

Special Issue Reprint

Hot Topics in Nutrition and Obesity

Edited by
Javier Gómez-Ambrosi and Victoria Catalán

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Hot Topics in Nutrition and Obesity

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Guest Editors

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About the Editors

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Preface

Obesity remains one of the most important health challenges worldwide and is closely linked to metabolic disease, chronic inflammation, impaired quality of life, and a growing burden for health systems. Its prevention and management require an integrated perspective that considers dietary habits, lifestyle, body composition, psychosocial factors, biological mechanisms, and the broad clinical consequences of excess adiposity.

This Reprint brings together contributions that reflect the diversity and relevance of current research in nutrition and obesity. The included studies address population based aspects of overweight and obesity, nutritional status and systemic inflammation, emotional eating in adolescence, and experimental approaches focused on obesity related metabolic dysfunction. Together, they illustrate how research across clinical, epidemiological, behavioral, and mechanistic fields can contribute to a better understanding of obesity and its associated complications.

The purpose of this Reprint is to provide researchers, clinicians, dietitians, nutritionists, public health professionals, and students with a useful overview of emerging evidence in this rapidly evolving field. We hope that the work collected here will encourage multidisciplinary dialogue and support the development of more effective, personalized, and sustainable strategies for obesity prevention and treatment.

Javier Gómez-Ambrosi and Victoria Catalán

Guest Editors

Editorial

Hot Topics in Nutrition and Obesity: Integrating Epidemiology, Mechanisms, and Therapeutic Perspectives

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1. Introduction: The Global Burden of Obesity

Obesity remains one of the most pressing global public health challenges, contributing significantly to the development of metabolic disorders, including type 2 diabetes, cardiovascular disease, dyslipidemia, metabolic dysfunction-associated steatotic liver disease (MASLD/MASH), and certain types of cancer [1,2]. Despite substantial advances in our understanding of obesity biology and the emergence of innovative therapeutic strategies, its global prevalence continues to rise, underscoring the need for comprehensive and multidisciplinary approaches.

On the 15th anniversary of *Nutrients*, this Special Issue, “Hot Topics on Nutrition and Obesity 2024”, was launched to provide an updated overview of key developments in the field. The aim was to gather high-quality contributions spanning nutritional epidemiology, mechanistic research, clinical and lifestyle interventions, and emerging technologies, reflecting the multifactorial nature of obesity. This collection includes eight articles—seven original research papers and one review—that collectively illustrate the complexity of obesity from population-level determinants to molecular mechanisms and therapeutic perspectives.

2. Epidemiological Insights and Population-Level Trends

Understanding the distribution and determinants of obesity at the population level is essential for informing effective prevention strategies. Epidemiological studies provide critical insights into prevalence patterns, temporal trends, and sociodemographic disparities, helping to identify high-risk groups and guide targeted public health interventions [3,4]. At the population level, the study by Traczyk et al. (contribution 4) provides a comprehensive analysis of the prevalence of overweight, general obesity, and abdominal obesity in a large sample of Polish adults, based on data from the National Health Program (2016–2020). Importantly, the authors not only quantify the burden of excess body weight but also examine its distribution across sociodemographic groups, revealing disparities associated with age, sex, and socioeconomic status. These findings reinforce the uneven distribution of obesity and highlight the need for targeted public health strategies.

Complementing these epidemiological insights, Jakše et al. (contribution 5) present a longitudinal assessment of body composition changes in Slovene adults over a two-year period. By incorporating both BMI and body fat percentage classifications, they demonstrate that reliance on BMI alone may underestimate true adiposity and related health risks. The longitudinal design further allows the authors to capture dynamic changes

in body composition, offering valuable evidence on the progression of obesity and the importance of continuous monitoring.

3. Advances in Body Composition Assessment

Accurate assessment of body composition is fundamental for the proper diagnosis and management of obesity [5,6]. Beyond conventional anthropometric indices, emerging methodologies allow for a more precise evaluation of adiposity and its distribution, particularly in populations where standard measures may be limited or misleading [7–10]. The importance of accurate body composition assessment is further emphasized in the study by Bagińska-Chyży and Korzeniecka-Kozerska (contribution 6), which focuses on children with spina bifida. In this population, standard anthropometric measures are often inadequate due to altered growth patterns and body proportions. The authors demonstrate that bioelectrical impedance analysis provides a more reliable tool for assessing adiposity and guiding weight management interventions, underscoring the need for tailored approaches in vulnerable and clinically complex populations.

4. Mechanistic Insights into Obesity and Metabolic Dysfunction

Elucidating the biological mechanisms underlying obesity is key to understanding its associated complications and identifying novel therapeutic targets [11,12]. Experimental and translational studies continue to uncover the complex interactions between the dietary components, cellular processes, and organ systems involved in metabolic dysfunction. The mechanistic studies in this Special Issue shed light on the biological underpinnings of obesity and its complications. Sivasubramanian et al. (contribution 7) investigate the effects of palmitic acid, a common saturated fatty acid, on human brainstem astrocytes. Their findings reveal that it induces oxidative stress and cellular senescence while impairing glutamate transporter expression, suggesting a potential link between dietary fat intake, neuroinflammation, and obesity-associated sympathetic overactivity. This work contributes to a growing body of evidence implicating central nervous system mechanisms in obesity-related cardiometabolic dysfunction.

In a complementary experimental study, Kang and Cao (contribution 3) explore the therapeutic potential of ursolic acid in a high-fat diet-induced obesity model. Their results show that ursolic acid supplementation improves muscle strength, enhances lipid metabolism, and reduces hepatic steatosis. These findings are particularly relevant in the context of sarcopenic obesity and metabolic liver disease, highlighting the potential of naturally occurring bioactive compounds as adjunctive treatments.

5. Inflammation and Immune–Nutritional Interactions

Chronic low-grade inflammation is a hallmark of obesity and a major contributor to its metabolic consequences [13–15]. Investigating the interplay between nutritional status and immune responses provides valuable insights into disease progression and highlights potential biomarkers and intervention targets. The role of inflammation as a key mediator of obesity-related complications is further explored by Uzun et al. (contribution 2), who examine the relationship between nutritional status and systemic immune–inflammation indices across BMI categories. By integrating multiple validated nutritional and inflammatory markers, the authors demonstrate a clear association between worsening nutritional status, increased adiposity, and heightened systemic inflammation. This reinforces the concept of obesity as a chronic low-grade inflammatory state and highlights the value of composite indices in risk stratification.

6. Gut Microbiota and Emerging Therapeutic Targets

The gut microbiota has emerged as a central player in the regulation of host metabolism and energy balance [16–18]. Advances in this field are shedding light on how diet–microbe interactions influence obesity development and how microbiota-targeted strategies may offer innovative therapeutic opportunities. Positioned at the interface of diet, microbiota, and metabolic health, the review by Yi et al. (contribution 8) provides a comprehensive synthesis of microbiota-based interventions in the context of high-fat diet-induced metabolic alterations. The authors discuss how changes in gut microbial composition, bile acid metabolism, and host–microbe interactions contribute to obesity pathogenesis. Importantly, they also evaluate potential therapeutic strategies aimed at restoring microbial balance, offering insights into a rapidly evolving and promising area of research.

7. Psychosocial and Behavioral Determinants of Obesity

Obesity is not only driven by biological factors but is also strongly influenced by behavioral and psychosocial determinants [19–21]. Understanding the role of stress, emotional regulation, and lifestyle behaviors is crucial for developing comprehensive and sustainable prevention and intervention strategies. The psychosocial dimension of obesity is addressed by Głabska et al. (contribution 1), who investigate the influence of stress and emotional eating on body mass in a population of Polish female adolescents. Their findings highlight the significant role of emotional and behavioral factors in shaping dietary habits and weight outcomes. The study underscores the importance of early-life interventions that incorporate psychological support and behavioral strategies to prevent the development of obesity.

8. Conclusions

Taken together, the contributions in this collection underscore the multifactorial nature of obesity, encompassing biological, behavioral, and environmental determinants. They highlight the need for integrated approaches that combine precise phenotyping, mechanistic understanding, and targeted interventions. Future research should aim to elucidate causal relationships, improve the precision of nutritional assessments, and develop personalized and scalable interventions. In particular, emerging fields such as nutrigenomics, digital health technologies, and microbiome research offer promising avenues for innovation.

In conclusion, this Special Issue provides a comprehensive overview of current “hot topics” in nutrition and obesity. By bringing together diverse lines of evidence, it contributes to advancing our understanding of this complex condition and supports the development of more effective strategies to address the ongoing obesity pandemic.

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1. Głabska, D.; Skolmowska, D.; Guzek, D. Analysis of Influence of Experienced Stress and Emotional Eating on Body Mass in a Population of Polish Female Adolescents: PLACE-19 Study. *Nutrients* **2026**, *18*, 85. <https://doi.org/10.3390/nu18010085>.
2. Uzun, H.; Fenercioglu, A.K.; Senyigit, A.; Can, G. Relationship Between Nutritional Status and Systemic Immune–Inflammation Indices Across BMI Categories. *Nutrients* **2025**, *17*, 3799. <https://doi.org/10.3390/nu17233799>.
3. Kang, D.; Cao, L. Muscle Strength, Lipid Metabolism and Hepatic Steatosis Are Improved with Ursolic Acid Treatment in High-Fat Diet-Induced Obese Mice. *Nutrients* **2025**, *17*, 3158. <https://doi.org/10.3390/nu17193158>.

4. Traczyk, I.; Kucharska, A.; Sińska, B.I.; Panczyk, M.; Samel-Kowalik, P.; Kłak, A.; Raciborski, F.; Wyleżół, M.; Samoliński, B.; Szostak-Węgierek, D. Prevalence of Overweight, Obesity, and Abdominal Obesity in Polish Adults: Sociodemographic Analysis from the 2016–2020 National Health Program. *Nutrients* **2024**, *16*, 4248. <https://doi.org/10.3390/nu16234248>.
5. Jakše, B.; Fras, Z.; Godnov, U. Body Composition Trend in Slovene Adults: A Two-Year Follow-Up. *Nutrients* **2024**, *16*, 4123. <https://doi.org/10.3390/nu16234123>.
6. Bagińska-Chyży, J.; Korzeniecka-Kozerska, A. Application of Bioelectrical Impedance Analysis in Weight Management of Children with Spina Bifida. *Nutrients* **2024**, *16*, 3222. <https://doi.org/10.3390/nu16183222>.
7. Sivasubramanian, M.K.; Monteiro, R.; Jagadeesh, M.; Balasubramanian, P.; Subramanian, M. Palmitic Acid Induces Oxidative Stress and Senescence in Human Brainstem Astrocytes, Down-regulating Glutamate Reuptake Transporters—Implications for Obesity-Related Sympathoexcitation. *Nutrients* **2024**, *16*, 2852. <https://doi.org/10.3390/nu16172852>.
8. Yi, L.; Li, Z.; Xu, H.; Shi, D.; Huang, Y.; Pan, H.; Zhao, Y.; Zhao, H.; Yang, M.; Wei, H.; et al. Microbiota-Based Intervention Alleviates High-Fat Diet Consequences Through Host-Microbe Environment Remodeling. *Nutrients* **2025**, *17*, 1402. <https://doi.org/10.3390/nu17091402>.

References

1. Burki, T. European Commission classifies obesity as a chronic disease. *Lancet Diabetes Endocrinol.* **2021**, *9*, 418. [CrossRef] [PubMed]
2. Hagberg, C.E.; Spalding, K.L. White adipocyte dysfunction and obesity-associated pathologies in humans. *Nat. Rev. Mol. Cell Biol.* **2024**, *25*, 270–289. [CrossRef] [PubMed]
3. NCD Risk Factor Collaboration. Worldwide trends in underweight and obesity from 1990 to 2022: A pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. *Lancet* **2024**, *403*, 1027–1050. [CrossRef] [PubMed]
4. Collaborators GBD. Global, regional, and national prevalence of adult overweight and obesity, 1990–2021, with forecasts to 2050: A forecasting study for the Global Burden of Disease Study 2021. *Lancet* **2025**, *405*, 813–838. [CrossRef] [PubMed]
5. Frühbeck, G.; Busetto, L.; Dicker, D.; Yumuk, V.; Goossens, G.H.; Hebebrand, J.; Halford, J.G.C.; Farpour-Lambert, N.J.; Blaak, E.E.; Woodward, E.; et al. The ABCD of obesity: An EASO position statement on a diagnostic term with clinical and scientific implications. *Obes. Facts* **2019**, *12*, 131–136. [CrossRef] [PubMed]
6. Salmón-Gómez, L.; Catalán, V.; Frühbeck, G.; Gómez-Ambrosi, J. Relevance of body composition in phenotyping the obesities. *Rev. Endocr. Metab. Disord.* **2023**, *24*, 809–823. [CrossRef] [PubMed]
7. Busetto, L.; Dicker, D.; Frühbeck, G.; Halford, J.C.G.; Sbraccia, P.; Yumuk, V.; Goossens, G.H. A new framework for the diagnosis, staging and management of obesity in adults. *Nat. Med.* **2024**, *30*, 2395–2399. [CrossRef] [PubMed]
8. Gómez-Ambrosi, J.; González-Alva, M.U.; Silva, C.; Salvador, J.; Frühbeck, G. A critical appraisal of emerging obesity diagnostic frameworks to bridge gaps and refine clinical stratification. *Commun. Med.* **2026**, *in press*.
9. Rubino, F.; Cummings, D.E.; Eckel, R.H.; Cohen, R.V.; Wilding, J.P.H.; Brown, W.A.; Stanford, F.C.; Batterham, R.L.; Farooqi, I.S.; Farpour-Lambert, N.J.; et al. Definition and diagnostic criteria of clinical obesity. *Lancet Diabetes Endocrinol.* **2025**, *13*, 221–262. [CrossRef] [PubMed]
10. Schiborn, C.; Hu, F.B.; Stefan, N.; Schulze, M.B. Preclinical and clinical obesity: Prevalence, associations to cardiometabolic risk and response to lifestyle intervention in NHANES and the EPIC-Potsdam and TULIP studies. *Nat. Commun.* **2026**, *17*, 1935. [CrossRef] [PubMed]
11. Heymsfield, S.B.; Wadden, T.A. Mechanisms, pathophysiology, and management of obesity. *N. Engl. J. Med.* **2017**, *376*, 254–266. [CrossRef] [PubMed]
12. Wen, X.; Zhang, B.; Wu, B.; Xiao, H.; Li, Z.; Li, R.; Xu, X.; Li, T. Signaling pathways in obesity: Mechanisms and therapeutic interventions. *Signal Transduct. Target. Ther.* **2022**, *7*, 298. [CrossRef] [PubMed]
13. Reilly, S.M.; Saltiel, A.R. Adapting to obesity with adipose tissue inflammation. *Nat. Rev. Endocrinol.* **2017**, *13*, 633–643. [CrossRef] [PubMed]
14. Rohm, T.V.; Meier, D.T.; Olefsky, J.M.; Donath, M.Y. Inflammation in obesity, diabetes, and related disorders. *Immunity* **2022**, *55*, 31–55. [CrossRef] [PubMed]
15. Sotak, M.; Clark, M.; Suur, B.E.; Borgeson, E. Inflammation and resolution in obesity. *Nat. Rev. Endocrinol.* **2025**, *21*, 45–61. [CrossRef] [PubMed]
16. Cani, P.D.; Van Hul, M. Gut microbiota in overweight and obesity: Crosstalk with adipose tissue. *Nat. Rev. Gastroenterol. Hepatol.* **2024**, *21*, 164–183. [CrossRef] [PubMed]

17. Tilg, H.; Zmora, N.; Adolph, T.E.; Elinav, E. The intestinal microbiota fuelling metabolic inflammation. *Nat. Rev. Immunol.* **2020**, *20*, 40–54. [CrossRef] [PubMed]
18. Van Hul, M.; Cani, P.D. From microbiome to metabolism: Bridging a two-decade translational gap. *Cell Metab.* **2026**, *38*, 14–32. [CrossRef] [PubMed]
19. Catalán, V.; Avilés-Olmos, I.; Rodríguez, A.; Becerril, S.; Fernández-Formoso, J.A.; Kiortsis, D.; Portincasa, P.; Gómez-Ambrosi, J.; Frühbeck, G. Time to consider the “Exposome hypothesis” in the development of the obesity pandemic. *Nutrients* **2022**, *14*, 1597. [CrossRef] [PubMed]
20. Graham, H.E.; Madigan, C.D.; Gokal, K.; Large, J.F.; Sanders, J.; McLeod, C.J.; Pearson, N.; Daley, A.J. A systematic review and meta-analysis of randomized controlled trials of behavioral interventions for the management of overweight and obesity in children that are delivered or referred to by health providers in primary care. *Obes. Rev.* **2026**, *27*, e70119. [CrossRef] [PubMed]
21. Jiménez-Pelaez, C.C.; Fernández-Aparicio, A.; Montero-Alonso, M.A.; González-Jimenez, E. Effect of dietary and physical activity interventions combined with psychological and behavioral strategies on preventing metabolic syndrome in adolescents with obesity: A meta-analysis of clinical trials. *Nutrients* **2025**, *17*, 2051. [CrossRef] [PubMed]

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Article

Analysis of Influence of Experienced Stress and Emotional Eating on Body Mass in a Population of Polish Female Adolescents: PLACE-19 Study

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Abstract

Background/Objectives: Emotional eating is defined as a coping mechanism characterized by food consumption in response to negative emotions, and it typically involves over-consumption and a preference for energy-dense and highly palatable foods. The aim of this study was to analyze the influence of experienced stress and emotional eating on body mass in a population of Polish female adolescents. **Methods:** This study included 816 participants (aged 15–20 years) recruited within the nationwide PLACE-19 Study using random quota sampling of Polish secondary schools, and data were collected by the Computer-Assisted Web Interview (CAWI). Perceived stress was measured with the Adolescent Stress Questionnaire (ASQ), emotional eating was measured with the Emotional Eating Subscale (EE-3) of the Three-Factor Eating Questionnaire (TFEQ), and self-reported body mass was also recorded. Body mass was interpreted either using standard BMI values for adults or growth reference values for minors. **Results:** Adolescents with excessive body weight reported higher levels of stress on the peer pressure ($p = 0.0011$ for continuous variables; $p = 0.0016$ for categories) and financial pressure component scales ($p = 0.0319$ for continuous variables) than their normal-weight and underweight counterparts. They also displayed higher emotional eating scores across all subscales and for the total emotional eating score ($p < 0.05$ for continuous variables), particularly for anxiety ($p = 0.0345$ for categories). The association was confirmed within mediation analysis, as the direct influence explained 79% of the influence of stress on body mass, and the indirect influence, mediated by emotional eating, explained 21% of the influence of stress on body mass. **Conclusions:** Adolescents with excessive body weight are more prone to stress and emotional eating. The stress itself affects body weight not only directly, but also by affecting emotional eating; therefore, adolescent girls should be taught how to cope with negative emotions using strategies other than increasing food consumption in response to negative emotions. Further studies should assess the mediating role of emotional eating among adolescent girls and evaluate the impact of stress management interventions on body weight.

Keywords: stress; emotional eating; body mass; adolescents; Adolescent Stress Questionnaire (ASQ); Emotional Eating Subscale (EE-3); Three-Factor Eating Questionnaire (TFEQ); PLACE-19 Study

1. Introduction

According to the World Health Organization (WHO), stress is defined as an individually experienced worry or mental tension resulting from a situation perceived as difficult [1]. This global problem is gradually escalating and is currently indicated as one of the health epidemics of the 21st century, which is reflected in rising stress levels; e.g., in the United States of America, its prevalence increased from 10% in 1983 to 30% in 2009 [2]. Before the COVID-19 pandemic, multiple stressors were identified at the individual level (e.g., abuse, educational problems), relationship level (e.g., conflicts with partner, parents, or friends), sociocultural level (e.g., socioeconomic status, norms and values), and community level (e.g., war, neighborhood environment, healthcare access) [3]. The COVID-19 pandemic emerged as a specific and novel stressor, exacerbating previously existing stressors [4].

The umbrella review by Amini-Rarani et al. [5] focusing on the COVID-19 pandemic identified patients, healthcare workers, pregnant women (populations particularly prone to infection or its consequences), and students as the groups experiencing the highest stress levels. This may result from the fact that responses to various stressors in adolescents differ from those in adults, including heightened stress-induced hormonal responses and an increased risk of stress-mediated psychological dysfunction [6]. Similarly, it has been emphasized that the COVID-19 pandemic was a more serious stressor for females, resulting in a higher prevalence of anxiety and depression compared to their male counterparts [7]. Moreover, in an analysis of the psychological and cognitive reactions to the COVID-19-related stress, young females were defined as an especially vulnerable group [8].

The influence of perceived stress on eating behavior may differ, depending on the circumstances, the physiological reaction of the organism, and individual factors [9]. This results from the multidimensional influence of stress on appetite and eating behaviors, a phenomenon observed at both the physiological and psychological levels [10]. Acute stress triggers a ‘fight-or-flight’ reaction, which is associated with an increased heart rate, blood glucose release, and the suppression of non-essential body functions, including hunger [11]. On the other hand, chronic stress is associated with elevated levels of appetite-related hormones, resulting in enhanced food cravings and reward-driven eating behavior [12]. This phenomenon is associated with so-called emotional eating, which is commonly defined as a coping mechanism involving food consumption in response to negative emotions (e.g., stress, upset, furiousness). In such cases, overconsumption is often observed, and energy-dense and palatable food products are chosen [13].

Emotional eating has been identified as an important variable to be taken into account in body mass management, as emphasized in a recent systematic review and meta-analysis by Smith et al. [13]. However, the level of emotional eating may differ depending on the studied group and individual characteristics within that group. In a national study conducted in the United States of America, named the Family Health Habits Survey [14], the groups with the highest emotional eating levels were defined as females, non-Hispanic Caucasians, and younger respondents. The role of the female gender was confirmed in a study of adolescents, also conducted in the United States of America, which indicated that a higher number of stimuli triggers emotional eating in girls than in boys [15]. At the same time, the role of age has been confirmed in several studies, indicating that emotional eating increases from early adolescence [16] and decreases with age in adulthood [17]. Consequently, in the case of adolescent girls, the frequency of emotional eating may reach 43%, compared to 15% for boys in the same population, as indicated for 16-year-olds in the Northern Finland Birth Cohort 1986 (NFBC1986) [18].

