. Average dietary index scores among concordant/discordant groups in PAI (physical activity liking index) vs. reported frequency of physical activity in college women. Dietary index scores (diet quality) were the sum of weighted consumption fruits, vegetables, sweets/salty snacks, and sugary beverages where >4 = healthier scores and <−7 = less healthy scores. Matching letters denote significant difference ($p < 0.05$).

Overall, dietary restraint was not significantly different among the concordant/discordant groups ($F(3, 251) = 1.628$, $p = 0.184$) (Figure 6). However, the greatest dietary restraint was seen in those with high physical activity liking and low frequency of physical activity. Post hoc analysis showed that this group significantly differed from the group with both low liking and frequency of physical activity.

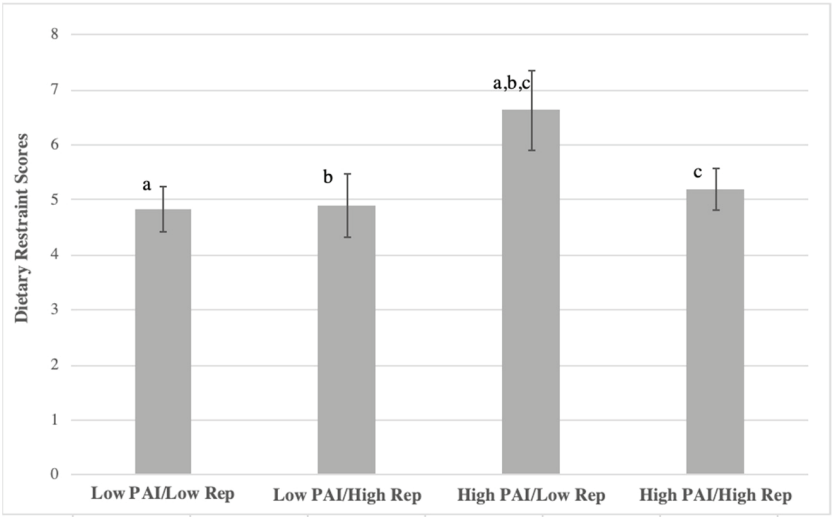

Figure 6. Average dietary restraint scores among concordant/discordant groups in PAI (physical activity liking index) vs. reported frequency of physical in college women. Scores were the sum of responses to three restraint questions, ranging from 0 = never to 12 = daily on each. Matching letters denote significant difference ($p < 0.05$).

4. Discussion

This paper describes an innovative approach to studying physical activity and diet behaviors through asking what is liked. We aimed to describe how liking of physical activity is associated with body size perception and dietary behaviors as well as interactions with reported frequency of physical activity. The convenience sample of collegiate women reported a low level of liking of physical activity, being lightly to moderately physically active, having a moderate level of dietary restraint, fair diet quality, and nearly 30% perceiving an overweight/obese body size. Serial mediation modeling revealed that women who perceived greater body size reported tradeoffs between physical activity liking and dietary behaviors—heathier diets were associated with lower liking of physical activity, whereas reasonable levels of dietary restraint were associated with greater liking of physical activity. By examining the agreement and disagreement between liking and frequency of physical activities, women who reported high liking and high frequency of physical activities had the lowest perceived body size and the healthiest diet quality. The highest level of dietary restraint was seen in women with high liking but low frequency of physical activities. These findings support the need to promote enjoyable physical activities at all body sizes, to encourage enjoyable physical activities coupled with healthy dietary behaviors, and to identify barriers faced by women who like but do not participate in physical activity.

Our physical activity liking measure was practical and novel. Recall of liking is cognitively simpler than recall of behaviors [62], which allowed a relatively quick assessment of multiple physical activities as well as foods and beverages. The novel physical activity liking index (PAI) acknowledges that a variety of physical activities support physical health, which is in accordance with the Physical Activity Guidelines for Americans, 2nd edition [2]. To our knowledge, there is no single physical activity measure that encompasses liking for a variety of physical activities. The PAI had acceptable psychometric properties as demonstrated by good variability and normal distribution in this sample of college women, acceptable internal reliability, and more than one theme in construct validity testing. The PAI was correlated with frequency of physical activity, which agrees with previous research [34,35] and supports the benefit of measuring liking of physical activity to address reasons for physical inactivity [34]. Comparing liking with the reported frequency of physical activities identified those with the healthiest dietary behaviors and potentially with the most sustainable physical activity behaviors.

The intersecting relationships between liking of physical activity (PAI), body size perception, and dietary behaviors in the present study are consistent with those observed in previous reports [23]. We found that women with greater body size perception had lower liking of physical activity partially explained by higher, but reasonable levels of dietary restraint, as well as lower diet quality. Appropriate levels of dietary restraint may prevent unwanted weight gain and support healthy diet quality on college campuses that provide students with unlimited access to less healthy food throughout the day and night [8,26,27]. However, we observed opposite relationships between dietary behaviors and liking of physical activity. Dietary restraint positively predicted greater physical activity liking, suggesting the women were consistent with their health behaviors, while diet quality predicted lesser liking of physical activity, suggesting a disconnect in health behaviors. The highest level of dietary restraint and lowest diet quality were found among women who reported that they liked but did not engage in physical activity, identifying the need to balance the healthiness of diet and physical activity behaviors. College women tend to have compensatory health behaviors [30,31], as observed in the present study, where one behavior is used to replace another, especially when physical activity is not easily accessible [28].