Stress-induced body mass changes may include either a decrease or an increase in weight, as indicated in the systematic review by Haidar et al. [19] for a population of students. Moreover, stress-related body mass change may be associated with initial

body weight; the Whitehall II Study [20] revealed that for men in the lowest Body Mass Index (BMI) quartile, stress caused body mass reduction, while for those in the highest BMI quartile, it caused weight gain. However, when emotional eating is included as a stress-induced reaction, the typical consequence is weight gain, leading to increased BMI [21] and waist circumference [22]. The role of emotional eating, as a mediator between stress and body mass, has been recently highlighted by several authors, based on studies conducted in populations of children [23], adolescents [24], young adults [25], adults [26], and parents [27].

Little is known about the phenomenon of emotional eating [28], as well as the neural pathways involved, including interactions between neural and neuroendocrine pathways, which are not fully understood [29]. Moreover, a recent review of the current clinical evidence by Dakanalis et al. [30] emphasized that, in spite of the fact that the influence of psychological distress on emotional eating and resultant weight gain is documented, more studies are needed, especially to define the coping mechanisms that are still unknown.

Despite the fact that emotional eating is indicated as an important phenomenon influencing body mass, and stress as a factor commonly activating it, little is still known about the complex influence of experienced stress and emotional eating on body mass. The previous Polish analysis conducted within the PLACE-19 Study (Polish Adolescents' COVID-19 Experience Study) assessed various psychological aspects of eating behaviors, but so far, it focused on the self-regulation of eating behaviors [31], emotional eating [32], emotional overeating [33], and the association between stress and emotional eating [34]. However, the combined influence of stress and emotional eating on body mass has not yet been addressed. Taking into account that young females are an especially stress-vulnerable group with the highest level of emotional eating, the aim of this study was to analyze the influence of experienced stress and emotional eating on body mass in a population of Polish female adolescents.

2. Materials and Methods

2.1. General Information

The analysis was conducted within the third phase of the PLACE-19 Study, assessing the psychological aspects of eating behaviors (conducted between 21 January and 17 February 2021). The first phase of the PLACE-19 Study assessed hygienic and personal protective behaviors, while the second phase assessed nutritional behaviors.

The PLACE-19 Study was conducted by the Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (WULS-SGGW). This study was approved by the Ethics Committee of the Central Clinical Hospital of the Ministry of Interior and Administration in Warsaw (No. 2/2021). It was conducted in agreement with the guidelines of the Declaration of Helsinki, and all the participants of this study, as well as their parents/legal guardians, expressed informed consent for participation.

2.2. Studied Population of Polish Female Adolescents

Recruitment within the PLACE-19 Study was conducted based on the random quota sampling of Polish secondary schools (separately conducted for each phase of this study). Based on the administrative division of Poland into 16 voivodeships and the division of voivodeships into counties, from each voivodeship, five counties were randomly selected ($16 \times 5 = 80$ counties). Afterwards, based on the national register of the secondary schools, from each county, five secondary schools were randomly selected ($80 \times 5 = 400$ secondary schools). The procedure of gathering a national sample within the secondary schools was chosen due to the high Net Enrollment Rate (NER) in Poland for this level of education (89.38% for December 2019, based on the statistics of the Central Statistical Office (CSO)).

in Poland [35]). As a consequence, the population of adolescents aged 15–20 years was targeted (typical age for secondary school students in Poland).

The principal of each selected secondary school was contacted and informed about this study. If the principal agreed to the participation of his school in this study, the students, as well as parents/legal guardians (in case of minor participants), were informed about this study, and their informed consents were gathered. The students, providing their informed consent (and the informed consent of their parents/legal guardians), received an electronic link to the study questionnaire to gather the necessary data within the Computer-Assisted Web Interview (CAWI).

The following inclusion criteria were applied: female individuals; age 15–20 years; being a student of a secondary school randomly chosen within a procedure of random quota sampling; informed consent provided; informed consent of parents/legal guardians provided by minor participants. The following exclusion criteria were applied: participation within any previous phase of the PLACE-19 Study; any missing/unreliable data within the questionnaires used for the analysis. The final sample for the conducted study was 816 female participants meeting the inclusion criteria and not excluded due to the exclusion criteria.

2.3. Questionnaire Applied Within This Study

To assess the level of perceived stress, the Adolescent Stress Questionnaire (ASQ) [36] was applied and to assess the emotional eating, the Emotional Eating Subscale (EE-3) [37] of the Three-Factor Eating Questionnaire (TFEQ) [38] was applied.

The ASQ was developed and validated by Byrne et al. [36]. Afterwards, it was used within large-scale studies, e.g., the international HELENA Study [39], and other studies in various countries—either in a full or in a shortened version [40–42], as it is stated to be the primary questionnaire of choice when measuring adolescent stress [43].

In the conducted study, ASQ was used in its full version, including 58 questions about stressors (including events, emotions, processes) relevant for adolescents. The 58 items are grouped within 10 stress component scales, based on the specific situations (contexts of stressors) associated with (1) home life, (2) school performance, (3) school attendance, (4) romantic relationships, (5) peer pressure, (6) teacher interaction, (7) future uncertainty, (8) school/leisure conflict, (9) financial pressure, and (10) emerging adult responsibility. For each question, a respondent is asked about the previous year and should define how stressful the potential stressor was, while using a 5-point Likert scale, using the categories as follows: ‘not stressful at all’ (1 point on a Likert scale), ‘a little stressful’ (2 points), ‘moderately stressful’ (3 points), ‘quite stressful’ (4 points), and ‘very stressful’ (5 points) [36]. Taking into account the total number of points (maximum: 58 questions $\times$ 5 points = 290 points), the stress level for each individual was defined based on the division of the studied group into quartiles of the stress level into lowest stress level within the studied group (first quartile), medium stress level within the studied group (second quartile and third quartile) and highest stress level within the studied group (fourth quartile).

The TFEQ was developed and validated by Stunkard & Messick [38] to measure cognitive restraint of eating (control over consumed food to influence body mass and/or body image), disinhibition (loss of control over food consumption), and hunger (subjective feeling of hunger and food cravings), based on 51 questions about eating behaviors. The following authors broadened the possibility of interpreting the obtained data, presenting the results and validations not only for the total TFEQ, but also for newly defined sub-scales (revised scales), including the Emotional Eating Subscale (EE-3), based on three questions

only [37]. The newly defined sub-scales, including EE-3, were validated and used in various studies [44–46], including the study conducted for a Polish population [47].

For each question, the respondent is asked about a specific statement and should define whether it is true or false for them, while the statements for EE-3 are as follows: (1) ‘When I feel anxious, I find myself eating’, (2) ‘When I feel blue, I often overeat’, (3) ‘When I feel lonely, I console myself by eating’. Respondent should assess each sentence while using a 4-point response scale, as follows: ‘definitely true’ (4 points), ‘mostly true’ (3 points), ‘mostly false’ (2 points), and ‘definitely false’ (1 point). Based on the total number of points obtained for the sub-scales, the raw scores for individuals were transformed into a result expressed in percent, based on the following equation: raw score minus lowest possible raw score (namely, 3 for EE-3), divided by possible raw score range (namely, 9 for EE-3) and multiplied by 100 [48]. Taking into account the total number of points (maximum: 100% after recalculation), the emotional eating level for each individual was defined based on the division of the studied group into sub-groups, as follows: emotional eating (recalculated result $\geq 50\%$), and no emotional eating (recalculated result $< 50\%$).

In order to adapt the tools for use in a Polish population, the applied questionnaires were adopted, according to the WHO recommendations [49]. The procedure of adaptation included: (1) forward translation from English to Polish, (2) backward translation from Polish to English, and (3) final polishing conducted by the panel of experts. Afterwards, the validation of the questionnaires included assessment of their internal consistency.

Except for the questions of the ASQ and EE-3, the study questionnaire included the questions allowing to verify the inclusion and exclusion criteria, as well as the questions about body mass and height (self-reported data).

In order to assess body mass, the BMI was calculated by dividing body mass in kilograms by the square of the body height in meters [50]. Afterwards, adult individuals (age > 18 years) were classified, based on the standard BMI cutoffs, as those with underweight ($< 18.5 \text{ kg/m}^2$), normal body weight ($18.5\text{--}25.0 \text{ kg/m}^2$), overweight ($25.0\text{--}30.0 \text{ kg/m}^2$), and obesity ($> 30.0 \text{ kg/m}^2$) [51]. At the same time, for minor individuals, the Polish gender- and age-specific growth reference values were used [52], while for each individual values were calculated using the Polish OLAF growth charts [53], and they were classified based on the standard WHO cutoffs, as those with underweight ($< 5\text{th}$ percentile), normal body weight ($5\text{th}\text{--}85\text{th}$ percentile), overweight ($85\text{th}\text{--}95\text{th}$ percentile) and obesity ($> 95\text{th}$ percentile) [54].

In order to assess body mass change, an additional question about the body mass and height one year before was asked, while to interpret existing change, the BMI from one year before was calculated separately and compared with the current value (using either the standard BMI value for adults, or growth reference values for minors).

2.4. Statistical Analysis

The normality of the data distribution was verified using two different methods depending on the group size—for large samples ($n > 100$), the Kolmogorov–Smirnov test with Lilliefors correction was applied, and for smaller samples, the Shapiro–Wilk test was applied. To compare subgroups, the *t*-Student test or Mann–Whitney U test were used for continuous variables (based on distribution), or the χ^2 test was used for categorical variables. The linear regression analysis and mediation analysis were conducted to verify the hypothesis of the mediating role of emotional eating for the influence of stress on body mass. The internal consistency of the ASQ component scales and EE-3 subscale was evaluated using Cronbach’s alpha coefficient, with a value of ≥ 0.70 considered acceptable [55].

The $p \leq 0.05$ was considered statistically significant. The statistical analysis was conducted using Statistica 8.0 (Statsoft Inc., Tulsa, OK, USA) and Jamovi (The Jamovi project, version 2.6.44, Sydney, NSW, Australia).

3. Results

The general characteristics and body mass measures of the Polish female adolescents in the PLACE-19 Study are presented in Table 1. Because the study population included participants under 18 years of age, as well as individuals up to 20 years old, BMI was reported as BMI values and as BMI percentiles for minors. The majority of adolescents were of normal weight; however, the proportion of participants with excessive weight was higher than that of those who were underweight. The distribution of recent body weight change was diverse, with similar proportions of participants reporting weight loss and weight gain, but the majority declaring no recent change in body mass.

Table 1. General characteristics and body mass measures of the Polish female adolescents in the PLACE-19 Study ($n = 816$).

Characteristics		Median ² (IQR)
Age (years)		17 (2)
Body Mass Index (BMI) (kg/m ²)		20.8 (4.1)
BMI percentile ¹		56 (54)
		<i>n</i> (%)
Body mass	Underweight	28 (3.4%)
	Normal body weight	619 (75.8%)
	Overweight	106 (13.0%)
	Obesity	63 (7.7%)
Body mass change	Decrease	237 (29.0%)
	No change	357 (43.7%)
	Increase	223 (27.3%)

¹—reported for minors ($n = 771$); ²—non-parametric distribution confirmed by Kolmogorov–Smirnov test with Lilliefors correction; IQR—Interquartile range.

Stress scores of the Polish female adolescents in the PLACE-19 Study, assessed with the ASQ, are presented in Table 2. The highest stress burden was observed for home life and school performance component scales, while the lowest levels were reported for school attendance and emerging adult responsibilities. The Cronbach’s alpha values exceeded 0.80 for all domains, indicating good internal reliability, while, for school performance, peer pressure, future uncertainty, school/leisure conflict, and emerging adult responsibility component scales, values exceeded 0.90, indicating very good internal reliability.

Emotional eating scores of the Polish female adolescents in the PLACE-19 Study, assessed with the EE-3 of the TFEQ, are presented in Table 3. Emotional eating was most pronounced in response to loneliness, followed by depressive symptoms and anxiety. The Cronbach’s alpha value for the EE-3 exceeded 0.84, indicating good internal consistency.

Associations between stress continuous variable scores and body mass categories of the Polish female adolescents in the PLACE-19 Study are presented in Table 4. Most stress domains did not differ significantly between underweight, normal body weight, and excessive body weight subgroups. However, adolescents with excessive body weight reported significantly higher scores for peer pressure ($p = 0.0011$) and financial pressure ($p = 0.0319$) than adolescents with normal weight. This suggests that, while overall stress may not be related to body mass status, specific psychosocial stressors, particularly interpersonal and financial ones, may affect adolescents with excessive body weight more than other adolescents.

Table 2. Stress scores of the Polish female adolescents in the PLACE-19 Study ($n = 816$), assessed with the Adolescent Stress Questionnaire (ASQ).

Stress Component Scales of the ASQ	Details	
	Cronbach's Alpha	Median ¹ (IQR)
Home life	0.90	23 (18)
School performance	0.93	21 (13)
School attendance	0.81	6 (4)
Romantic relationships	0.82	8 (8)
Peer pressure	0.92	14 (13)
Teacher interaction	0.87	14 (11)
Future uncertainty	0.91	11 (8)
School/leisure conflict	0.92	15 (11)
Financial pressure	0.90	8 (9)
Emerging adult responsibility	0.92	7 (8)
Total for general stress	-	134 (66)

¹—non-parametric distribution confirmed by Kolmogorov–Smirnov test with Lilliefors correction; IQR—Interquartile range.

Table 3. Emotional eating scores of the Polish female adolescents in the PLACE-19 Study ($n = 816$), assessed with the Emotional Eating Subscale (EE-3) of the Three-Factor Eating Questionnaire (TFEQ).

Emotional Eating Stimuli Within EE-3	Median ¹ (IQR)
Anxiety	33.3 (50.0)
Depression	33.3 (66.7)
Loneliness	33.3 (66.7)
Total for general emotional eating	33.3 (55.6)

¹—non-parametric distribution confirmed by Kolmogorov–Smirnov test with Lilliefors correction; IQR—Interquartile range.

Table 4. Associations between stress continuous variable scores, assessed with the Adolescent Stress Questionnaire (ASQ), and body mass categories of the Polish female adolescents in the PLACE-19 Study ($n = 816$).

Stress Component Scales of the ASQ	Details	Body Mass Sub-Groups			<i>p</i>
		Underweight ($n = 28$)	Normal Body Weight ($n = 619$)	Excessive Body Weight ($n = 169$)	
Home life	Median (IQR)	20.5 ¹ (19.5)	23 ² (17)	25 ² (18)	0.2650
	Mean $\pm$ SD	21.7 $\pm$ 8.1	-	-	
School performance	Median (IQR)	-	20 ² (13)	22 ² (14)	0.2369
School attendance	Median (IQR)	6 ¹ (5)	6 ² (4)	6 ² (5)	0.7659
Romantic relationships	Median (IQR)	9 ¹ (9)	8 ² (8)	8 ² (8)	0.2212
Peer pressure	Median (IQR)	13 ¹ (13) ^{ab}	14 ² (12) ^a	19 ² (14) ^b	0.0011
Teacher interaction	Median (IQR)	14 ¹ (12)	13 ² (11)	14 ² (10)	0.6303
Future uncertainty	Median (IQR)	11 ¹ (8.5)	10 ² (8)	11 ² (7)	0.8270
School/leisure conflict	Median (IQR)	15 ¹ (11)	14 ² (11)	16 ² (11)	0.3433
Financial pressure	Median (IQR)	6.5 ¹ (6.5) ^{ab}	8 ² (8) ^a	10 ² (9) ^b	0.0319
Emerging adult responsibility	Median (IQR)	6.5 ¹ (6.5)	7 ² (8)	7 ² (8)	0.8812
Total for general stress	Median (IQR)	126.5 ¹ (70.5)	131 ² (65)	142 ² (72)	0.1549

¹—non-parametric distribution confirmed by the Shapiro–Wilk test; ²—non-parametric distribution confirmed by Kolmogorov–Smirnov test with Lilliefors correction; ^{a,b}—different letters in rows indicate statistically significant differences; SD—standard deviation; IQR—Interquartile range.

Associations between emotional eating continuous variable scores and body mass categories of the Polish female adolescents in the PLACE-19 Study are presented in Table 5. Significant differences between weight groups were found for all emotional eating stimuli and for the total emotional eating score ($p < 0.05$). Adolescents with excessive body weight

reported higher emotional eating scores than those with normal weight or underweight. These findings highlight a consistent relationship between excessive body mass and the tendency to eat in response to negative emotional states.

Table 5. Associations between emotional eating continuous variable scores, assessed with the Emotional Eating Subscale (EE-3) of the Three-Factor Eating Questionnaire (TFEQ), and body mass categories of the Polish female adolescents in the PLACE-19 Study ($n = 816$).

Emotional Eating Stimuli Within EE-3	Details	Body Mass Sub-Groups			<i>p</i>
		Underweight ($n = 28$) ¹	Normal Body Weight ($n = 619$) ²	Excessive Body Weight ($n = 169$) ²	
Anxiety	Median (IQR)	0 (16.7) ^a	33.3 (33.3) ^b	33.3 (66.7) ^b	0.0008
Depression	Median (IQR)	0 (33.3) ^a	33.3 (66.7) ^a	33.3 (66.7) ^b	0.0006
Loneliness	Median (IQR)	0 (66.7) ^a	33.3 (66.7) ^a	33.3 (66.7) ^b	0.0199
Total for general emotional eating	Median (IQR)	0 (44.4) ^a	33.3 (55.6) ^a	44.4 (55.6) ^b	0.0004

¹—non-parametric distribution confirmed by the Shapiro–Wilk test; ²—non-parametric distribution confirmed by Kolmogorov–Smirnov test with Lilliefors correction; ^{a,b}—different letters in rows indicate statistically significant differences; IQR—Interquartile range.

Associations between stress categories and body mass categories of the Polish female adolescents in the PLACE-19 Study are presented in Table 6. For the majority of subscales, the proportions of adolescents classified into low, medium, and high stress categories did not differ between body weight groups. The only exception was the peer pressure category ($p = 0.0016$), where individuals with excessive body weight were more frequently represented in the highest stress category than their normal-weight and underweight counterparts. This pattern underscores the importance of peer-related stress in understanding weight-related psychological vulnerabilities in this population.

Table 6. Associations between stress categories, assessed with the Adolescent Stress Questionnaire (ASQ), and body mass categories of the Polish female adolescents in the PLACE-19 Study ($n = 816$).

Stress Component Scales of the ASQ	Body Mass Sub-Groups									<i>p</i>
	Underweight (<i>n</i> = 28)			Normal Body Weight (<i>n</i> = 619)			Excessive Body Weight (<i>n</i> = 169)			
	Lowest SL	Medium SL	Highest SL	Lowest SL	Medium SL	Highest SL	Lowest SL	Medium SL	Highest SL	
Home life	12 (42.9%)	8 (28.6%)	8 (28.6%)	147 (23.7%)	296 (47.8%)	176 (28.4%)	37 (21.9%)	80 (47.3%)	52 (30.8%)	0.1447
School performance	5 (17.9%)	12 (42.9%)	11 (39.3%)	143 (23.1%)	314 (50.7%)	162 (26.2%)	30 (17.8%)	81 (47.9%)	58 (34.3%)	0.1472
School attendance	8 (28.6%)	11 (39.3%)	9 (32.1%)	139 (22.5%)	331 (53.5%)	149 (24.1%)	40 (23.7%)	78 (46.2%)	51 (30.2%)	0.2750
Romantic relationships	5 (17.9%)	13 (46.4%)	10 (35.7%)	188 (30.4%)	269 (43.5%)	162 (26.2%)	52 (30.8%)	74 (43.8%)	43 (25.4%)	0.6475
Peer pressure	4 (14.3%)	14 (50%)	10 (35.7%)	129 (20.8%)	328 (53%)	162 (26.2%)	32 (18.9%)	66 (39.1%)	71 (42%)	0.0016
Teacher interaction	5 (17.9%)	15 (53.6%)	8 (28.6%)	145 (23.4%)	306 (49.4%)	168 (27.1%)	29 (17.2%)	88 (52.1%)	52 (30.8%)	0.4812
Future uncertainty	9 (32.1%)	10 (35.7%)	9 (32.1%)	141 (22.8%)	302 (48.8%)	176 (28.4%)	38 (22.5%)	79 (46.7%)	52 (30.8%)	0.6608
School/leisure conflict	5 (17.9%)	15 (53.6%)	8 (28.6%)	133 (21.5%)	325 (52.5%)	161 (26%)	33 (19.5%)	81 (47.9%)	55 (32.5%)	0.5553
Financial pressure	9 (32.1%)	14 (50%)	5 (17.9%)	161 (26%)	287 (46.4%)	171 (27.6%)	32 (18.9%)	81 (47.9%)	56 (33.1%)	0.1881
Emerging adult responsibility	7 (25%)	15 (53.6%)	6 (21.4%)	148 (23.9%)	300 (48.5%)	171 (27.6%)	41 (24.3%)	76 (45%)	52 (30.8%)	0.8314
Total for general stress	7 (25%)	11 (39.3%)	10 (35.7%)	126 (20.4%)	321 (51.9%)	172 (27.8%)	29 (17.2%)	83 (49.1%)	57 (33.7%)	0.4001

SL—stress level—the lowest stress level (first quartile), the medium stress level (the second and third quartiles merged), and the highest stress level (fourth quartile).

Associations between emotional eating categories and body mass categories of the Polish female adolescents in the PLACE-19 Study are presented in Table 7. For the majority of emotional eating stimuli, the proportions of adolescents classified as presenting or not presenting emotional eating did not differ between body weight groups. A significant relationship was found only for anxiety ($p = 0.0345$), with adolescents with excessive body weight more likely to be classified as emotional eaters than their normal-weight and underweight counterparts.

Table 7. Associations between emotional eating categories, assessed with the Emotional Eating Subscale (EE-3) of the Three-Factor Eating Questionnaire (TFEQ), and body mass categories of the Polish female adolescents in the PLACE-19 Study ($n = 816$).

Emotional Eating Stimuli Within EE-3	Body Mass Sub-Groups						<i>p</i>
	Underweight (<i>n</i> = 28)		Normal Body Weight (<i>n</i> = 619)		Excessive Body Weight (<i>n</i> = 169)		
	Emotional Eating	No Emotional Eating	Emotional Eating	No Emotional Eating	Emotional Eating	No Emotional Eating	
Anxiety	4 (14.3%)	24 (85.7%)	146 (23.6%)	473 (76.4%)	54 (32%)	115 (68%)	0.0345
Depression	6 (21.4%)	22 (78.6%)	198 (32%)	421 (68%)	64 (37.9%)	105 (62.1%)	0.1499
Loneliness	8 (28.6%)	20 (71.4%)	253 (40.9%)	366 (59.1%)	78 (46.2%)	91 (53.8%)	0.1708
Total for general emotional eating	6 (21.4%)	22 (78.6%)	189 (30.5%)	430 (69.5%)	65 (38.5%)	104 (61.5%)	0.0707

The linear regression analysis to predict body mass of the Polish female adolescents in the PLACE-19 Study, based on stress and emotional eating, is presented in Table 8. Regression analysis showed that stress and emotional eating are positive predictors of body mass, and the model explained 17% of the body mass variance ($R^2 = 0.028$; $F(2, 768) = 11.1$; $p < 0.001$).

Table 8. Linear regression analysis to predict body mass of the Polish female adolescents in the PLACE-19 Study based on the stress and emotional eating ($n = 771$).

Variable	B	SE B	β	t	<i>p</i>	95% CI		Collinearity Statistics	
						LL	UL	Tolerance	VIF
(Intercept)	42.4608	3.4525		12.30	<0.001				
Stress	0.0566	0.0240	0.0851	2.36	0.019	0.0142	0.156	1.03	0.971
Emotional eating	0.1237	0.0343	0.1304	3.61	<0.001	0.0595	0.201	1.03	0.971

SE—standard error; CI—confidence interval; LL—lower level; UL—upper level; VIF—variance inflation factor.

The mediation analysis for emotional eating and the association between stress and body mass of the Polish minor female adolescents in the PLACE-19 Study is presented in Table 9 and Figure 1. Stress was found to be a significant positive predictor of emotional eating, and emotional eating was a significant positive predictor of body mass. The total direct and indirect effects were significant, which indicated that emotional eating partially mediates the relationship between stress and body mass among Polish female adolescents, while the direct influence explained 79% of the influence of stress on body mass, and the indirect influence, mediated by emotional eating, explained 21% of the influence of stress on body mass.

Table 9. The mediation analysis for the emotional eating and the association between stress and body mass of the Polish minor female adolescents in the PLACE-19 Study ($n = 771$).

Type	Effect	b	SE	95% CI		β	z	<i>p</i>
				LL	UL			
Indirect Component	Stress $\Rightarrow$ Emotional eating $\Rightarrow$ Body mass	0.0147	0.00510	0.00471	0.0247	0.0221	2.88	0.004
	Stress $\Rightarrow$ Emotional eating	0.1189	0.02487	0.0701	0.1676	0.1696	4.78	<0.001
	Emotional eating $\Rightarrow$ Body mass	0.1237	0.03418	0.0567	0.1907	0.1304	3.62	<0.001
Direct	Stress $\Rightarrow$ Body mass	0.0566	0.02396	0.0096	0.1035	0.0851	2.36	0.018
Total	Stress $\Rightarrow$ Body mass	0.0713	0.02382	0.0246	0.1180	0.1072	2.99	0.003

SE—standard error; CI—confidence interval; LL—lower level; UL—upper level.

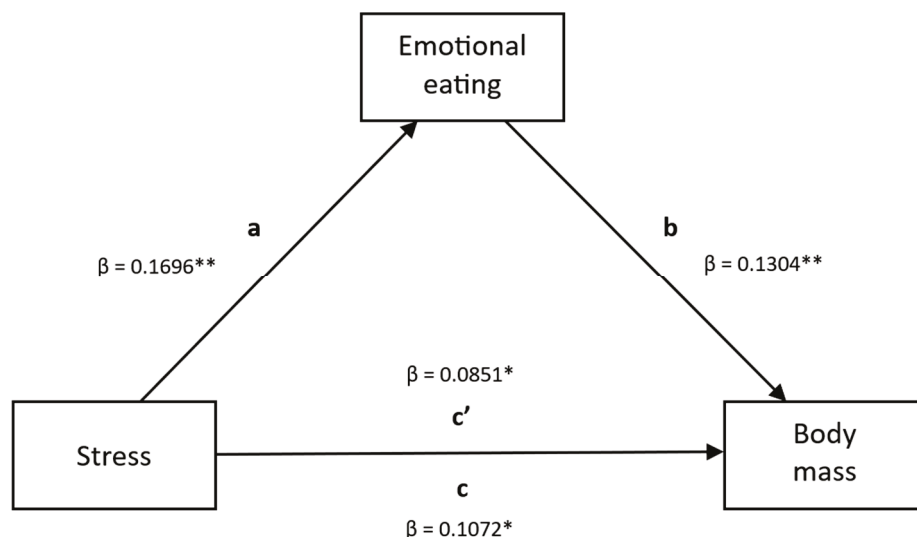


Figure 1. Path model of the mediation analysis for emotional eating and the association between stress and body mass of the Polish minor female adolescents in the PLACE-19 Study. Paths *a* and *b* represent the standardized regression coefficients for the indirect effect of stress on body mass via emotional eating. Path *c* and *c'* are the standardized regression coefficients for the total effect and direct effect, respectively. * $p < 0.05$; ** $p < 0.001$.

4. Discussion

The results of this study indicate that, while most adolescents presented a normal body weight, psychological factors, particularly emotional eating, were strongly associated with excessive body mass. Emotional eating emerged as a mediating factor for the association between stress and body mass, in spite of the fact that the direct influence of stress explained 79% of its influence on body mass, and for the indirect influence, mediated by emotional eating, the 21% of the influence of stress on body mass was indicated. These findings suggest that affect-driven eating behaviors, even if they are not the most important, may play a role in shaping weight status among Polish female adolescents.

In their review of emotional eating and weight in adults, Frayn & Knäuper [56] emphasized that emotional eating has a significant negative association with weight loss and should not be ignored when developing weight loss programs for adults. However, adolescence is a critical developmental period marked by increased vulnerability to stress and the maturation of neural systems involved in stress processing [57], meaning that analyses in this age group may yield different results. Indeed, the systematic review by Limbers & Summers [58], which included 13 eligible studies on emotional eating and weight among adolescents, concluded that the available evidence does not consistently support an association between emotional eating and excessive body weight or reduced weight-loss success in this age group. On the other hand, the more recent systematic review and meta-analysis by Demir & Bektaş [59], based on 16 eligible studies, found that emotional eating in adolescents had a positive and medium-sized effect and a significant impact on obesity risk. These findings align with the results obtained in the present study, indicating the most prominent role of stress, but also the mediating role of emotional eating.

This is confirmed by the results of other authors, focusing on stress as a factor contributing to excessive body mass in adolescents, but with improper nutritional habits exacerbating the problem [60]. Moreover, the studies conducted for adolescent girls indicated that the role of stress is observed not only for the body mass during this period, but also 12 months later, which indicates that it is a serious factor potentially associated with early cardiometabolic risk [61].

However, emotional eating behavior, as a moderating factor, must be indicated, as it may contribute to the development of overweightness or obesity, which is emphasized particularly in adolescents [59]. However, in adults, it can be observed not only among individuals with overweight or obesity but also among those with normal body weight or who are underweight [62]. Although emotional eating is most commonly defined as a coping mechanism associated with consumption in response to negative emotions [13], some researchers suggest that emotional eating may also be triggered by positive emotions. For example, underweight adults have been shown to eat less during negative emotional states but more during positive emotional states [62].

Emotional eating, defined as eating in response to emotions rather than hunger, is often associated with higher levels of perceived stress. This was confirmed in the study by Bell et al. [24], who found that emotional eating partially mediated the relationship between perceived stress and eating behavior in adolescents. However, not all adolescents respond to stress with changes in eating behavior. In the study by Sato et al. [63], the authors found that neither chronic stress nor subjective or objective stress reactivity was associated with emotional eating following stress induction. Moreover, it is worth emphasizing that specific stressors, such as peer pressure, appearance concerns, and social relationships, may trigger various eating-related behaviors. The study by Cohrdes et al. [64] revealed that weight- and appearance-related discrimination was associated with a higher risk of eating disorder symptoms in adolescents, whereas interpersonal pressure to be thin and criticism about appearance were associated with increases in disordered eating over time [65].

Moreover, the emotional coping strategies of adolescent girls are still underdeveloped, potentially leading to compensatory eating behaviors. As demonstrated by Lu et al. [66], higher levels of emotional suppression may increase the risk of emotional eating. In girls, higher suppression or a lack of cognitive reappraisal was independently associated with greater consumption of energy-dense foods, which may predispose them to weight gain.

This study has several strengths, including a well-defined population of Polish female adolescents and the use of validated questionnaires to assess stress and emotional eating. However, it also has some limitations. First, its cross-sectional design does not allow for conclusions about direct causality between experienced stress, emotional eating, and body mass. Second, both stress and emotional eating were assessed using self-reported questionnaires, which may be associated with subjective responses, and the instruments were not fully validated in the study population. Third, no data were collected on physical activity or diet quality, both of which could influence body mass and eating behaviors; additionally, body mass and height were assessed based on the self-reported data. Finally, these findings highlight the need for longitudinal studies to better understand the dynamics and causal relationships between stress, emotional eating, and body weight.

5. Conclusions

Adolescents with excessive body weight are more prone to stress and emotional eating. The stress itself affects body weight not only directly, but also by affecting emotional eating; therefore, adolescent girls should be taught how to cope with negative emotions using strategies other than increasing food consumption in response to negative emotions. Further studies should assess the mediating role of emotional eating among adolescent girls and evaluate the impact of stress management interventions on body weight.

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Abbreviations

The following abbreviations are used in this manuscript:

ASQ	Adolescent Stress Questionnaire
BMI	Body Mass Index
CI	Confidence Interval
EE-3	Emotional Eating Subscale
IQR	Interquartile range
LL	Lower Level
SE	Standard Error
SD	Standard Deviation
TFEQ	Three-Factor Eating Questionnaire
UL	Upper Level
VIF	Variance Inflation Factor
WHO	World Health Organization

References

1. World Health Organization. Stress. Available online: <https://www.who.int/news-room/questions-and-answers/item/stress> (accessed on 6 December 2025).
2. Fink, G. Stress, Definitions, Mechanisms, and Effects Outlined: Lessons from Anxiety. In *Stress: Concepts, Cognition, Emotion, and Behavior*; Fink, G., Ed.; Elsevier Inc.: San Diego, CA, USA, 2016; Volume 1, pp. 3–11.
3. Premji, S.S.; Yim, I.S.; Dosani Mawji, A.; Kanji, Z.; Sulaiman, S.; Musana, J.W.; Samia, P.; Shaikh, K.; Letourneau, N.; MiGHT Group. Psychobiobehavioral Model for Preterm Birth in Pregnant Women in Low- and Middle-Income Countries. *Biomed. Res. Int.* **2015**, *2015*, 450309. [CrossRef]
4. Manchia, M.; Gathier, A.W.; Yapici-Eser, H.; Schmidt, M.V.; de Quervain, D.; van Amelsvoort, T.; Bisson, J.I.; Cryan, J.F.; Howes, O.D.; Pinto, L.; et al. The impact of the prolonged COVID-19 pandemic on stress resilience and mental health: A critical review across waves. *Eur. Neuropsychopharmacol.* **2022**, *55*, 22–83. [CrossRef]
5. Amini-Rarani, M.; Rezaei, S.; Azami-Aghdash, S.; Bashzar, S.; Allahverdi, S.; Mohseni, M. The prevalence of stress during the COVID-19 pandemic: An umbrella review. *BMC Public Health* **2025**, *25*, 4022. [CrossRef]
6. Romeo, R.D. The Teenage Brain: The Stress Response and the Adolescent Brain. *Curr. Dir. Psychol. Sci.* **2013**, *22*, 140–145. [CrossRef] [PubMed]
7. Purvis, R.S.; Ayers, B.L.; Rowland, B.; Moore, R.; Hallgren, E.; McElfish, P.A. “Life is hard”: How the COVID-19 pandemic affected daily stressors of women. *Dialogues Health* **2022**, *1*, 100018. [CrossRef]
8. Maggi, G.; Baldassarre, I.; Barbaro, A.; Cavallo, N.D.; Cropano, M.; Nappo, R.; Santangelo, G. Age- and gender-related differences in the evolution of psychological and cognitive status after the lockdown for the COVID-19 outbreak: A follow-up study. *Neurol. Sci.* **2022**, *43*, 1521–1532. [CrossRef]
9. Beydoun, M.A. The interplay of gender, mood, and stress hormones in the association between emotional eating and dietary behavior. *J. Nutr.* **2014**, *144*, 1139–1141. [CrossRef]

10. Carpio-Arias, T.V.; Solís Manzano, A.M.; Sandoval, V.; Vinueza-Veloz, A.F.; Rodríguez Betancourt, A.; Betancourt Ortiz, S.L.; Vinueza-Veloz, M.F. Relationship between perceived stress and emotional eating. A cross sectional study. *Clin. Nutr. ESPEN* **2022**, *49*, 314–318. [CrossRef] [PubMed]
11. Herhaus, B.; Bastianon, C.; Ghassabei, S.; Petrowski, K. Fight or flight: The effect of heart rate reactivity on acute stress-induced food intake. *Physiol. Behav.* **2022**, *246*, 113704. [CrossRef]
12. Chao, A.M.; Jastreboff, A.M.; White, M.A.; Grilo, C.M.; Sinha, R. Stress, cortisol, and other appetite-related hormones: Prospective prediction of 6-month changes in food cravings and weight. *Obesity* **2017**, *25*, 713–720. [CrossRef] [PubMed]
13. Smith, J.; Ang, X.Q.; Giles, E.L.; Traviss-Turner, G. Emotional Eating Interventions for Adults Living with Overweight or Obesity: A Systematic Review and Meta-Analysis. *Int. J. Environ. Res. Public Health* **2023**, *20*, 2722. [CrossRef]
14. Elran Barak, R.; Shuval, K.; Li, Q.; Oetjen, R.; Drope, J.; Yaroch, A.L.; Fennis, B.M.; Harding, M. Emotional Eating in Adults: The Role of Sociodemographics, Lifestyle Behaviors, and Self-Regulation-Findings from a U.S. National Study. *Int. J. Environ. Res. Public Health* **2021**, *18*, 1744. [CrossRef] [PubMed]
15. Nguyen-Rodriguez, S.T.; Unger, J.B.; Spruijt-Metz, D. Psychological determinants of emotional eating in adolescence. *Eat. Disord.* **2009**, *17*, 211–224. [CrossRef]
16. Webb, H.J.; Kerin, J.L.; Zimmer-Gembeck, M.J. Increases in Emotional Eating During Early Adolescence and Associations With Appearance Teasing by Parents and Peers, Rejection, Victimization, Depression, and Social Anxiety. *J. Early Adolesc.* **2020**, *41*, 754–777. [CrossRef]
17. Samuel, L.; Cohen, M. Expressive suppression and emotional eating in older and younger adults: An exploratory study. *Arch. Gerontol. Geriatr.* **2018**, *78*, 127–131. [CrossRef]
18. Jääskeläinen, A.; Nevanperä, N.; Remes, J.; Rahkonen, F.; Järvelin, M.R.; Laitinen, J. Stress-related eating, obesity and associated behavioural traits in adolescents: A prospective population-based cohort study. *BMC Public Health* **2014**, *14*, 321. [CrossRef] [PubMed]
19. Haidar, S.A.; de Vries, N.K.; Karavetian, M.; El-Rassi, R. Stress, Anxiety, and Weight Gain among University and College Students: A Systematic Review. *J. Acad. Nutr. Diet.* **2018**, *118*, 261–274. [CrossRef] [PubMed]
20. Kivimäki, M.; Head, J.; Ferrie, J.E.; Shipley, M.J.; Brunner, E.; Vahtera, J.; Marmot, M.G. Work stress, weight gain and weight loss: Evidence for bidirectional effects of job strain on body mass index in the Whitehall II study. *Int. J. Obes.* **2006**, *30*, 982–987. [CrossRef]
21. Hunot-Alexander, C.; Arellano-Gómez, L.P.; Smith, A.D.; Kaufer-Horwitz, M.; Vásquez-Garibay, E.M.; Romero-Velarde, E.; Fildes, A.; Croker, H.; Llewellyn, C.H.; Beeken, R.J. Examining the validity and consistency of the Adult Eating Behaviour Questionnaire-Español (AEBQ-Esp) and its relationship to BMI in a Mexican population. *Eat. Weight Disord.* **2022**, *27*, 651–663. [CrossRef]
22. Konttinen, H.; Van Strien, T.; Männistö, S.; Jousilahti, P.; Haukkala, A. Depression, emotional eating and long-term weight changes: A population-based prospective study. *Int. J. Behav. Nutr. Phys. Act.* **2019**, *16*, 28. [CrossRef]
23. Ohrt, T.K.; Perez, M.; Liew, J.; Hernández, J.C.; Yu, K.Y. The influence of temperament on stress-induced emotional eating in children. *Obes. Sci. Pract.* **2020**, *6*, 524–534. [CrossRef]
24. Bell, B.M.; Spruijt-Metz, D.; Naya, C.H.; Lane, C.J.; Wen, C.K.F.; Davis, J.N.; Weigensberg, M.J. The mediating role of emotional eating in the relationship between perceived stress and dietary intake quality in Hispanic/Latino adolescents. *Eat. Behav.* **2021**, *42*, 101537. [CrossRef]
25. Lazarevich, I.; Irigoyen Camacho, M.E.; Velázquez-Alva, M.D.C.; Zepeda, M.Z. Relationship among obesity, depression, and emotional eating in young adults. *Appetite* **2016**, *107*, 639–644. [CrossRef]
26. Barcın-Güzeldere, H.K.; Devrim-Lanpir, A. The Association Between Body Mass Index, Emotional Eating and Perceived Stress during COVID-19 Partial Quarantine in Healthy Adults. *Public Health Nutr.* **2022**, *25*, 43–50. [CrossRef]
27. van Strien, T.; Konttinen, H.; Homberg, J.R.; Engels, R.C.; Winkens, L.H. Emotional eating as a mediator between depression and weight gain. *Appetite* **2016**, *100*, 216–224. [CrossRef] [PubMed]
28. Evers, C.; Dingemans, A.; Junghans, A.F.; Boevé, A. Feeling bad or feeling good, does emotion affect your consumption of food? A meta-analysis of the experimental evidence. *Neurosci. Biobehav. Rev.* **2018**, *92*, 195–208. [CrossRef]
29. Godet, A.; Fortier, A.; Bannier, E.; Coquery, N.; Val-Laillet, D. Interactions between emotions and eating behaviors: Main issues, neuroimaging contributions, and innovative preventive or corrective strategies. *Rev. Endocr. Metab. Disord.* **2022**, *23*, 807–831. [CrossRef]
30. Dakanalis, A.; Mentzelou, M.; Papadopoulou, S.K.; Papandreou, D.; Spanoudaki, M.; Vasios, G.K.; Pavlidou, E.; Mantzorou, M.; Giaginis, C. The Association of Emotional Eating with Overweight/Obesity, Depression, Anxiety/Stress, and Dietary Patterns: A Review of the Current Clinical Evidence. *Nutrients* **2023**, *15*, 1173. [CrossRef] [PubMed]
31. Guzek, D.; Skolmowska, D.; Głabska, D. Analysis of Self-Regulation of Eating Behaviors within Polish Adolescents' COVID-19 Experience (PLACE-19) Study. *Nutrients* **2022**, *14*, 1679. [CrossRef]