Our findings are also consistent with the negative health impacts of perceiving an elevated weight [42,43]. That is, one being a higher weight or overweight does not always motivate behavior change [21,22]. It was suggested in a recent review that greater body perception causes psychological distress resulting from internal and societal weight stigmatization, which negatively impacts health promoting behaviors [42]. Simply perceiving oneself to have a greater body size can fuel feelings of shame and discourage physical activity engagement in young adults [43]. Decreased comfortability in public places [28] and internalized weight stigma [44] further hinder physical activity. Some women in the present study may have relied more on dietary restraint than physical activity to manage their greater perceived body size. However, dietary restraint efforts do not always translate to improved diet quality [26], which is consistent with the negative association between body size perception and diet quality in our sample. Compensatory behaviors, especially when influenced by body size misperception [17], do not sustain healthy behaviors [29] and may lead to unwanted weight gain [42]. Promoting education about healthy body size perception is important for the cultivation of healthy behaviors in young women [63]. Frequent physical activity participation promotes increased body satisfaction [64], a healthier body image [23,24], and reduced risk of disordered eating [23]. In our sample, women who both liked and participated in physical activity reported the lowest perceived body size and healthiest diets.

There are some limitations to this study that are worth noting. Statistical limitations include the removal of outliers (<2%) and violation of the ANOVA assumption for normality. The sample was homogenous in race/ethnicity, limiting the generalizability of the findings. Body size perception may vary across different racial and ethnic groups [65], as may cultural beliefs about physical activity and dietary behaviors in women [66]. The survey was based on self-reported data. Although we suggest that liking is a reasonable proxy of behavior, bias and inaccuracy always exist in self-reported data. Social desirability may also be a limitation. Even through a discrete online platform, participants may have answered what they thought researchers wanted and in relation to the social norms of their peers reported on social media. The specific physical activities and exercises asked about on the liking survey may not capture the range of types that collegiate women engage in, which could have falsely lowered the PAI. However, the behavioral inclinations sub-score of the PAI were not specific to an activity type and showed significant correlation with the PAI. Only three questions from the Dutch Eating Behavior questionnaire were used, limiting the comparability to other studies. Lastly, our diet quality index score was based on a limited number of items and had a Cronbach's α of 0.54, which is below the acceptable range and lower than the Healthy Eating Index, which is based on multiple items (Cronbach's $\alpha = 0.68$) [51]. However, diet quality is known to be multidimensional, and reliability may

not be a necessary characteristic [51]. Our diet quality index score demonstrated a strong correlation with the reported diet quality from participants, suggesting further confidence in this index.

Despite the limitations, our findings can be applied to support physical activity and healthy dietary behaviors among collegiate women. While our sample of women was a convenience sample, the participants represented multiple college majors and displayed sufficient variability in physical activity liking, dietary behaviors, and perceived body size to test the study aims and hypotheses. The perceived level of overweight/obese and reported dietary intake as well as the level of physical activity were consistent with U.S. pre-pandemic surveys of college women [6,7]. Measuring the liking and frequency of physical activity can promote sustainable physical activity engagement [34]. Emerging evidence from our laboratory suggests that motivating and reinforcing health promotion messages can be delivered online to college students based on their reported liking of diet and physical activities; these messages are reported to be relevant and useful [67]. This evidence expands the tailored message program that we previously conducted with children and parents [68,69]. These tailored messages can highlight specific areas of change prior to an intervention or counseling session and promote motivation and self-efficacy to change health behaviors [67,70,71]. Focusing tailored counseling and health messages on body misconceptions, healthy dietary behaviors, and enjoyable physical activity supports the overarching goal of health promotion.

5. Conclusions

This study utilized liking to feasibly measure physical activity in college women with a novel, valid, and reliable index that captured a variety of activity types. Multivariate modeling showed that women who perceived greater body size reported less liking of physical activity as well as less healthy dietary behaviors. Women who both liked and engaged in physical activities had a lower body size perception and healthier diet quality. The COVID-19 pandemic saw increases in both physical activity and sedentary behaviors among college students [10]. Assessing physical activity liking could help to improve our understanding of drivers and barriers of physical activity for tailored health counseling and interventions.

Author Contributions: Conceptualization, P.A.H., M.M. and V.B.D.; methodology, P.A.H., M.M. and V.B.D.; formal analysis, P.A.H. and T.B.H.-M.; investigation, P.A.H. and M.M.; resources, V.B.D.; data curation, M.M.; writing—original draft preparation, P.A.H. and M.M.; writing—review and editing, P.A.H., M.M., T.B.H.-M., T.M.L. and V.B.D.; visualization, P.A.H.; supervision, T.B.H.-M., T.M.L. and V.B.D.; project administration, V.B.D.; funding acquisition, V.B.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the United States Department of Agriculture National Institute of Food and Agriculture, Hatch project 1001056.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of The University of Connecticut (protocol code Exemption #Xl 7-085 and 1 December 2017).

Informed Consent Statement: The study is exempt under 45 CFR 46.101 (b) (2). An approved, validated information sheet (with the IRB's stamp) was used to obtain the consent of each subject.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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ISBN 978-3-0365-6086-1