32. Skolmowska, D.; Głabska, D.; Guzek, D. Body Mass and Emotional Eating: Emotional Eater Questionnaire (EEQ) in the Polish Adolescents' COVID-19 Experience (PLACE-19) Study. *Nutrients* **2022**, *14*, 828. [CrossRef]
33. Głabska, D.; Skolmowska, D.; Guzek, D. Emotional Overeating during the COVID-19 Pandemic: Polish Adolescents' COVID-19 Experience (PLACE-19) Study. *Nutrients* **2023**, *15*, 3818. [CrossRef]
34. Skolmowska, D.; Głabska, D.; Guzek, D. Stress during the COVID-19 Pandemic and Emotional Eating Scale Adapted for Children and Adolescents (EES-C) Results in Girls: Polish Adolescents' COVID-19 Experience (PLACE-19) Study. *Nutrients* **2023**, *15*, 4197. [CrossRef]
35. The Central Statistical Office in Poland December 2019. Available online: <https://bdl.stat.gov.pl/BDL/dane/podgrup/temat> (accessed on 15 June 2020).
36. Byrne, D.G.; Davenport, S.C.; Mazanov, J. Profiles of adolescent stress: The development of the adolescent stress questionnaire. (ASQ). *J. Adolesc.* **2007**, *30*, 393–416. [CrossRef] [PubMed]
37. Karlsson, J.; Persson, L.O.; Sjöström, L.; Sullivan, M. Psychometric properties and factor structure of the Three-Factor Eating Questionnaire (TFEQ) in obese men and women. Results from the Swedish Obese Subjects (SOS) study. *Int. J. Obes.* **2000**, *24*, 1715–1725. [CrossRef] [PubMed]
38. Stunkard, A.J.; Messick, S. The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger. *J. Psychosom. Res.* **1985**, *29*, 71–83. [CrossRef] [PubMed]
39. De Vriendt, T.; Clays, E.; Moreno, L.A.; Bergman, P.; Vicente-Rodriguez, G.; Nagy, E.; Dietrich, S.; Manios, Y.; De Henauw, S.; HELENA Study Group. Reliability and validity of the Adolescent Stress Questionnaire in a sample of European adolescents—the HELENA study. *BMC Public Health* **2011**, *11*, 717. [CrossRef]
40. Kallianta, M.K.; Katsira, X.E.; Tsitsika, A.K.; Vlachakis, D.; Chrousos, G.; Darviri, C.; Bacopoulou, F. Stress management intervention to enhance adolescent resilience: A randomized controlled trial. *EMBnet J.* **2021**, *26*, e967. [CrossRef]
41. Efthymiou, V.; Kotsikogianni, D.; Tsitsika, A.K.; Vlachakis, D.; Chrousos, G.P.; Charmandari, E.; Bacopoulou, F. Validation of the Greek version of the Adolescent Sleep Hygiene Scale (ASHS). *EMBnet J.* **2021**, *26*, e979. [CrossRef]
42. McKay, M.; Andretta, J.; Perry, J. The shortened version of the Adolescent Stress Questionnaire (ASQ-S.; Sweden): A validation study in United Kingdom adolescents. *Scand. J. Child Adolesc. Psychiatr. Psychol.* **2019**, *7*, 81–87. [CrossRef]
43. Kokka, I.; Chrousos, G.P.; Darviri, C.; Bacopoulou, F. Measuring Adolescent Chronic Stress: A Review of Established Biomarkers and Psychometric Instruments. *Horm. Res. Paediatr.* **2023**, *96*, 74–82. [CrossRef]
44. Swartz, M.C.; Basen-Engquist, K.M.; Markham, C.; Lyons, E.J.; Cox, M.; Chandra, J.; Ater, J.L.; Askins, M.A.; Scheurer, M.E.; Lupo, P.J.; et al. Psychometric Analysis of the Three-Factor Eating Questionnaire-R18V2 in Adolescent and Young Adult-Aged Central Nervous System Tumor Survivors. *J. Adolesc. Young Adult Oncol.* **2016**, *5*, 278–285. [CrossRef]
45. Alhebshi, S.; Hilary, S.; Safi, S.K.H.; Ali, H.I.; Cheikh Ismail, L.; Al Dhaheri, A.; Stojanovska, L. Validity and Reliability of the Arabic Version of the Three-Factor Eating Questionnaire-R18. *Heliyon* **2023**, *9*, e17623. [CrossRef] [PubMed]
46. Martins, B.G.; da Silva, W.R.; Maroco, J.; Campos, J.A.D.B. Psychometric characteristics of the Three-Factor Eating Questionnaire-18 and eating behavior in undergraduate students. *Eat. Weight Disord.* **2021**, *26*, 525–536. [CrossRef] [PubMed]
47. Dzielska, A.; Mazur, J.; Małkowska-Szkućnik, A.; Nałęcz, H. Adaptation of the Three-Factor Eating Questionnaire (TFEQ-13) for school-aged adolescents in a population study [Adaptacja polskiej wersji kwestionariusza Three-Factor Eating Questionnaire (TFEQ-13) wśród młodzieży szkolnej w badaniach populacyjnych]. *Probl. Hig. Epidemiol.* **2009**, *90*, 362–369.
48. de Lauzon, B.; Romon, M.; Deschamps, V.; Lafay, L.; Borys, J.M.; Karlsson, J.; Ducimetière, P.; Charles, M.A.; Fleurbaey Laventie Ville Sante Study Group. The Three-Factor Eating Questionnaire-R18 is able to distinguish among different eating patterns in a general population. *J. Nutr.* **2004**, *134*, 2372–2380. [CrossRef]
49. World Health Organization (WHO). Process of Translation and Adaptation of Instruments. Available online: <https://www.coursehero.com/file/30372721/WHO-Process-of-translation-and-adaptation-of-instrumentspdf/> (accessed on 4 August 2023).
50. World Health Organization (WHO). Obesity and Overweight. Available online: <https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight> (accessed on 22 December 2025).
51. World Health Organization (WHO). Body Mass Index—BMI. Available online: <https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/body-mass-index> (accessed on 4 August 2023).
52. Kulaga, Z.; Litwin, M.; Tkaczyk, M.; Rózdzyńska, A.; Barwicka, K.; Grajda, A.; Swiader, A.; Gurzkowska, B.; Napieralska, E.; Pan, H. The height-, weight-, and BMI-for-age of Polish school-aged children and adolescents relative to international and local growth references. *BMC Public Health* **2010**, *10*, 109. [CrossRef]
53. OLAF. Calculator from OLAF Study. Available online: <https://www.surf.nl/en/online-laboratory-calculus-with-automaticinstant-feedback-olaf> (accessed on 4 August 2023).
54. De Onis, M.; Onyango, A.W.; Borghi, E.; Siyam, A.; Nishida, C.; Siekmann, J. Development of a WHO growth reference for school-aged children and adolescents. *Bull. World Health Organ.* **2007**, *85*, 660–667. [CrossRef] [PubMed]
55. Nunnally, J.C.; Bernstein, I.H. *Psychometric Theory*; McGraw-Hill: New York, NY, USA, 1994.

56. Frayn, M.; Knäuper, B. Emotional Eating and Weight in Adults: A Review. *Curr. Psychol.* **2018**, *37*, 924–933. [CrossRef]
57. Sisk, L.M.; Gee, D.G. Stress and adolescence: Vulnerability and opportunity during a sensitive window of development. *Curr. Opin. Psychol.* **2022**, *44*, 286–292. [CrossRef]
58. Limbers, C.A.; Summers, E. Emotional Eating and Weight Status in Adolescents: A Systematic Review. *Int. J. Environ. Res. Public Health* **2021**, *18*, 991. [CrossRef]
59. Demir Kösem, D.; Bektaş, M. The effect of emotional eating in adolescents on obesity: A systematic review and meta-analysis study. *J. Pediatr. Nurs.* **2025**, *81*, e39–e46. [CrossRef] [PubMed]
60. Tajik, E.; Zulkefli, N.A.; Baharom, A.; Minhat, H.S.; Latiff, L.A. Contributing factors of obesity among stressed adolescents. *Electron. Physician* **2014**, *6*, 771–778. [CrossRef]
61. Lee, S.Y.; Jao, N.C.; Gaffey, A.E.; Reid, B.M.; Vergara-Lopez, C.; Bublitz, M.H.; Stroud, L.R. Female adolescents' early life stress and body mass index: Differential effects of anger and anxiety in response to rejection. *J. Adolesc.* **2024**, *96*, 830–840. [CrossRef]
62. Geliebter, A.; Aversa, A. Emotional eating in overweight, normal weight, and underweight individuals. *Eat. Behav.* **2003**, *3*, 341–347. [CrossRef]
63. Sato, A.F.; Darling, K.E.; Ruzicka, E.B.; Fahrenkamp, A.J.; Shields, C.V.; West, C.E.; Schuler, S.M. A biobehavioral examination of emotional eating in adolescents: Evaluating the role of acute cortisol stress reactivity in the context of a multi-method assessment of eating in response to stress. *Appetite* **2023**, *184*, 106486. [CrossRef]
64. Cohrdes, C.; Santos-Hövenner, C.; Kajikhina, K.; Hölling, H. The role of weight- and appearance-related discrimination on eating disorder symptoms among adolescents and emerging adults. *BMC Public Health* **2021**, *21*, 1751. [CrossRef] [PubMed]
65. Shomaker, L.B.; Furman, W. Interpersonal influences on late adolescent girls' and boys' disordered eating. *Eat. Behav.* **2009**, *10*, 97–106. [CrossRef]
66. Lu, Q.; Tao, F.; Hou, F.; Zhang, Z.; Ren, L.L. Emotion regulation, emotional eating and the energy-rich dietary pattern. A population-based study in Chinese adolescents. *Appetite* **2016**, *99*, 149–156. [CrossRef] [PubMed]

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Article

Relationship Between Nutritional Status and Systemic Immune–Inflammation Indices Across BMI Categories

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Abstract

Background: This study aimed to determine the relationship between nutritional status and systemic inflammation using four validated nutrition indices—Hemoglobin, Albumin, Lymphocyte, and Platelet (HALP) score, Prognostic Nutritional Index (PNI), Controlling Nutritional Status (CONUT) score, and Nutritional Risk Index (NRI)—and three immune–inflammation biomarkers—Systemic Immune–Inflammation Index (SII), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR)—in healthy adults with varying body mass index (BMI) levels. **Methods:** This retrospective study included 290 clinically healthy adults aged 18–60 years, categorized by BMI. Individuals with chronic diseases, medication use, or morbid obesity (BMI ≥ 40 kg/m²) were excluded. Nutrition scores (HALP, PNI, NRI, CONUT) and systemic immune–inflammation indices (SII, NLR, PLR) were calculated from laboratory data. For the comparisons of SII, PLR, NLR, PNI, HALP, NRI, and CONUT values between groups, age was adjusted for, and an ANCOVA test was performed. **Results:** Among the systemic immune–inflammation indices, SII and NLR were significantly higher in both the overweight and obesity groups. The CONUT score, a negative indicator of nutritional status, demonstrated positive correlations with SII, NLR, and PLR in the overweight group, and with PLR in the obesity group. Although PNI showed significant inverse correlations with SII, PLR, and NLR in both groups, the mean PNI values remained above 50, indicating overall normal nutritional status in the study population. HALP was inversely correlated with SII, PLR, and NLR in both groups. **Conclusions:** The HALP score appears to be the most reliable marker, as it reflects the inverse relationship between nutritional status and systemic immune–inflammation indices.

Keywords: BMI; HALP score; PNI; NRI; CONUT; SII; NLR; PLR

1. Introduction

Maintaining a healthy body mass index (BMI) is crucial for preventing premature death from high blood pressure, cardiovascular disorders, cerebrovascular diseases, and certain cancers. Overweight individuals or those with obesity are also at an increased risk of other chronic diseases such as type 2 diabetes, psychiatric disorders, respiratory diseases, and skeletal disorders [1–3]. A high BMI is associated with chronic low-grade inflammation.

Excess adipose tissue promotes the secretion of pro-inflammatory cytokines, including tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6). This leads to macrophage infiltration into adipose tissue and decreases adiponectin synthesis, creating a proinflammatory environment and oxidative stress. These inflammatory factors can trigger a systemic inflammatory response, leading to diseases associated with obesity [1,3].

A complete blood count (CBC) is an affordable and easily accessible blood test. Biomarkers like the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and Systemic Immune-Inflammation Index (SII) are derived from CBC and have been shown to help in the diagnosis, monitoring, and assessment of various systemic inflammatory conditions [1–5]. Neutrophils and lymphocytes are key players in inflammation. A lower NLR is linked to better outcomes in different cancer types and coronary artery disease [4–8]. In a Spanish study, an inflammatory state measured by NLR was positively associated with abdominal obesity and negatively associated with diet quality [9]. Another study conducted in adolescents with obesity found that NLR and C-reactive protein (CRP) levels were significantly higher in the obesity group compared to healthy controls [10]. Blood platelets also considerably contribute to the immune response in chronic inflammation caused by obesity [11]. The SII measures the relationship between platelet count, neutrophil count, and lymphocyte count and is considered the most effective tool to reflect the balance between inflammatory factors and immune responses in the body [1–5]. Studies have demonstrated a significant positive correlation between SII and obesity [1,2].

Malnutrition is often perceived to affect only underweight individuals. In fact, there is a large burden of malnutrition in the overweight and individuals with obesity due to inadequate micronutrient consumption and poor food quality [12]. Another reason for malnutrition in obesity is the inability to preserve body composition and performance due to sarcopenia [13]. The quality of diet affects systemic inflammation status in obesity [9]. Therefore, beyond BMI alone, routine nutritional screening is recommended for the management of most acute and chronic conditions, particularly in hospitalized patients. A validated and widely utilized nutritional screening tool includes the Controlling Nutritional Status (CONUT) score, the Nutritional Risk Index (NRI), and the Prognostic Nutritional Index (PNI). This is a nutritional assessment tool based on easily accessible laboratory indicators. Its superior prognostic value has been demonstrated [14]. Another scoring system for assessing a patient's nutritional status and prognosis is the Hemoglobin, Albumin, Lymphocyte, and Platelet (HALP) score. The HALP score has recently emerged as a novel biomarker that reflects both nutritional reserves and immune-inflammatory activity [15,16].

The present study aimed to investigate the association between nutritional status and systemic inflammation according to BMI categories in a healthy population. For this purpose, individuals with normal and high BMI were evaluated for their nutritional status using HALP, PNI, CONUT, and NRI scores. The relationship between these nutritional indices and systemic immune-inflammatory markers (SII, NLR, PLR) was then examined separately in normal- and high-BMI groups.

2. Materials and Methods

2.1. Ethical Approval

This study was conducted as a retrospective case-control study at the Department of Internal Medicine, Istanbul Atlas University Medical Faculty Hospital. The study was designed in accordance with the Declaration of Helsinki and good clinical practice guidelines. It was approved by Istanbul Atlas University Non-Interventional Scientific Research Ethics Committee (Approval number: E-22686390-050.99-61546, date: 17 February 2025). The Ethics

Committee waived the requirement for written informed consent due to the retrospective design of the study.

2.2. Study Design

This retrospective study included individuals who attended the Internal Medicine Outpatient Clinic of Istanbul Atlas University for their annual health check-up between March and July 2024. During this period, a total of 290 healthy individuals were evaluated, of whom 156 had a normal body mass index, 71 were classified as overweight, and 63 were classified as individuals with obesity. Individuals with morbid obesity ($\text{BMI} \geq 40.0 \text{ kg/m}^2$) were excluded from the study, as systemic inflammation is commonly present in this population and could confound the results. According to the WHO classification, individuals were categorized as normal weight with a BMI of $18.5\text{--}24.9 \text{ kg/m}^2$, overweight with a BMI of $25.0\text{--}29.9 \text{ kg/m}^2$, and obesity with a BMI $\geq 30.0 \text{ kg/m}^2$. Individuals with a BMI $\geq 40.0 \text{ kg/m}^2$ were classified as morbid obesity, corresponding to WHO Obesity Class III [17]. Individuals with normal BMI constituted the control group, while individuals who were overweight or with obesity constituted the study group.

The study included individuals between the ages of 18–60 who are completely healthy, do not use any medication that could affect inflammation, and do not smoke or consume alcohol. The sociodemographic characteristics of the participants, as well as their height, weight, waist circumference, physical examination findings, and laboratory test results, were obtained retrospectively from their medical records.

2.2.1. Inclusion Criteria

Individuals aged 18 to 60 years who did not have a known chronic illness, did not use any medication that could affect inflammation, did not smoke, or consume alcohol were included in the study. Only those with a BMI between 18.5 and 39.99 kg/m^2 were included in the study.

2.2.2. Exclusion Criteria

Individuals with any chronic illness, such as diabetes, chronic renal insufficiency, thyroid dysfunction, hepatic dysfunction, metabolic syndrome, autoimmune disease, malignancy, active infection, or a recent history of trauma or surgery, were excluded from the study. Additional exclusion criteria included the use of steroids, anti-inflammatory drugs, antiepileptic medications, antioxidant supplements, or vitamin preparations; smoking or alcohol consumption; and pregnancy. Furthermore, individuals over the age of 60 and with a BMI $\geq 40.0 \text{ kg/m}^2$ were excluded, as systemic inflammation is commonly present in this population and could confound the results.

2.3. Assessments

Comprehensive medical histories—including information on smoking and alcohol use, physical examination findings such as weight, height, and waist circumference, and laboratory test results—were obtained from the medical records of the subjects. Waist circumference was measured with the participant standing upright, feet shoulder-width apart, arms relaxed at the sides, and breathing normally. A non-stretchable, flexible measuring tape was used to measure the waist circumference at the midpoint between the lower margin of the last palpable rib and the top of the iliac crest. Body weight was measured with a margin of error of 0.1 kg using an electronic scale (Seca digital scale, 0.1 precision, Hamburg, Germany), allowing only underwear to remain. Height was measured with a Harpenden stadiometer (Seca mod. 240 ce 0123, made in Hamburg, Germany) with an error margin of 0.1 cm. Height measurements were performed in the vertical position with bare feet, feet together and parallel, and the shoulder and gluteal

region in contact with the wall. BMI was calculated by dividing weight (kg) by height squared (m^2).

Fasting venous blood samples were drawn between 8 and 10 a.m. after the subjects fasted overnight (10–12 h). Blood samples were drawn from the brachial veins in the brachial fossa and placed into plain tubes and anticoagulant-free tubes. The samples were centrifuged for 10 min at $4000 \times g$ rpm at 4°C . Biochemical tests were performed immediately. The result of CBC was recorded with an automatic hematology analyzer (Sysmex, Sysmex XN-1000, Norderstedt, Germany).

Biochemical parameters such as fasting blood glucose (FBG), low-density lipoprotein cholesterol (LDL-C), very-low-density lipoprotein cholesterol (VLDL-C), high-density lipoprotein cholesterol (HDL-C), triglyceride, total cholesterol, total protein, albumin, uric acid, and creatinine were determined using enzymatic methods (Architect I2000, Abbott Park, IL, USA). Insulin levels were measured by the electrochemiluminescence immunoassay (ECLIA) method on Roche-Hitachi E170 (Roche/Hitachi MODULAR Analytics Combination Systems, Roche Diagnostics, Indianapolis, IN, USA). Hemoglobin A1c (HbA1c) determination was based on High-Performance Liquid Chromatography (HPLC) (Variant Turbo II, Bio-Rad Laboratories, Inc., Hercules, CA, USA). Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated by using the following formula:

$$\text{HOMA-IR} = \text{Fasting glucose (mg/dL)} \times \text{Fasting insulin (mU/L)} / 405$$

Nutrition Scores

Nutritional indices, including the PNI score, CONUT score, and NRI score, and systemic immune–inflammation indices were calculated and interpreted based on established formulas from the literature [14–20].

The **PNI score** was calculated using the following formula:

$$\text{PNI} = (10 \times \text{serum albumin [g/dL]}) + (0.005 \times \text{peripheral lymphocyte count [}/\text{mm}^3\text{]})$$

The Prognostic Nutritional Index (PNI) is a clinical tool originally developed to assess nutritional and immunological status, particularly in patients undergoing surgery or with chronic illnesses such as cancer, cardiovascular disease, and chronic inflammatory conditions. Lower PNI scores indicate a higher risk of malnutrition. A PNI score > 50 indicates normal nutritional and immune status, whereas scores between 45 and 50 suggest a mild risk of malnutrition, 40–45 indicate moderate risk, and scores < 40 reflect severe malnutrition or a high nutritional risk [18].

The **CONUT score** was determined using serum albumin level, total lymphocyte count, and total cholesterol level. The scoring system ranges from 0 to 12, with higher scores indicating worse nutritional status. The point allocation for each parameter is as follows:

Serum albumin (g/dL):

$\geq 3.5 = 0$ points; $3.0\text{--}3.49 = 2$ points; $2.5\text{--}2.99 = 4$ points; $< 2.5 = 6$ points.

Lymphocyte count ($/\mu\text{L}$):

$\geq 1600 = 0$ points; $1200\text{--}1599 = 1$ point; $800\text{--}1199 = 2$ points; $< 800 = 3$ points.

Total cholesterol (mg/dL):

$\geq 180 = 0$ points; $140\text{--}179 = 1$ point; $100\text{--}139 = 2$ points; $< 100 = 3$ points.

Higher CONUT scores indicate nutritional deficiency and immunosuppression. The total CONUT score ranges from 0 to 12 and is interpreted as follows: 0–1 point: normal nutritional status, 2–4 points: mild risk of malnutrition, 5–8 points: moderate malnutrition, 9–12 points: severe malnutrition [19].

The **NRI score** was calculated using the formula:

$$\text{NRI} = (1.519 \times \text{serum albumin [g/dL]}) + (41.7 \times \text{actual body weight [kg]}/\text{ideal body weight [kg]})$$

Lower NRI scores indicate a higher risk of malnutrition. An NRI score of 100 or higher is considered indicative of the absence of nutritional risk. An NRI score between 97.5 and 99.9 suggests mild risk, 83.5 to 97.4 suggests moderate risk, and below 83.5 indicates severe risk of malnutrition [20].

The **HALP score** was calculated using the following formula:

$$\text{HALP} = [\text{hemoglobin (g/L)} \times \text{albumin (g/L)} \times \text{lymphocyte count (/L)}]/\text{platelet count (/L)}$$

This composite index reflects the interplay between nutritional and inflammatory status [15,16]. There is no universally accepted cut-off, as HALP values may vary by population and disease context. However, lower HALP scores suggest malnutrition, inflammation, or poor prognosis

Systemic Immune–Inflammation Indices:

SII is a new and integrated inflammatory biomarker derived from CBC parameters that reflects the balance between the host's immune and inflammatory status. SII was calculated using the formula [1]:

$$\text{SII} = (\text{platelet count [/L]} \times \text{neutrophil count [/L]})/\text{lymphocyte count [/L]}$$

The SII index reflects both innate (neutrophils, platelets) and adaptive (lymphocytes) immune responses.

In addition to SII, NLR and PLR were also calculated for each subject using their respective neutrophil, lymphocyte, and platelet counts obtained from the CBC.

Neutrophil-to-Lymphocyte Ratio (NLR) and Platelet-to-Lymphocyte Ratio (PLR) are calculated using the following formulas [9,11]:

$$\text{NLR} = \text{absolute neutrophil count [/L]}/\text{absolute lymphocyte count [/L]}$$

$$\text{PLR} = \text{platelet count [/L]}/\text{absolute lymphocyte count [/L]}$$

2.4. Statistical Analysis

The statistical analyses were performed using SPSS version 27 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA). Quantitative variables were presented as mean, standard deviation, median, minimum, and maximum values, while qualitative variables were expressed as frequency and percentage. The normality of data distribution was assessed using the Shapiro–Wilk test, skewness and kurtosis values, and Box Plot graphics.

For the comparison of more than two normally distributed quantitative variables, the One-Way ANOVA test was applied, and the Bonferroni test was used for post hoc pairwise comparisons. For non-normally distributed variables, the Kruskal–Wallis test was used, and the Dunn test was applied to identify the source of the difference (Table 1).

For the comparisons of SII, PLR, NLR, PNI, HALP, NRI, and CONUT values between groups, age-adjusted ANCOVA analyses were conducted. Bonferroni post hoc tests were subsequently applied to identify the specific groups contributing to the observed differences (Table 2).

To evaluate the relationships between variables, partial correlation analyses were performed for SII, PLR, NLR, PNI, HALP, NRI, and CONUT values, while adjusting for the effect of age (Tables 3 and 4).

Table 1. The comparison of baseline characteristics and laboratory findings of the study population by BMI.

	Normal (n = 156)		Overweight (n = 71)		Obesity (n = 63)		p-Value
	Mean $\pm$ SD	Median (Min–Max)	Mean $\pm$ SD	Median (Min–Max)	Mean $\pm$ SD	Median (Min–Max)	
Age (years)	39.91 $\pm$ 10.45	40 (21–65)	42.51 $\pm$ 10.72	43 (17–59)	45.56 $\pm$ 9.63	47 (26–69)	^a 0.001 **
Waist circumference (cm)	83.51 $\pm$ 9.89	84 (60–101)	92.73 $\pm$ 10.38	92 (72–119)	106.81 $\pm$ 9.60	107 (86–128)	^a 0.001 **
WBC (/ μ L)	7108.33 $\pm$ 1629.43	7000 (3400–12,300)	6964.23 $\pm$ 1574.45	6800 (3700–12,400)	7522.54 $\pm$ 1626.82	7500 (3550–10,900)	^a 0.114
CRP (mg/L)	1.98 $\pm$ 1.56	1.8 (0.1–7)	3.14 $\pm$ 4.03	2 (0.3–27)	4.19 $\pm$ 3.26	2.9 (0.4–16)	^b 0.001 **
FBG (mg/dL)	86.58 $\pm$ 9.88	85.7 (60–119)	89.23 $\pm$ 15.46	85 (64–130)	95.06 $\pm$ 16.89	93 (64–158)	^b 0.001 **
HbA1c (%)	5.52 $\pm$ 0.38	5.6 (4.6–6.3)	5.59 $\pm$ 0.40	5.6 (4.6–6.4)	5.83 $\pm$ 0.45	5.9 (4.6–6.5)	^a 0.001 **
Insulin (mU/L)	8.10 $\pm$ 3.72	7.1 (2.5–28)	13.61 $\pm$ 6.82	12.5 (2–50)	18.92 $\pm$ 11.98	17 (5.1–67)	^b 0.001 **
HOMA-IR	1.72 $\pm$ 0.78	1.6 (0.5–5.6)	3.02 $\pm$ 1.50	2.8 (0.4–8.1)	4.39 $\pm$ 2.46	4.3 (1–12.4)	^b 0.001 **
Creatinine (mg/dL)	0.75 $\pm$ 0.46	0.7 (0.4–6)	0.75 $\pm$ 0.15	0.7 (0.5–1.1)	0.73 $\pm$ 0.15	0.7 (0.5–1.1)	^b 0.628
Urea (mg/dL)	18.48 $\pm$ 7.93	17 (5.5–39)	26.63 $\pm$ 6.72	25 (16–44)	25.61 $\pm$ 7.18	24 (13–44)	^a 0.001 **
Uric acid (mg/dL)	4.62 $\pm$ 1.25	4.6 (1.9–7.7)	4.68 $\pm$ 1.16	4.5 (2.7–8.2)	5.38 $\pm$ 1.29	5.4 (2.2–8.2)	^a 0.001 **
Total Protein (g/dL)	7.17 $\pm$ 0.38	7.2 (6.3–8.2)	8.24 $\pm$ 8.53	7.3 (6–79)	7.26 $\pm$ 0.43	7.2 (6–8.4)	^b 0.148
Albumin (g/dL)	4.59 $\pm$ 0.33	4.6 (4–5.5)	4.58 $\pm$ 0.32	4.6 (3.8–5.3)	4.49 $\pm$ 0.37	4.6 (3.5–5.1)	^a 0.127
Total Cholesterol (mg/dL)	185.33 $\pm$ 31.69	181 (84.8–310)	205.12 $\pm$ 34.9	206.8 (128.6–288.6)	213.76 $\pm$ 34.81	214 (138.8–302.6)	^a 0.001 **
Triglyceride (mg/dL)	105.10 $\pm$ 106.53	93 (11–1290)	124.92 $\pm$ 77.46	108 (38–535)	173.54 $\pm$ 109.47	153 (11–628)	^b 0.001 **
VLDL-C (mg/dL)	21.02 $\pm$ 21.31	18.6 (2.2–258)	24.98 $\pm$ 15.49	21.6 (7.6–107)	34.71 $\pm$ 21.89	30.6 (2.2–125.6)	^b 0.001 **
HDL-C (mg/dL)	54.31 $\pm$ 13.99	50.2 (15–106)	54.22 $\pm$ 16.55	51 (29–106)	46.29 $\pm$ 9.30	45 (32–68)	^a 0.001 **
LDL-C (mg/dL)	106.79 $\pm$ 28.95	101.8 (0–236)	125.92 $\pm$ 27.61	130 (69–189)	132.76 $\pm$ 25.76	138 (77–188)	^a 0.001 **

CRP: C-reactive Protein, FBG: Fasting Blood Glucose, HbA1c: Hemoglobin A1c, HDL-C: High-Density Lipoprotein Cholesterol, HOMA-IR: Homeostasis model assessment for insulin resistance, LDL-C: Low-Density Lipoprotein Cholesterol, VLDL-C: Very-Low-Density Lipoprotein Cholesterol, WBC: White Blood Cells. ^a One-Way Anova Test and Bonferroni Test, ^b Kruskal–Wallis Test and Dunn Bonferroni Test; ** $p < 0.01$. Bolded values denote statistically significant results.

Table 2. The association of BMI with nutrition scores and systemic immune–inflammatory indices.

	Normal (n = 156)		Overweight (n = 71)		Obesity (n = 63)		p-Value
	Mean $\pm$ SD	Median (Min–Max)	Mean $\pm$ SD	Median (Min–Max)	Mean $\pm$ SD	Median (Min–Max)	
PNI	58.18 $\pm$ 0.69	58 (6.5–74.2)	56.33 $\pm$ 0.90	57 (46.5–73)	56.48 $\pm$ 1.00	58 (45.5–65.5)	^c 0.18
NRI	114.05 $\pm$ 0.50	114.33 (104.48–128.12)	123.17 $\pm$ 0.74	123.21 (111.64–137.82)	135.04 $\pm$ 0.79	134.49 (107.66–155.62)	^c 0.001 **
CONUT	0.63 $\pm$ 0.05	1 (0–3)	0.48 $\pm$ 0.08	0 (0–3)	0.32 $\pm$ 0.90	0 (0–4)	^c 0.01 *
SII ($\times 100$)	441.33 $\pm$ 24.48	420.8 (44.1–1820.2)	617.82 $\pm$ 36.28	503.9 (157.6–2356)	569.59 $\pm$ 38.52	526.2 (189.3–1663.2)	^c 0.001 **
PLR	126.05 $\pm$ 3.97	122.9 (17.6–388.1)	139.21 $\pm$ 5.89	124.1 (64.2–380)	126.47 $\pm$ 6.26	119.5 (59.2–308)	^c 0.16
NLR	1.55 $\pm$ 0.07	1.4 (0.2–5.6)	2.13 $\pm$ 0.11	1.7 (0.8–7)	2.01 $\pm$ 0.11	1.8 (0.7–5.4)	^c 0.001 **
HALP	55.15 $\pm$ 4.46	50.21 (13.22–370.01)	67.24 $\pm$ 6.55	50.71 (19.99–628.33)	54.17 $\pm$ 6.95	49.39 (17.92–112.56)	^c 0.261

CONUT: Controlling Nutritional Status. HALP: Hemoglobin. Albumin. Lymphocyte. And Platelet Score. NLR: Neutrophil Lymphocyte Ratio. NRI: Nutritional Risk Index. PLR: Platelet Lymphocyte Ratio. PNI: Prognostic Nutritional Index. SII: Systemic Immune–Inflammation Index. ^c ANCOVA Test and Bonferroni Test; ** $p < 0.01$, * $p < 0.05$. Bolded values denote statistically significant results.

Table 3. The correlation of nutrition status with systemic immune–inflammatory indices in overweight participants.

	SII		PLR		NLR		PNI		HALP		NRI	
	r	p	r	p	r	p	r	p	r	p	r	p
PLR	0.837	<0.001 ***	-	-	-	-	-	-	-	-	-	-
NLR	0.844	<0.001 ***	0.589	<0.001 **	-	-	-	-	-	-	-	-
PNI	-0.295	0.013 *	-0.392	0.001 **	-0.462	<0.001 ***	-	-	-	-	-	-
HALP	-0.23	0.046 *	-0.307	0.01 *	-0.170	0.160	0.342	0.004 **	-	-	-	-
NRI	-0.101	0.403	0.069	0.571	-0.149	0.219	-0.144	0.235	-0.226	0.06	-	-
CONUT	0.307	0.01 *	0.302	0.011 *	0.449	<0.001 ***	-0.292	0.014 *	-0.112	0.355	-0.096	0.432

CONUT: Controlling Nutritional Status, HALP: Hemoglobin, Albumin, Lymphocyte, and Platelet Score, NLR: Neutrophil Lymphocyte Ratio, NRI: Nutritional Risk Index, PLR: Platelet Lymphocyte Ratio, PNI: Prognostic Nutritional Index, SII: Systemic Immune–Inflammation Index, Partial Correlation test; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. Bolded values denote statistically significant results.

Table 4. The correlation of nutrition status with systemic immune–inflammatory indices in participants with obesity.

	SII		PLR		NLR		PNI		HALP		NRI	
	r	p	r	p	r	p	r	p	r	p	r	p
PLR	0.6	<0.001 ***	-	-	-	-	-	-	-	-	-	-
NLR	0.887	<0.001 ***	0.371	0.003 **	-	-	-	-	-	-	-	-
PNI	−0.337	0.007 **	−0.363	0.004 **	−0.498	<0.001 ***	-	-	-	-	-	-
HALP	−0.522	<0.001 ***	−0.809	<0.001 ***	−0.358	0.004 **	0.601	<0.001 ***	-	-	-	-
NRI	−0.162	0.209	0.183	0.155	−0.299	0.018 *	−0.062	0.632	−0.256	0.045 *	-	-
CONUT	−0.014	0.912	0.378	0.002 **	0.048	0.713	−0.166	0.198	−0.236	0.064	0.057	0.658

CONUT: Controlling Nutritional Status. HALP: Hemoglobin. Albumin. Lymphocyte and Platelet Score. NLR: Neutrophil Lymphocyte Ratio. NRI: Nutritional Risk Index. PLR: Platelet Lymphocyte Ratio. PNI: Prognostic Nutritional Index. SII: Systemic Immune–Inflammation Index. Partial Correlation test; *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$. Bolded values denote statistically significant results.

3. Results

The study population was divided into three groups based on BMI: normal weight ($n = 156$), overweight ($n = 71$), and obesity ($n = 63$). A statistically significant difference in mean age was observed across the groups, with individuals in the obesity group being older on average ($p = 0.001$). Although these differences were statistically significant, the observed age ranges suggest that they are unlikely to be clinically meaningful. Moreover, age-adjusted covariance analyses for the relevant parameters did not alter the level of statistical significance, indicating that age did not materially influence the results. As expected, waist circumference increased progressively with BMI and was significantly higher among participants with obesity ($p = 0.001$) (Table 1).

3.1. Comparison of Metabolic Parameters Across BMI Categories

Among the inflammatory and metabolic parameters, CRP, FBG, HbA1c, insulin, HOMA-IR, urea, uric acid, total cholesterol, triglyceride, VLDL-C, HDL-C, and LDL-C levels showed significant differences across BMI groups. In particular, differences in CRP, insulin, and HOMA-IR levels were significantly elevated in the obesity group, indicating a potential increase in systemic inflammation and insulin resistance ($p = 0.001$) (Table 1). No significant differences were observed for white blood cell (WBC) or creatinine levels (Table 1).

3.2. Relationship Between Nutrition Scores and Systemic Immune–Inflammatory Indices Across BMI Categories

Table 2 shows the association of BMI with nutrition scores and systemic immune–inflammatory indices. Although the NRI increased significantly with BMI ($p = 0.001$), it remained above 100 in all BMI categories, indicating no apparent nutritional risk across groups. No significant differences were found in PNI scores among the groups ($p = 0.18$). The mean CONUT scores in all BMI groups were <1 , indicating a normal nutritional status across the study population. Similarly, the PNI scores were >50 in all groups, indicating a normal nutritional status across the study population (Table 2). There were no statistically significant differences in HALP scores among the BMI groups ($p = 0.261$). Regarding systemic immune–inflammatory indices, SII and NLR were significantly higher in the obesity and overweight groups compared to normal-weight individuals ($p = 0.001$ for both), suggesting heightened inflammatory activity. There were no statistically significant differences observed in PLR ($p = 0.16$) (Table 2). Figure 1 demonstrates the distribution of SII and NLR across BMI categories.

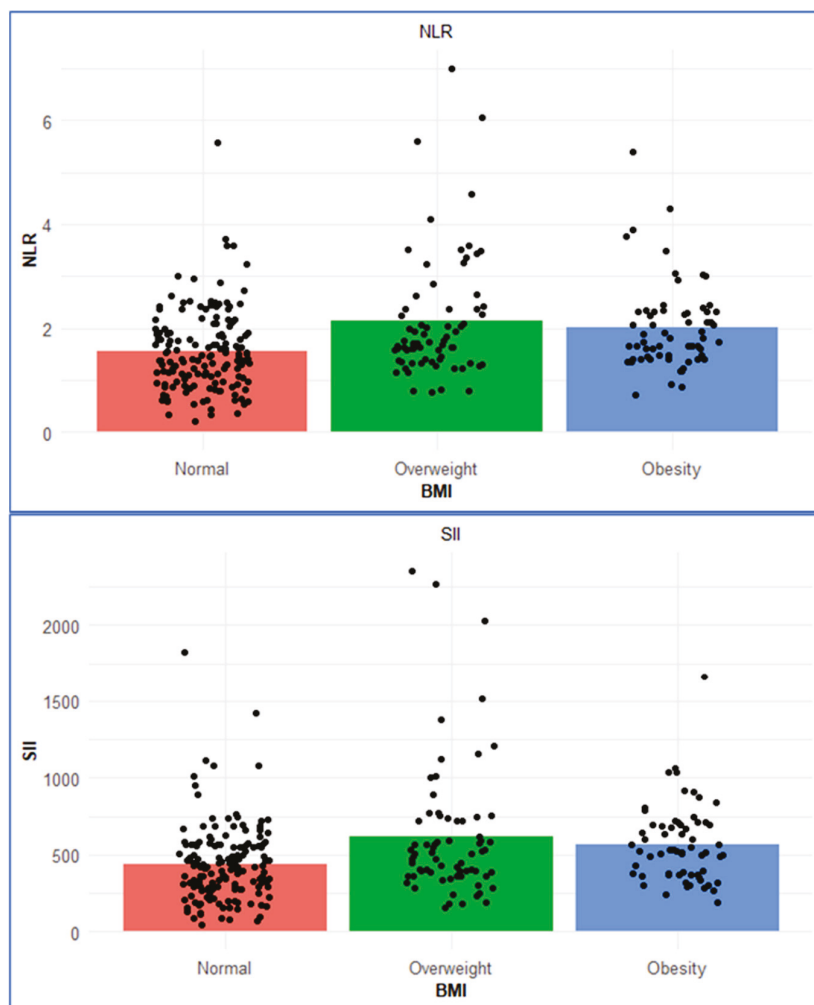


Figure 1. SII and NLR across BMI Categories.

3.3. Correlation of Nutrition Scores with Systemic Immune–Inflammatory Indices in High BMI Groups

In overweight individuals, significant positive correlations were observed among the systemic inflammatory indices. Strong positive correlations were found between SII and PLR ($r = 0.837$; $p < 0.001$) and between SII and NLR ($r = 0.844$; $p < 0.001$). SII showed significant negative correlations with PNI ($r = -0.295$; $p = 0.013$) and the HALP score ($r = -0.23$; $p = 0.046$). Additionally, SII demonstrated a significant positive correlation with CONUT, a negative indicator of nutritional status ($r = 0.307$; $p = 0.01$) (Table 3).

Strong negative correlations were found between PLR and PNI ($r = -0.392$; $p = 0.001$) and between PLR and HALP ($r = -0.307$; $p = 0.01$). No significant correlation was observed between PLR and NRI. PLR showed a statistically significant positive correlation with CONUT ($r = 0.302$; $p = 0.011$) (Table 3).

No significant correlations were observed between NLR and HALP or NRI. A statistically significant positive correlation was observed between NLR and CONUT ($r = 0.449$; $p < 0.001$), and a strong negative correlation was observed between NLR and PNI ($r = 0.462$; $p < 0.001$) (Table 3).

Among the nutritional indices, PNI showed a positive correlation with HALP ($r = 0.342$, $p = 0.004$). A statistically significant negative correlation was found between PNI and CONUT ($r = -0.292$; $p = 0.014$) (Table 3).

In participants with obesity, strong positive correlations were observed among systemic inflammatory indices. SII showed strong positive correlations with PLR ($r = 0.6$,

$p < 0.001$) and NLR ($r = 0.887$, $p < 0.001$). PLR was also positively correlated with NLR ($r = 0.371$, $p = 0.003$). SII correlated negatively with HALP ($r = -0.522$, $p < 0.001$). No significant correlations were observed between SII and NRI or CONUT (Table 4).

A strong positive correlation was observed between PLR and NLR ($r = 0.371$; $p = 0.003$). PLR also showed significant negative correlations with PNI ($r = -0.363$, $p = 0.004$) and HALP ($r = -0.809$, $p < 0.001$). No statistically significant correlation was observed between PLR and NRI, while a strong positive correlation was found between PLR and the CONUT score ($r = 0.378$; $p = 0.002$) (Table 3).

NLR was inversely correlated with PNI ($r = -0.498$, $p < 0.001$) and HALP ($r = -0.358$, $p = 0.004$). No statistically significant correlation was found between NLR and the CONUT score, while a significant negative correlation was observed between NLR and NRI ($r = -0.299$; $p = 0.018$) (Table 4).

Among the nutritional indices, PNI demonstrated a strong positive correlation with HALP ($r = 0.601$, $p < 0.001$). No statistically significant correlation was found between PNI and NRI or CONUT. A significant negative correlation was observed between HALP and NRI ($r = -0.256$; $p = 0.045$). The CONUT score did not show significant correlations with any of the inflammatory or nutritional indices (all $p > 0.05$) (Table 4).

These findings indicate that higher inflammatory burden (particularly SII, PLR, and NLR) is generally associated with poorer nutritional indices, whereas better nutritional status tends to accompany reduced systemic inflammation.

4. Discussion

This study examined the relationship between nutritional status and systemic immune-inflammatory indices across BMI categories in an adult population without any chronic diseases. Our findings show that as BMI increases, there is a corresponding rise in inflammation-related biomarkers (e.g., CRP, SII, NLR) (Tables 1 and 2). Regarding nutritional scores, although the NRI score increased significantly with BMI ($p = 0.001$), it remained ≥ 100 across all BMI categories, indicating no apparent nutritional risk in any group. No significant differences were found in PNI or HALP scores among the groups (Table 2). The mean CONUT scores in all BMI groups were < 1 , indicating a normal nutritional status across the study population. Similarly, the PNI scores were > 50 in all groups, indicating a normal nutritional status across the study population (Table 2). However, correlational analysis provided additional insights. In overweight participants, the CONUT score—a negative indicator of nutritional status—showed significant positive correlations with SII, PLR, and NLR, whereas PNI and HALP scores demonstrated significant inverse correlations with SII, PLR, and NLR. In the obesity group, the CONUT positively correlated with PLR, while PNI and HALP scores inversely correlated with SII, PLR, and NLR (Tables 3 and 4) (Figures 2 and 3).

These results support the growing body of evidence suggesting that adiposity contributes to a persistent low-grade inflammatory state [1,2]. Adipose tissue functions not only as a reservoir for energy storage but also as an immune-active organ, secreting a range of pro-inflammatory mediators collectively referred to as adipokines, including adiponectin, leptin, tumor necrosis factor-alpha (TNF- α), interleukin (IL)-1 β , and IL-6 [21]. Dysregulation of adipokine production contributes to the establishment of a chronic inflammatory milieu within adipose tissue. As adipocytes undergo hypertrophy and lipid accumulation increases, immune cell infiltration—particularly by macrophages—intensifies, thereby amplifying both local and systemic inflammatory responses. In addition to macrophages, other immune and hematologic cells—including neutrophils, platelets, and lymphocytes—contribute significantly to the chronic low-grade inflammation associated with obesity [22,23]. Neutrophils are among the earliest immune

cells to infiltrate adipose tissue in early obesity, releasing inflammatory mediators that amplify macrophage recruitment and maintain a chronic inflammatory state, thereby contributing to increased NLR and SII levels [24]. Neutrophils exacerbate both local and systemic inflammation by releasing pro-inflammatory mediators such as $\text{TNF-}\alpha$ and $\text{IL-1}\beta$. Platelets facilitate immune cell recruitment by secreting a variety of cytokines and chemokines, thereby amplifying the inflammatory response. Meanwhile, adaptive immunity is also altered: obesity induces a decline in regulatory T-cells and an increase in effector T-cell activity, shifting the balance toward pro-inflammatory T-cell subsets and further amplifying chronic low-grade inflammation—all of which may be captured by markers like PLR [25].

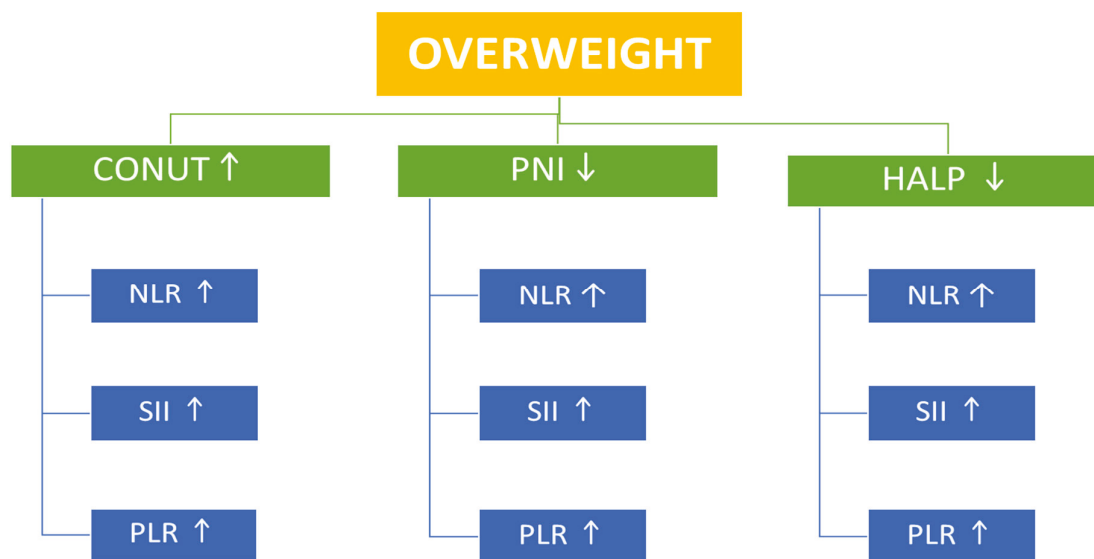


Figure 2. Associations of Nutritional Indices with Systemic Immune–Inflammatory Markers in the Overweight Group. ↑ = increase, ↓ = decrease.

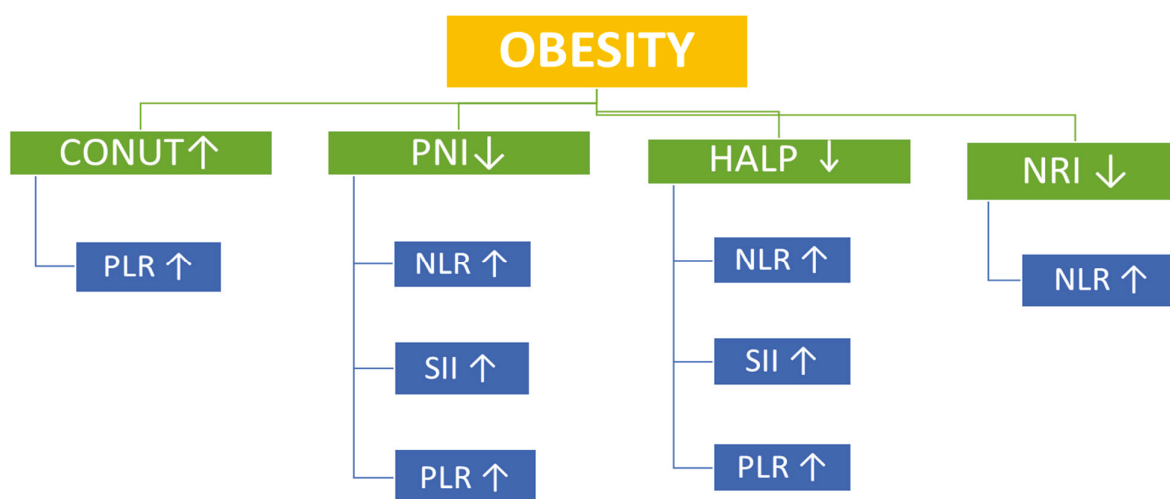


Figure 3. Associations of Nutritional Indices with Systemic Immune–Inflammatory Markers in the Obesity Group. ↑ = increase, ↓ = decrease.

Zhang et al. demonstrated a U-shaped relationship between the systemic immune–inflammation index (SII) and adolescent obesity, suggesting that both low and high SII levels may be associated with an increased risk of obesity in the population [1]. In contrast, Yu et al. reported a significant positive correlation between SII and body mass index (BMI), particularly among adults under 60 years of age who did not have hypertension or diabetes [2]. Similarly,

in our study population, which included individuals aged 18–60 years without any chronic disease, SII was found to increase significantly with rising BMI ($p = 0.001$).

Consistent with previous research, individuals with obesity in our study exhibited significantly higher levels of CRP, insulin, and triglycerides (Table 1), all of which are established markers of metabolic dysfunction and inflammation [9,22]. Although BMI is the conventional metric for classifying obesity, waist circumference also increases significantly with BMI and may provide additional insight into visceral adiposity and cardiometabolic risk [4,9]. In our study, waist circumference was significantly elevated in both the overweight and obesity groups ($p = 0.001$). Rodríguez-Rodríguez et al. reported that abdominal obesity was associated with a heightened inflammatory state, as indicated by NLR, in a Spanish population over 50 years of age. Furthermore, the study reported that better diet quality, assessed using the Spanish Healthy Eating Score, was significantly associated with reduced systemic inflammation [9]. Aydin et al. revealed that NLR and CRP levels were higher in children with obesity compared to healthy controls, whereas PLR levels were not significantly different between these two groups [10]. Similarly, in our study, NLR was significantly higher in the obesity and overweight groups compared to normal weight individuals ($p = 0.001$), while PLR did not show a statistically significant difference across BMI categories.

The NRI is a widely used tool for assessing nutritional status, particularly in clinical settings, and is calculated using serum albumin levels. Since albumin is a negative acute-phase reactant. Its synthesis is influenced not only by nutritional status but also by systemic inflammation. In individuals with obesity, despite a high BMI, protein–energy malnutrition may still occur—a condition referred to as “sarcopenic obesity” [26]. In patients with sarcopenic obesity, inadequate protein and calorie intake, together with chronic low-grade inflammation, may jointly contribute to reduced albumin levels despite excess body weight. While BMI tends to correlate positively with systemic inflammatory markers, NRI may correlate inversely, largely due to the inflammatory suppression of albumin synthesis and alterations in body composition [27]. Therefore, NRI may provide complementary insights to BMI in evaluating the complex relationship between nutritional status and inflammation in populations with obesity. In our study, although the NRI score increased significantly with BMI ($p < 0.001$), it remained ≥ 100 across all BMI categories, indicating no apparent nutritional risk in any group (Table 2). In addition, no significant correlations were found between NRI and systemic inflammation markers in the overweight group (Table 3), which may be consistent with the possibility of sarcopenic obesity. In individuals with sarcopenia, NRI may provide a falsely reassuring assessment of nutritional status, particularly in cases of sarcopenic obesity, where reduced muscle mass coexists with increased fat mass [28].

The HALP and PNI scores did not differ significantly across BMI groups, and CONUT scores were < 1 in all BMI groups, indicating a normal nutritional status across the study population. These findings highlight that higher weight may not equate to better nutritional quality [12,13]. However, correlation analysis provided additional insights into the present study. In both overweight individuals and those with obesity, HALP scores showed inverse correlations with SII, NLR, and PLR, suggesting that reduced nutritional status is associated with increased inflammation (Tables 3 and 4). Among the markers, HALP stood out as a particularly consistent and sensitive integrative index, inversely associated with inflammation and positively with PNI, similar to findings in previous cancer and cardiovascular studies [14,15].

Initially proposed in oncology settings, the HALP score has shown prognostic value in several malignancies, including gastric, colorectal, and hepatocellular carcinomas [15,16]. More recently, its utility has been explored in non-cancer populations, such as patients with cardiovascular disease and chronic kidney disease [29,30]. Its simplicity—requiring

only routinely available blood parameters—makes it particularly attractive for widespread clinical use. Despite growing interest, data on the HALP score in healthy individuals and its variation across BMI categories are limited. Given that obesity is characterized by a state of chronic low-grade inflammation and altered hematologic and nutritional parameters, evaluating HALP in relation to BMI may provide novel insights into the subclinical inflammatory–nutritional burden of excess weight. The HALP score may reflect the imbalance between nutritional status and inflammation in individuals with obesity. A low HALP score can serve as a significant indicator of underlying complications such as metabolic syndrome, insulin resistance, or sarcopenic obesity. Given its integrative nature, HALP may provide valuable insights into the interplay between nutritional deficiencies and chronic low-grade inflammation in the context of obesity.

Strengths and Limitations of the Study

Our study makes a unique contribution in several ways. First, to our knowledge, it is among the few to examine the relationship between multiple composite nutritional indices (HALP, PNI, CONUT, NRI) and systemic immune–inflammatory markers (SII, NLR, PLR) in an adult population stratified by BMI. Unlike many prior studies that focused on hospitalized or chronically ill patients, our inclusion of individuals without known chronic diseases allows for a clearer evaluation of subclinical inflammatory and nutritional dynamics related to adiposity. Second, the use of a wide array of validated indices—both nutritional and inflammatory—provides a comprehensive and multidimensional assessment of immune–nutritional interactions in the context of increasing BMI. We believe that these contributions broaden the existing knowledge and lay an essential groundwork for future prospective studies involving individuals without known chronic diseases.

This study has several limitations. One limitation of our study is that a priori sample size estimation was not performed, as the retrospective design required the inclusion of all eligible patients within a specific time frame. The absence of formal sample size calculation may have limited the statistical power and the generalizability of subgroup findings—particularly in the obesity category where greater heterogeneity exists (e.g., sarcopenic obesity). In addition, the retrospective nature and relatively limited sample size precluded subgroup analyses with additional variables or the calculation of sample size estimates for each subgroup, which may have restricted the ability to detect more nuanced associations. Nevertheless, a post hoc power analysis for the SII values—representing the parameter with the lowest level of statistical significance—demonstrated a power of 81.7%. Thus, the current sample size is considered sufficient for the present stage. Future prospective studies with larger cohorts and predefined subgroup stratifications are needed to validate and expand upon our results.

The retrospective and cross-sectional design of this study precludes causal inferences. While exclusion criteria aimed to remove confounders such as chronic disease and medication use, subclinical inflammation or undiagnosed conditions may still have influenced the biomarker profiles. Although we included only individuals without known chronic diseases, the BMI interval of 18.5–39.99 kg/m² covers distinct categories—normal weight, overweight, and obesity—with different health implications. According to WHO definitions, overweight and obesity do not represent a healthy weight status. Therefore, even though the participants were free of chronic disease, the inclusion of individuals with overweight and obesity may inherently reflect varying metabolic and inflammatory profiles. In addition, smoking status and alcohol consumption were obtained from medical records, which may be subject to reporting inaccuracies and potential misclassification. These factors should be considered when interpreting our findings.

Another limitation of our study is that BMI does not differentiate between visceral and non-visceral adiposity or account for variations in muscle mass. Body composition parameters such as fat mass, muscle mass, or waist-to-hip ratio were not directly measured. This limits the ability to distinguish between visceral adiposity and sarcopenia, both of which influence inflammation and nutritional markers independently of BMI. Dietary intake, physical activity, and socioeconomic status—potential confounders affecting both inflammation and nutritional status—were not assessed.

Finally, the retrospective design of our study may compromise internal validity, as data collection relied on existing records rather than standardized prospective assessments. Furthermore, the lack of subgroup analyses and the absence of certain variables, such as measures of visceral adiposity, muscle mass, and detailed lifestyle factors, may limit the external validity and generalizability of our findings.

5. Conclusions

In conclusion, our study demonstrates that higher BMI, even among individuals without known chronic diseases, is associated with increased levels of systemic immune-inflammatory markers. Among the nutritional indices examined, the HALP score appears to reflect the inverse relationship between nutrition and inflammation most effectively. This index may be useful for early identification of subclinical inflammation in overweight and/or obesity populations. Routine use of simple blood-based scores like HALP in clinical settings could support preventive strategies by identifying individuals at risk for inflammation-related complications before overt disease manifests in individuals with high BMI.

We recommend that future cohort studies incorporate body composition measurements and detailed assessments of dietary intake and physical activity, and be conducted with larger sample sizes with predefined subgroup analysis. Investigating the association between nutritional scores and systemic immune-inflammatory indices in obesity remains a promising area for further research.

Author Contributions: H.U. and A.K.F. conceptualized and designed this study; H.U. and A.S. performed data acquisition, G.C. carried out statistical analysis; H.U. and A.K.F. drafted the manuscript. Both authors finalized the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted as a retrospective case-control study at the Department of Internal Medicine, Istanbul Atlas University Medical Faculty Hospital. The study was designed in accordance with the Declaration of Helsinki and good clinical practice guidelines. It was approved by Istanbul Atlas University Non-Interventional Scientific Research Ethics Committee (Approval number: E-22686390-050.99-61546, date: 17 February 2025).

Informed Consent Statement: Patient consent was waived due to the retrospective design of the study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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

Abbreviations

BMI	Body Mass Index
CBC	Complete Blood Count
CONUT	Controlling Nutritional Status
CRP	C-reactive protein

ECLIA	Electrochemiluminescence Immunoassay
FBG	Fasting Blood Glucose
HALP	Hemoglobin, Albumin, Lymphocyte and Platelet
HbA1c	Hemoglobin A1c (HbA1c)
HDL	Low-Density Lipoprotein Cholesterol
HOMA-IR	Homeostasis Model Assessment for Insulin Resistance
HPLC	High-Performance Liquid Chromatography
IL-6	Interleukin-6
LDL-C	Low-Density Lipoprotein Cholesterol
NLR	Neutrophil–Lymphocyte Ratio
NRI	Nutritional Risk Index
PLR	Platelet–Lymphocyte Ratio
PNI	Prognostic Nutritional Index
SII	Systemic Immune–Inflammation Index
TNF- α	Tumor Necrosis Factor- α
VLDL-C	Very-Low-Density Lipoprotein Cholesterol
WBCs	White Blood Cells

References

1. Zhang, Y.; Ma, R.; Bhandari, S.; Xie, J.; Zhang, X.; Xie, C.; Duan, H.; Meng, J.; Wu, Q.-Y.; Liu, K.; et al. Association between systemic immune inflammation index and adolescent obesity in a cross-sectional analysis. *Sci. Rep.* **2025**, *15*, 6439. [CrossRef]
2. Yu, Y.; Tan, T.; Yang, W.; Xu, Z.; Liu, Y. Association between the systemic immune-inflammation index and obesity among adults: Insights from the NHANES 2017–2018. *PLoS ONE* **2024**, *19*, e0308288. [CrossRef] [PubMed]
3. Ramezankhani, A.; Tohidi, M.; Hadaegh, F. Association between the systemic immune-inflammation index and metabolic syndrome and its components. *Cardiovasc. Diabetol.* **2025**, *24*, 78. [CrossRef] [PubMed]
4. Dong, C.H.; Wang, Z.M.; Chen, S.Y. Neutrophil to lymphocyte ratio predicts mortality and major adverse cardiac events in acute coronary syndrome. *Clin. Biochem.* **2018**, *52*, 131–136. [CrossRef] [PubMed]
5. Yang, Y.L.; Wu, C.H.; Hsu, P.F.; Chen, S.C.; Huang, S.S.; Chan, W.L.; Lin, S.-J.; Chou, C.-Y.; Chen, J.-W.; Pan, J.-P.; et al. Systemic immune-inflammation index predicted clinical outcome in coronary artery disease. *Eur. J. Clin. Investig.* **2020**, *50*, e13230. [CrossRef]
6. Templeton, A.J.; Knox, J.J.; Lin, X.; Simantov, R.; Xie, W.; Lawrence, N.; Broom, R.; Fay, A.P.; Rini, B.; Donskov, F.; et al. Change in neutrophil-to-lymphocyte ratio in metastatic renal cell carcinoma. *Eur. Urol.* **2016**, *70*, 358–364. [CrossRef]
7. Miura, T.; Matsumoto, S.; Sato, A.; Kojima, S.; Sasaki, G.; Morino, M.; Matsumoto, K.; Kashima, H.; Koito, Y.; Ishii, T.; et al. Prognostic factors with endoscopic submucosal dissection in older adults. *DEN Open* **2025**, *6*, e70137. [CrossRef]
8. Cupp, M.A.; Cariolou, M.; Tzoulaki, I.; Aune, D.; Evangelou, E.; Berlanga-Taylor, A.J. Neutrophil to lymphocyte ratio and cancer prognosis. *BMC Med.* **2020**, *18*, 360. [CrossRef]
9. Rodríguez-Rodríguez, E.; López-Sobaler, A.M.; Ortega, R.M.; Delgado-Losada, M.L.; López-Parra, A.M.; Aparicio, A. Neutrophil-to-lymphocyte ratio and abdominal obesity. *Nutrients* **2020**, *12*, 855. [CrossRef]
10. Aydin, M.; Yilmaz, A.; Donma, M.M.; Tulubas, F.; Demirkol, M.; Erdogan, M.; Gurel, A. Neutrophil/lymphocyte ratio in obese adolescents. *North. Clin. Istanbul.* **2015**, *2*, 87–91. [CrossRef]
11. Yazaki, L.G.; Faria, J.C.P.; Souza, F.I.S.; Sarni, R.O.S. Neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios in overweight children. *Rev. Assoc. Med. Bras.* **2022**, *68*, 1006–1010. [CrossRef]
12. Kobylińska, M.; Antosik, K.; Decyk, A.; Kurowska, K. Malnutrition in Obesity. *Obes. Facts* **2022**, *15*, 19–25. [CrossRef]
13. Barazzoni, R.; Gortan Cappellari, G. Double burden of malnutrition in obesity. *Rev. Endocr. Metab. Disord.* **2020**, *21*, 307–313. [CrossRef] [PubMed]
14. Li, S.Y.; Wan, L.L.; Liu, Y.F.; Li, Y.W.; Huang, X.; Liu, R.J. Prognostic value of NRI, PNI, CONUT in prostate cancer. *Front. Nutr.* **2024**, *11*, 1436063. [CrossRef]
15. Farag, C.M.; Antar, R.; Akosman, S.; Ng, M.; Whalen, M.J. HALP score review. *Oncotarget* **2023**, *14*, 748. [CrossRef] [PubMed]
16. Chen, X.L.; Xue, L.; Wang, W.; Chen, H.N.; Zhang, W.H.; Liu, K.; Chen, X.-Z.; Yang, K.; Zhang, B.; Chen, Z.-X.; et al. HALP in gastric carcinoma. *Oncotarget* **2015**, *6*, 41370–41382. [CrossRef]
17. World Health Organization. *Obesity: Preventing and Managing the Global Epidemic*; WHO: Geneva, Switzerland, 2000; Available online: <https://iris.who.int/handle/10665/63854> (accessed on 28 August 2025).
18. Ekinci, I.; Uzun, H.; Utku, I.K.; Ozkan, H.; Buyukkaba, M.; Cinar, A.; Akarsu, M.; Kumbasar, A.; Tabak, O. Prognostic nutritional index in COVID-19. *Int. J. Vitam. Nutr. Res.* **2022**, *92*, 4–12. [CrossRef]

19. Kuroda, D.; Sawayama, H.; Kurashige, J.; Iwatsuki, M.; Eto, T.; Tokunaga, R.; Kitano, Y.; Yamamura, K.; Ouchi, M.; Nakamura, K.; et al. CONUT score in gastric cancer. *Gastric Cancer* **2018**, *21*, 204–212. [CrossRef]
20. Przekop, Z.; Szostak-Węgierek, D.; Milewska, M.; Panczyk, M.; Zaczek, Z.; Sobocki, J. Nutritional indices in head and neck cancer. *Nutrients* **2022**, *14*, 1268. [CrossRef]
21. Fantuzzi, G. Adipose tissue, adipokines, and inflammation. *J. Allergy Clin. Immunol.* **2005**, *115*, 911–919. [CrossRef]
22. Ouchi, N.; Parker, J.L.; Lugus, J.J.; Walsh, K. Adipokines in inflammation and metabolic disease. *Nat. Rev. Immunol.* **2011**, *11*, 85–97. [CrossRef]
23. Elgazar-Carmon, V.; Rudich, A.; Hadad, N.; Levy, R. Neutrophils infiltrate fat in early high-fat feeding. *J. Lipid Res.* **2008**, *49*, 1894–1903. [CrossRef]
24. Uribe-Querol, E.; Rosales, C. Neutrophils in obesity-related inflammation. *Cells* **2022**, *11*, 1883. [CrossRef]
25. Grant, R.W.; Dixit, V.D. Adipose tissue as an immunological organ. *Obesity* **2015**, *23*, 512–518. [CrossRef]
26. Prado, C.M.; Wells, J.C.; Smith, S.R.; Stephan, B.C.; Siervo, M. Sarcopenic obesity: A critical appraisal. *Clin. Nutr.* **2012**, *31*, 583–601. [CrossRef]
27. Don, B.R.; Kaysen, G. Serum albumin and inflammation. *Semin. Dial.* **2004**, *17*, 432–437. [CrossRef] [PubMed]
28. Donini, L.M.; Busetto, L.; Bauer, J.M.; Bischoff, S.; Boirie, Y.; Cederholm, T.; Cruz-Jentoft, A.J.; Dicker, D.; Frühbeck, G.; Giustina, A.; et al. Sarcopenic obesity definitions. *Clin. Nutr.* **2020**, *39*, 2368–2388. [CrossRef] [PubMed]
29. Zhang, C.; Peng, W.; Ning, M.; Liang, W.; Su, B.; Guo, T.; Hu, K.; Su, W.; Chen, Y.; Liu, Y. HALP and short-term mortality. *J. Health Popul. Nutr.* **2025**, *44*, 36. [CrossRef] [PubMed]
30. Yuan, Y.; Liang, X.; He, M.; Wu, Y.; Jiang, X. HALP as predictor in IgA nephropathy. *Front. Endocrinol.* **2024**, *15*, 1339921. [CrossRef]

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Article

Muscle Strength, Lipid Metabolism and Hepatic Steatosis Are Improved with Ursolic Acid Treatment in High-Fat Diet-Induced Obese Mice

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Abstract

Background/Objectives: The prevalence of obesity globally has increased steadily in the past decades. Obesity, sarcopenic obesity (SO) and nonalcoholic fatty liver disease (NAFLD) commonly coexist. Ursolic acid (UA), a natural pentacyclic triterpenoid, has demonstrated potential anti-obesity properties. This study was designed to evaluate the anti-obesity efficacy of UA in a mouse model of high-fat diet (HFD)-induced obesity, with a particular focus on its impact on muscle function and NAFLD. **Methods:** Male C57BL/6J mice (6 weeks old) were randomly assigned to three groups ($n = 20$ per group): a control group (CON) fed a normal chow diet, a high-fat diet group (HFD), and a UA treatment group (UA). The HFD and UA groups received a high-fat diet for 10 weeks to induce obesity. Thereafter, mice in the UA group were administered UA orally once daily for 6 weeks. **Results:** In HFD-induced obese mice, UA administration significantly reduced body weight (BW), abdominal fat weight and liver weight; improved grip strength and muscle weight; and enhanced lipid profiles, including triglycerides, total cholesterol, low-density lipoprotein cholesterol and free fatty acid levels in serum. UA also improved histological changes in the liver and abdominal adipose tissues, regulated serum GH, IGF-1, T3, T4 and leptin levels and downregulated the inflammation-associated gene expression of TNF- α and IL-1 β in abdominal adipose tissue. **Conclusions:** UA could enhance muscle strength, improve lipid metabolism and hepatic steatosis and might be considered a potential therapeutic agent for managing obesity and related metabolic diseases.

Keywords: ursolic acid; obesity; grip strength; lipid metabolism; hormone; mice

1. Introduction

Obesity is a major health problem worldwide. It is defined as abnormal or excessive fat accumulation with health risk by the World Health Organization (WHO) [1] and increases the risk of numerous diseases, including cardiovascular disease, type 2 diabetes, hyperlipidemia, fatty liver disease, cognitive impairments and various cancers [2–4]. The prevalence of obesity is dramatically increasing, and 20% of the world's population will be obese by 2030, according to projections [5]. Long-term over-nutrition, lack of exercise and an imbalance between energy intake and energy expenditure are factors that lead to obesity [6]. Obesity is also influenced by genetic factors [7]. Sarcopenia is defined as low muscle strength with the loss of skeletal muscle mass and function [8]. Meta-analyses have shown that sarcopenia and NAFLD have a positive association [9], and the cross-talk of cytokines produced by inflammatory reactions in skeletal muscle, liver and adipose tissue might

form a triangle of harmful events that trigger the development and progression of obesity, sarcopenia and NAFLD [10]. Although sarcopenia is commonly associated with the elderly, recent research has shown that children may also develop the condition [11]. Sarcopenic obesity (SO), the coexistence of obesity and sarcopenia, is defined as obesity with low skeletal muscle mass and function [12]. Both obesity and sarcopenia synergistically exert an additional hazardous impact on metabolic disease or amplify the health risks [13,14]. In line with sarcopenia, SO is linked not only to the elderly population, but also the pediatric population and possess negative health outcomes in children and adolescents [15–17].

Skeletal muscle, constituting approximately 40% of total body mass, is a critical tissue responsible for a range of key physiological functions, including energy metabolism, thermogenesis and joint locomotion [18,19]. Skeletal muscle is also endocrine tissue and participates in body weight regulation by different signaling pathways [20–22]. Existing studies have shown a strong association between muscle strength, adipose tissue and nonalcoholic fatty liver disease [23]. The complex network relationships among them might be constructed by myokines, adipokines and hormones [24]. Skeletal muscle mass is decreased with weight loss, implying that loss of muscle mass and function should be minimized during the management of weight reduction. Grip strength is often used to measure muscular strength and muscle mass [25]. A recent study revealed a close correlation between grip strength and NAFLD risk [26]. Grip strength has been proposed as a tool for identifying metabolic syndrome and sarcopenia in the elderly [26,27]. Therefore, understanding the relationship between obesity and muscular strength is important.

To improve obesity, available treatment approaches for obesity or overweight include lifestyle corrections and the use of medications. Additionally, currently, limited scientific evidence, the lack of standardization and the paucity of clinical studies contribute to the challenges in formulating definitive WHO general recommendations for the use of natural products in this clinical field, which necessitates the design and development of natural products as potential anti-obesity agents for the therapeutic management of obesity [28–30].

Ursolic acid (UA), a natural pentacyclic triterpenoid, is widely present in a variety of medicinal plants, fruits and herbs [31], such as ginseng, pear apples, olives, plums, cranberries, rosemary, oregano and eucalyptus, calendula rosemarie [32,33]. So, UA is considered a component of the standard human diet. UA has gained significant attention in recent years due to its diverse biological functions and multiple pharmacological characteristics, such as anti-inflammation, anti-oxidation and anticancer effects [34–36]. Kunkel and colleagues highlighted that UA increases skeletal muscle mass in a mouse model of diet-induced obesity, identifying that the Akt molecular pathway is involved in muscle growth [37]. Sun and colleagues found that UA treatment significantly reduced the weight of the gastrocnemius muscle and lipid droplets in the same muscle by increasing the secretion of irisin and the expression of genes involved in adipocyte differentiation and lipogenesis [38]. Several studies have also shown that UA improved adipose tissue insulin resistance in aged rats and ameliorated HFD-induced hepatic steatosis and liver injury [39,40]. These results indicate that UA possesses anti-obesity and anti-inflammatory effects. However, UA's mechanism of action has not been fully elucidated, and the effect of UA on muscle strength and NAFLD requires further verification during anti-obesity treatment. In addition, hormones play pivotal roles in maintaining skeletal muscle homeostasis, and further research is needed to confirm the regulatory effects of UA on hormones.

This study aimed to investigate anti-obesity effect of UA in HFD-induced obese mice with a specific dose for six weeks. In addition, this study aimed to examine the influence of UA on muscle performance and NAFLD. These data will provide evidence for the use of UA as a dietary supplement in preventing and treating obesity and its related metabolic syndrome.

2. Materials and Methods

2.1. Chemicals

Ursolic acid (UA, purity $\geq 90.0\%$) was obtained from Sigma-Aldrich (Shanghai, China) Trading Co., Ltd. The dose of UA (40 mg/kg body weight) was selected based on previous literature [41,42].

2.2. Animal Experiments

All animal experiments received approval from the Institutional Animal Care and Use Committee of Henan University of Science and Technology (Approval No: 202403017; Date: 8 March 2024) and were performed in accordance with the Guide for the Care and Use of Laboratory Animals: Eighth Edition [43].

2.3. Study Design

Male specific pathogen free C57BL/6J (6 weeks old) mice were selected from Si Pei Fu (Beijing) Biotechnology Co., Ltd. (Beijing, China). All animals were housed in cages, and they were acclimated to the environment with automatic temperature, light and humidity control for 1 week. The room temperature was maintained at 24 °C and the humidity was controlled at 55%, with a 12-h light/dark cycle.

A total of 60 mice were randomly assigned to three experimental groups ($n = 20$ per group): (1) control (CON), (2) high-fat diet (HFD) and (3) UA treatment (UA). The CON group received a standard chow diet (LAD3001M, Trophic Animal Feed High-Tech Co., Ltd., Nantong, China), with a nutritional composition of 67% carbohydrate, 18% protein and 5% fat, providing 7% of calories from fat. The HFD and UA groups were fed a high-fat diet (TP23400, Trophic Animal Feed High-Tech Co., Ltd, Nanning, China.) containing 23% protein, 32% carbohydrate, 31.5% fat (27.5% lard + 4.0% soybean oil), 6.8% fiber, 1.5% choline chloride, 4.0% mineral mix and 1.2% vitamin mix, with 60% of calories derived from fat. The mice were housed with five replicate cages per group (four mice per cage) and were allowed ad libitum access to food and tap water. After 10 weeks, the UA group was administered ursolic acid (40 mg/kg body weight) once daily via oral gavage, whereas the CON and HFD groups received an equivalent volume of saline for a duration of 6 weeks. Body weight and food intake were recorded weekly. The experimental timeline is summarized in Figure 1.

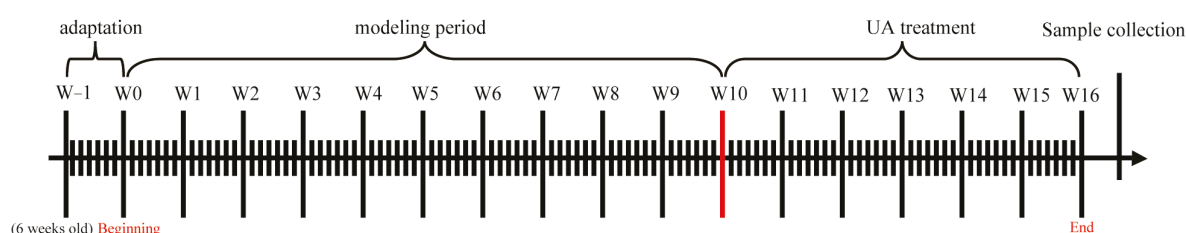


Figure 1. The time schedule of the procedures in this study.

2.4. Grip Strength Test

Prior to the endpoint of this study, forelimb grip strength was evaluated in all mice using a specialized grip strength meter (RWD Life Science Co., Ltd., Shenzhen, China). During testing, each mouse was allowed to grasp a metal grip with both forepaws, after which the tail was gently pulled backward horizontally until the animal released its grip. The maximum force exerted immediately before release was recorded. Each mouse underwent three consecutive trials separated by one-minute intervals, and the average of these measurements was calculated to represent grip strength.

2.5. Sample Collections

At the end of the experiment (after UA treatment for 6 weeks), the mice were anaesthetized with pentobarbital sodium after 12 h of overnight fasting. Blood from the hearts was collected using vacutainer tubes, and serum samples were obtained from the collected blood samples through 10 min of centrifugation ($3500 \times g$, 4°C). Then, the triceps, gastrocnemius, quadriceps and soleus muscles, abdominal adipose tissues and liver tissues were removed, weighed and processed for subsequent analyses. In addition, the liver index was calculated as follows:

$$\text{Liver index} = \text{liver weight/body weight}$$

2.6. Measurement of Serum Lipids

The levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and free fatty acid (FFA) in serum were determined using commercial assay ELISA kits, according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China).

2.7. Observation and Analysis of Histopathology

Liver and abdominal subcutaneous adipose tissue samples were collected and fixed in 10% neutral buffered formalin. After 48 h of fixation, the tissues were dehydrated, embedded in paraffin and sectioned at a thickness of $5\ \mu\text{m}$. Following deparaffinization in xylene and rehydration, the sections were stained with hematoxylin and eosin (H&E). Liver histopathology was examined using light microscopy, with hepatic steatosis defined as the presence of lipid droplets in 5% or more of hepatocytes [44]. In brief, steatosis was assayed in three zones of the classic lobules of liver in each field: zone I (port area), zone II (intermediate area) and zone III (central vein area). When the total steatosis area in the three zones was $\geq 5\%$, it was recorded. For adipose tissue, images of the slides were captured, twenty cells were randomly selected from each slide and the diameter was measured. ImageJ software (1.49v) was used for the analysis of steatosis area and the diameters of adipocytes.

2.8. Measurement of Serum Hormones

Serum levels of thyroxine (TH, T3, T4), leptin, growth hormone (GH) and insulin-like growth factor-1 (IGF-1) were measured using corresponding commercial ELISA kits from Nanjing Jiancheng Bioengineering Institute.

2.9. Real-Time PCR

Total RNA was extracted from abdominal adipose tissue using TRIzol reagent (TaKaRa Bio Inc, Otsu City, Japan), following the manufacturer's instructions. Subsequent cDNA synthesis was carried out with oligo-dT primers and PrimeScript RT enzyme (TaKaRa). Quantitative real-time PCR was performed on a Bio-Rad iQ5 detection system using SYBR Premix ExTaq II (TaKaRa) to evaluate the expression of inflammatory cytokines. All reactions were conducted in triplicate and normalized to the endogenous control, β -actin. The primer sequences utilized are listed in Table 1, and relative gene expression levels were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method [45].

Table 1. Primer sequences for quantitative real-time PCR.

Gene	Forward Primer (5'–3')	Reverse Primer (5'–3')
IL-1 β	TGCCACCTTTTGACAGTGATG	TTCTTGTGACCCTGAGCGAC
TNF- α	TCTTTCGGAGAAGGTTGCCC	AGAAGTCCTGCCACTTCACG
β -actin	AGAGGGAAATCGTGCGTGAC	CAATAGTGATGACCTGGCCGT

IL-1 β : Interleukin-1 β . TNF- α : Tumor necrosis factor- α .

2.10. Statistical Analysis

All data were assessed for normality using the Kolmogorov–Smirnov test and are presented as mean $\pm$ standard deviation (SD). Statistical comparisons were performed using one-way ANOVA with Tukey's post hoc test for multiple comparisons, using SPSS 22.0 software. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. The Effect of Ursolic Acid on Body Weight and Feed Intake

At the beginning of the experiment (week 0), no significant differences in body weight were observed among the different groups ($p > 0.05$). However, following 10 weeks of dietary intervention, mice fed a high-fat diet exhibited a significant increase in body weight compared to those maintained on a standard chow diet (Figure 2A). After treatment with UA for 6 weeks (Week 16), mice in the HFD group had higher body weight than mice in the CON group. However, the body weight of mice in the UA group was lower than that of mice in the HFD group (Figure 2B). No significant differences for feed intake were observed among the three experiment groups (Figure 2C).

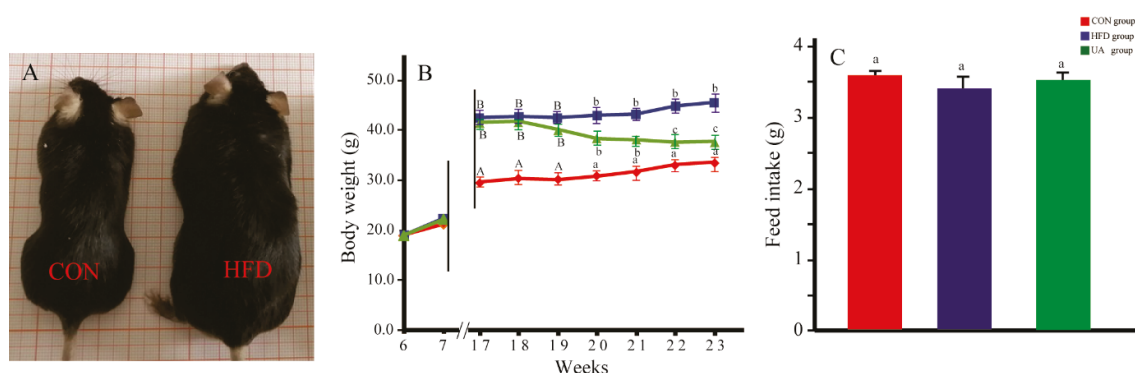


Figure 2. Changes in body weight and feed intake. (A): Representative images of C57BL/6J mice fed a standard chow diet and a high-fat diet for 10 weeks. (B): Body weight changes during the 6 weeks of UA administration. (C): Food intake during the 6 weeks of UA administration. Data are expressed as mean $\pm$ SD. Values with different capital letters are significant at the 0.01 level. Values with different lowercase letters are significant at the 0.05 level.

3.2. The Effect of Ursolic Acid on Abdominal Fat and Liver Weight

At the end of the experiment, the abdominal subcutaneous adipose tissue weight, liver weight and live index value of the mice in the HFD group were higher than those in the CON group. In contrast, the mice administered 40 mg/kg/day UA had lower liver weight, abdominal fat weight and liver index values than those in the HFD group (Figure 3C–E).

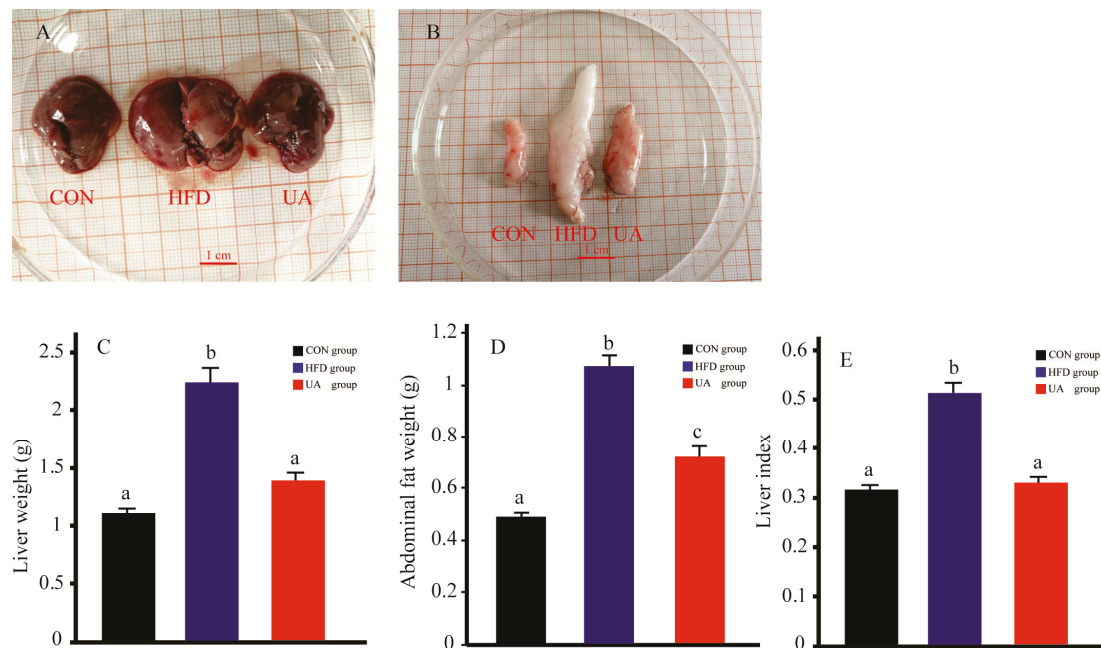


Figure 3. Changes in abdominal fat and liver weight. (A): Representative gross images of liver. (B): Representative gross images of abdominal subcutaneous adipose tissue. (C): Liver weight. (D): Abdominal fat weight. (E): Liver index. Data are expressed as mean $\pm$ SD. Different letters represent significant differences at the 0.05 level.

3.3. The Effect of Ursolic Acid on Grip Strength and Muscle Weight

At the end of the experiment, grip strength and muscle weights were measured in this study. The grip strength of mice in the HFD group was significantly lower than that in the CON group, and UA administration led to a significant increase compared with the HFD group ($p < 0.05$). Lower weights of the triceps, quadriceps and gastrocnemius, but not soleus, were observed in the HFD group compared with the CON group. UA administration significantly increased the weights of these muscles compared with those in the HFD group. These results are presented in Table 2.

Table 2. The effect of ursolic acid on grip strength and various muscle weights.

	Groups		
	CON	HFD	UA
Grip strength (g force (gf))	118.34 $\pm$ 13.24 ^a	99.68 $\pm$ 14.71 ^b	112.86 $\pm$ 11.52 ^a
Triceps (mg)	234.77 $\pm$ 25.14 ^a	198.64 $\pm$ 17.75 ^b	242.54 $\pm$ 15.68 ^a
Quadriceps (mg)	415.31 $\pm$ 39.03 ^a	369.61 $\pm$ 38.73 ^b	364.00 $\pm$ 27.38 ^b
Gastrocnemius (mg)	288.61 $\pm$ 21.89 ^a	271.64 $\pm$ 17.43 ^b	290.29 $\pm$ 21.82 ^a
Soleus (mg)	14.26 $\pm$ 1.06	16.58 $\pm$ 0.95	15.14 $\pm$ 1.32

Data are expressed as mean $\pm$ SD. Different letters represent significant differences at the 0.05 level.

3.4. The Effect of Ursolic Acid on Serum Lipids

We analyzed serum TG, TC, HDL-C, LDL-C and FFA levels. As shown in Table 3, these parameters in the HFD group showed significant increases compared with those in the CON group ($p < 0.05$). Treatment with UA significantly decreased TG, TC, LDL-C and FFA levels ($p < 0.05$), but not HDL-C.

Table 3. The effect of ursolic acid on plasma lipids.

	Groups		
	CON	HFD	UA
TG (mmol/L)	0.64 ± 0.04 ^a	1.18 ± 0.07 ^b	0.86 ± 0.05 ^a
TC (mmol/L)	2.53 ± 0.28 ^a	4.69 ± 0.23 ^b	3.07 ± 0.31 ^a
HDL-C (mmol/L)	2.34 ± 0.14 ^a	3.64 ± 0.17 ^b	3.24 ± 0.25 ^b
LDL-C (mmol/L)	0.21 ± 0.03 ^a	0.42 ± 0.05 ^b	0.26 ± 0.08 ^a
FFA (mmol/L)	0.37 ± 0.04 ^a	0.78 ± 0.11 ^b	0.44 ± 0.06 ^a

Data are expressed as mean ± SD. Different letters represent significant differences at the 0.05 level.

3.5. The Effect of Ursolic Acid on Histological Changes in Liver and Abdominal Adipose Tissues

Histopathological assessment of liver tissues indicated that mice in the HFD group developed severe hepatic steatosis, exhibiting significantly larger steatotic areas ($p < 0.01$), along with substantial lipid droplet accumulation and hepatocellular swelling relative to the CON group (Figure 4A). In contrast, UA treatment effectively ameliorated these pathological changes, resulting in a noticeable reduction in both the number and size of intra-cellular lipid vacuoles. The steatotic area was markedly decreased from 53% in the HFD group to 22% in the UA-treated group (Figure 4B).

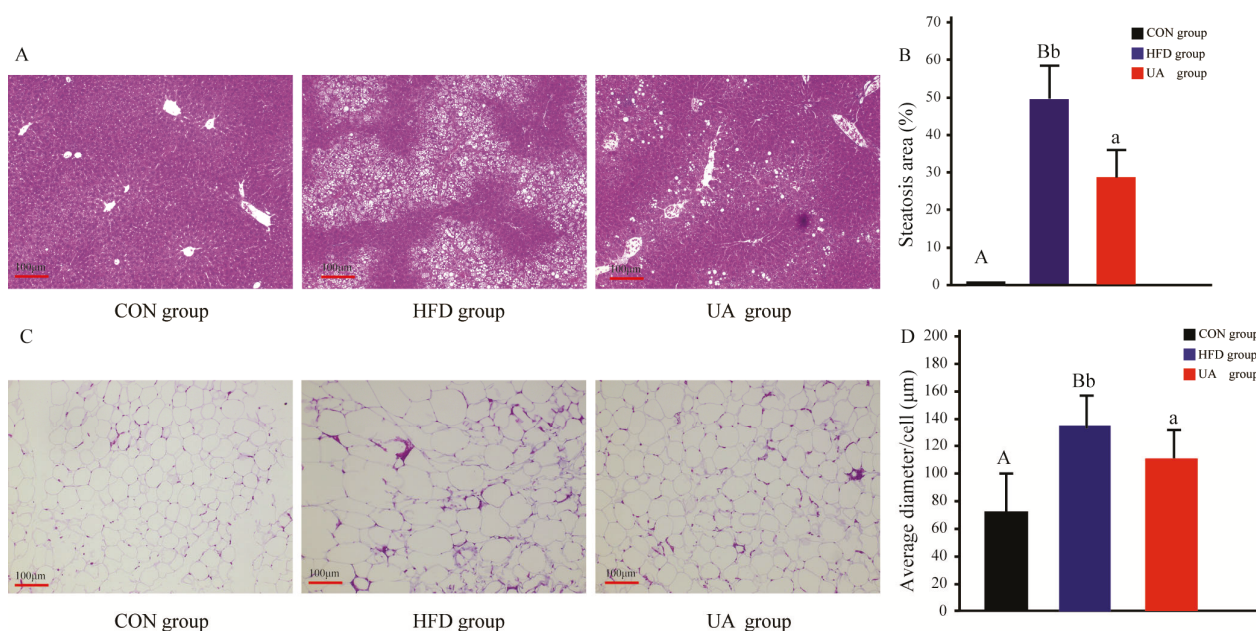


Figure 4. Histopathological changes in liver and abdominal adipose tissues. (A): Representative liver histology by H&E staining. (B): Steatosis area of the liver. (C): Representative adipose tissues. (D): Average diameter of adipocytes. Values with different capital letters are significant at the 0.01 level. Values with different lowercase letters are significant at the 0.05 level.

Morphometric analysis of abdominal adipose tissue further revealed adipocyte hypertrophy in the HFD group compared with the CON group. UA intervention significantly attenuated this increase in adipocyte size, as demonstrated by a clear reduction in the cross-sectional area of adipocytes in the UA group relative to the HFD group (Figure 4C,D).

3.6. The Effect of Ursolic Acid on Serum Hormones

To investigate the potential involvement of endocrine factors in obesity development, serum levels of leptin, T3, T4, GH and IGF-1 were measured. As shown in Figure 5, the HFD group exhibited notably reduced concentrations of GH, IGF-1 and T3 compared with the CON group ($p < 0.05$). In contrast, leptin levels were significantly elevated in HFD-fed

mice relative to the control group ($p < 0.05$). After UA treatment, GH, IGF-1 and T3 levels were increased, and leptin levels were decreased ($p < 0.05$). Additionally, no significant difference was observed for T4 levels between the CON group and the HFD group, but the UA group showed a notable increase.

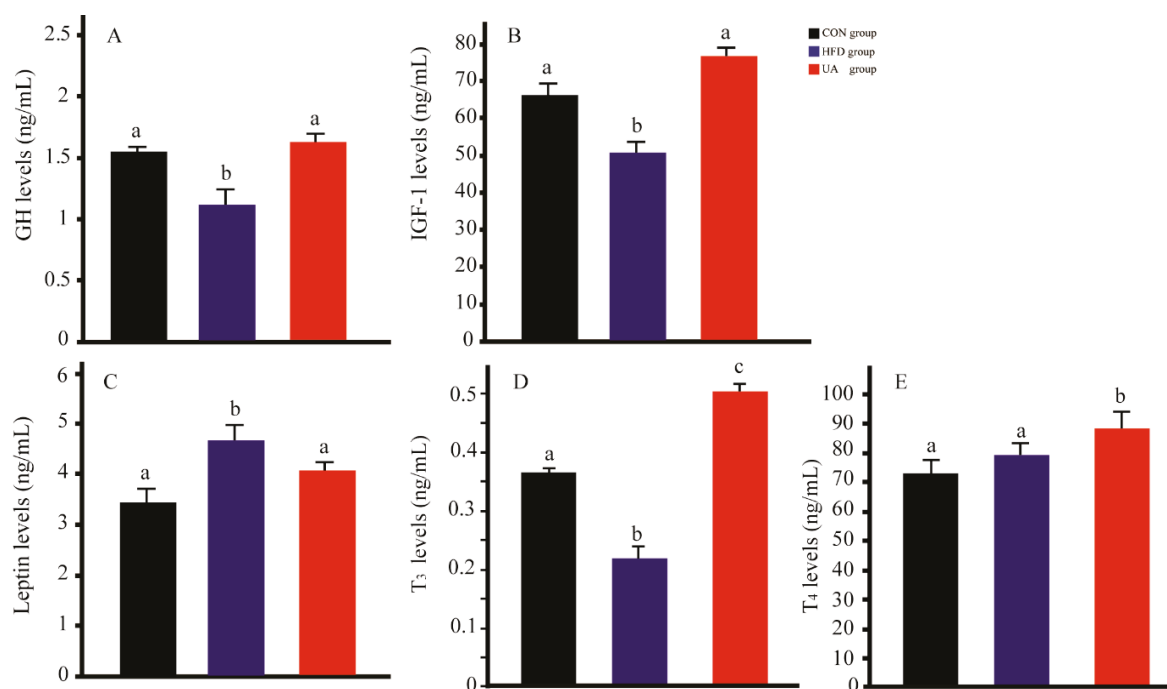


Figure 5. The effect of ursolic acid on serum hormone levels. (A) Serum GH levels. (B): Serum IGF-1 levels. (C): Serum leptin levels. (D): Serum T3 levels. (E): Serum T4 levels. Different letters represent significant differences at the 0.05 level.

3.7. The Effect of Ursolic Acid on the Expression of Inflammation-Associated Genes in Abdominal Adipose Tissue

We analyzed the expression of inflammation-associated genes TNF- α and IL-1 β . The expression of TNF- α and IL-1 β was significantly higher in the HFD group than in the CON group. UA administration significantly reduced their mRNA expression compared with the HFD group. The results are presented in Figure 6.

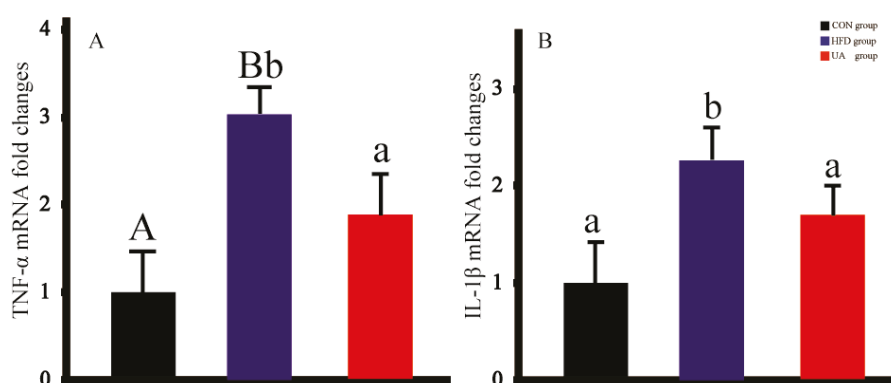


Figure 6. Changes in the expression of inflammation-associated genes. (A): TNF- α mRNA. (B): IL-1 β mRNA. Values with different capital letters are significant at the 0.01 level. Values with different lowercase letters are significant at the 0.05 level.

4. Discussion

Obesity represents a significant public health concern affecting elderly populations, children, and adolescents globally. Although body weight reduction is beneficial for managing obesity and mitigating associated metabolic disorders, it is frequently accompanied by a decline in skeletal muscle mass [46]. Skeletal muscle mass is closely related to function and metabolic capacity of the muscle [47], and skeletal muscle atrophy and dysfunction can further impair whole-body metabolism [48]. The desired outcomes for the management of people living with obesity are the maintenance or increase of muscle mass and function when body weight (fat mass) is reduced. Hand grip strength is a well-established indicator of muscle strength and is often used to assess muscle condition [49,50]. In the present study, the assessment of grip strength revealed a significant reduction in the HFD group. Notably, UA supplementation effectively restored grip strength and concurrently increased muscle mass in HFD-induced obese mice. Results regarding muscle strength and muscle mass are in line with human clinical studies, in which muscle strength was positively correlated with muscle mass [51–53].

Obesity is defined as a condition of excessive adipose tissue accumulation, characterized by adipose tissue expansion through adipocyte hypertrophy (increase in cell size) and/or hyperplasia (increase in cell number) [54,55]. The current study found that mice fed a HFD gained more body weight, abdominal fat mass and higher liver index values than those who received a control diet, and UA supplementation reduced body weight, abdominal fat mass and liver index values in HFD-fed mice. We also found that the adipocyte size in abdominal adipose tissue was decreased by UA, indicating that adipose tissue loss may have led to weight loss and that UA may regulate lipid metabolism in peripheral adipose tissues. The lipid abnormalities usually observed in obese patients are high levels of serum TG, TC, FFA and LDL-C [56,57]. Serum lipids were tested in the current study and our results demonstrated that UA effectively decreased TG, TC, LDL-C and FFA in serum in HFD-fed mice after 6 weeks of administration. This suggests that UA might be useful for treating and preventing hyperlipidemia.

Given the role of leptin and TH in the regulation of energy expenditure and lipid homeostasis, circulating leptin and TH levels were determined. Leptin is produced in adipose tissue and can regulate energy balance by inhibiting hunger and increasing energy expenditure in the body [58,59]. However, leptin levels seem to be increased in obese patients [60]. We also found that serum leptin levels are higher in HFD-fed mice. It is possible that high leptin levels lead to leptin resistance and blunt its positive effects [61,62]. TH was secreted by the thyroid gland. T3 is the most metabolically active form of TH, and T4 is considered a prohormone and constitutes a large reserve of T3. The regulation of energy expenditure is a critical role of TH [63]. Shao et al. reported that high-fat diets could induce hypothyroidism in mice by increasing thyrotropin and decreasing T3 and T4 levels, likely related to increased leptin signaling [64]. Consistent Shao et al.'s research, we found that T3 levels were lower in HFD-fed mice. UA treatment increased T3 and T4 levels. These data indicated that UA played a key role in energy homeostasis by regulating circulating hormone levels.

Both GH and IGF-1 are anabolic hormones related to skeletal muscle function and mass. Early and recent studies have shown that GH strongly affected skeletal muscle mass and performance [65,66]. GH can stimulate skeletal muscle protein synthesis and skeletal muscle growth in human and animals [67,68]. IGF-1 primarily regulates muscle mass through both the activation of protein synthesis and the inhibition of protein breakdown [69], and IGF-1 mediates the primary anabolic action of GH in skeletal muscle [70]. The reduction of GH/IGF-1 is markedly associated with a decrease in muscle growth and function in people with obesity [71]. In the present study, UA administration significantly

elevated serum GH and IGF-1 levels compared with the HFD group, suggesting a plausible role of UA in enhancing muscle mass and function. Further investigation is warranted to elucidate the underlying mechanisms involved.

Obesity is a chronic metabolic disease accompanying low-grade inflammatory responses [72]. Excessive fat leads to the expansion of adipose tissue. Under these conditions, macrophage infiltration is triggered and levels of inflammatory cytokines (e.g., TNF- α and IL-1 β) produced by activated macrophages are increased [73]. It has been demonstrated that TNF- α and IL-1 β are two important pro-inflammatory biomarkers involved in the development of obesity [74–76]. Previous research has shown that TNF- α possesses higher expression levels in adipose tissue in obese and diabetic rodents [77], and increased TNF- α production in human adipose tissue is positively correlated with obesity [78]. IL-1 β participates in the inflammatory process of obesity and obesity-related diseases [79] and contributes to the development of insulin resistance [80]. Our findings were consistent with previous research. Here, the HFD group showed a higher expression of TNF- α and IL-1 β mRNA, whereas UA treatment down-regulated their mRNA levels. This demonstrates that UA possesses anti-inflammatory effects.

Nonalcoholic fatty liver disease (NAFLD) is closely associated with obesity [81]. When adipose tissue storage capacity becomes limited, ectopic fat deposition occurs in organs such as the liver, which may contribute to the onset and progression of NAFLD [82]. Duan et al. showed that HFD induced rapid weight gain and obesity and expanded areas of liver steatosis in mice [83]. Similarly to their study, the current study indicated that the mice fed a HFD had higher body weight and liver weight and bigger areas of liver steatosis. To test the ability of UA to limit liver steatosis, we evaluated the histological appearance of this organ in mice. The results showed that UA reduced liver weight and improved the histological appearance of the liver, indicating that UA reduced liver steatosis.

This study has potential limitations. Muscle strength was assessed at only a single terminal point; data from both baseline and the end of the experiment should be included. Although a dose of 40 mg/kg body weight is effective under the conditions of this study, this cannot be directly translated to dietary intake in humans; more clinical studies are needed in the future. In addition, doses of UA should be tested in order to establish dose–response effects. Energy expenditure could not be tested due to limitations of the experimental conditions, which limits further understanding of the management of obesity with UA treatment. The mass and functions of brown adipose tissues were not evaluated in this study, so further examination of brown adipose tissue might provide a stronger scientific basis for the application of UA. In addition, the mechanism of action of UA on different target organs also needs to be clarified.

5. Conclusions

In this study, we showed that UA enhanced grip strength and improved body weight and hepatic steatosis by altering muscle mass, fat mass, hormone and inflammatory cytokines in HFD-induced obese mice, demonstrating its efficacy against obesity and improving muscle function and the metabolic environment.

These findings confirmed that UA administration enhanced muscle strength and improved lipid metabolism and hepatic steatosis induced by HFD. These data also show that UA acts on multiple target organs and could be considered a potential therapeutic agent for managing obesity, sarcopenia and other metabolic diseases, or might serve as a functional ingredient in the development of health-promoting foods. Based on these findings, future studies are required to understand the underlying mechanisms.

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Institutional Review Board Statement: The animal experiments received approval from the Institutional Animal Care and Use Committee of Henan University of Science and Technology (Approval No: 202403017; Date: 8 March 2024) and were performed in accordance with the Guide for the Care and Use of Laboratory Animals: Eighth Edition.

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

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

Abbreviations

The following abbreviations are used in this manuscript:

SO	Sarcopenic obesity
NAFLD	Nonalcoholic fatty liver disease
UA	Ursolic acid
HFD	High-fat diet
TC	Total cholesterol
TG	Triglyceride
HDL-C	High-density lipoprotein cholesterol
LDL-C	Low-density lipoprotein cholesterol
TH	Thyroxine
GH	Growth hormone
IGF-1	Insulin-like growth factor-1
IL-1 β	Interleukin-1 β
TNF- α	Tumor necrosis factor- α
FFA	Free fatty acid

References

1. The World Health Organization (WHO). *Obesity and Overweight*; WHO Media Center: Geneva, Switzerland, 2012.
2. Mopuri, R.; Ganjari, M.; Banavathy, K.S.; Parim, B.N.; Meriga, B. Evaluation of anti-obesity activities of ethanolic extract of *Terminalia paniculata* bark on high fat diet-induced obese rats. *BMC Complement. Altern. Med.* **2015**, *15*, 76. [CrossRef]
3. Naomi, R.; Teoh, S.H.; Embong, H.; Balan, S.S.; Othman, F.; Bahari, H.; Yazid, M.D. The role of oxidative stress and inflammation in obesity and its impact on cognitive impairments—a narrative review. *Antioxidants* **2023**, *12*, 1071. [CrossRef]
4. Yang, H.W.; Fernando, K.H.N.; Oh, J.Y.; Li, X.; Jeon, Y.J.; Ryu, B. Anti-obesity and anti-diabetic effects of *ishige okamurae*. *Mar. Drugs* **2019**, *17*, 202. [CrossRef]
5. Hruby, A.; Hu, F.B. The epidemiology of obesity: A big picture. *Pharmacoeconomics* **2015**, *33*, 673–689. [CrossRef] [PubMed]
6. Lee, E.S.; Choi, S.J.; Kim, H.D.; Kang, M.H.; Ji, Y.J.; Kim, G.S.; Jang, G.Y. Anti-obesity activity in 3T3-L1 cells of *Cornus officinalis* fruits harvested at different times. *Processes* **2022**, *10*, 2008. [CrossRef]
7. Goodarzi, M.O. Genetics of obesity: What genetic association studies have taught us about the biology of obesity and its complications. *Lancet Diabetes Endocrinol.* **2018**, *6*, 223–236. [CrossRef]
8. Cruz-Jentoft, A.J.; Bahat, G.; Bauer, J.; Boirie, Y.; Bruyère, O.; Cederholm, T.; Cooper, C.; Landi, F.; Rolland, Y.; Sayer, A.A.; et al. Writing group for the european working group on sarcopenia in older people 2 (EWGSOP2), and the extended group for EWGSOP2. sarcopenia: Revised european consensus on definition and diagnosis. *Age Ageing* **2019**, *48*, 16–31. [CrossRef]

9. Wijarnpreecha, K.; Panjawatanan, P.; Thongprayoon, C.; Jaruvongvanich, V.; Ungprasert, P. Sarcopenia and risk of nonalcoholic fatty liver disease: A metaanalysis. *Saudi J. Gastroenterol.* **2018**, *24*, 12–17. [CrossRef]
10. Polyzos, S.A.; Vachliotis, I.D.; Mantzoros, C.S. Sarcopenia, sarcopenic obesity and nonalcoholic fatty liver disease. *Metabolism* **2023**, *147*, 155676. [CrossRef]
11. Steffl, M.; Chrudimsky, J.; Tufano, J.J. Using relative handgrip strength to identify children at risk of sarcopenic obesity. *PLoS ONE* **2017**, *12*, e0177006. [CrossRef]
12. Barazzoni, R.; Bischoff, S.C.; Boirie, Y.; Busetto, L.; Cederholm, T.; Dicker, D.; Toplak, H.; Van Gossum, A.; Yumuk, V.; Vettor, R. Sarcopenic obesity: Time to meet the challenge. *Clin. Nutr.* **2018**, *37*, 1787–1793. [CrossRef] [PubMed]
13. Alizadeh Pahlavani, H. Exercise therapy for people with sarcopenic obesity: Myokines and adipokines as effective actors. *Front. Endocrinol.* **2022**, *13*, 811751. [CrossRef] [PubMed]
14. Koliaki, C.; Liatis, S.; Dalamaga, M.; Kokkinos, A. Sarcopenic obesity: Epidemiologic evidence, pathophysiology, and therapeutic perspectives. *Curr. Obes. Rep.* **2019**, *8*, 458–471. [CrossRef] [PubMed]
15. Kim, K.; Hong, S.; Kim, E.Y. Reference values of skeletal muscle mass for korean children and adolescents using data from the korean national health and nutrition examination survey 2009–2011. *PLoS ONE* **2016**, *11*, e0153383. [CrossRef]
16. Yodoshi, T.; Orkin, S.; Arce Clachar, A.C.; Bramlage, K.; Sun, Q.; Fei, L.; Beck, A.F.; Xanthakos, S.A.; Trout, A.T.; Mouzaki, M. Muscle mass is linked to liver disease severity in pediatric nonalcoholic fatty liver disease. *J. Pediatr.* **2020**, *223*, 93–99. [CrossRef]
17. Burrows, R.; Correa-Burrows, P.; Reyes, M.; Blanco, E.; Albala, C.; Gahagan, S. High cardiometabolic risk in healthy Chilean adolescents: Associations with anthropometric, biological and lifestyle factors. *Public Health Nutr.* **2016**, *19*, 486–493. [CrossRef]
18. Hope, D.C.D.; Tan, T.M. Skeletal muscle loss and sarcopenia in obesity pharmacotherapy. *Nat. Rev. Endocrinol.* **2024**, *20*, 695–696. [CrossRef]
19. Frontera, W.R.; Ochala, J. Skeletal muscle: A brief review of structure and function. *Calcif. Tissue Int.* **2015**, *96*, 183–195. [CrossRef]
20. Schiaffino, S.; Dyar, K.A.; Ciciliot, S.; Blaauw, B.; Sandri, M. Mechanisms regulating skeletal muscle growth and atrophy. *FEBS J.* **2013**, *280*, 4294–4314. [CrossRef]
21. Lee, S.H.; Park, S.Y.; Choi, C.S. Insulin resistance: From mechanisms to therapeutic strategies. *Diabetes. Metab. J.* **2022**, *46*, 15–37. [CrossRef]
22. Huang, X.; Liu, G.; Guo, J.; Su, Z. The PI3K/AKT pathway in obesity and type 2 diabetes. *Int. J. Biol. Sci.* **2018**, *14*, 1483–1496. [CrossRef] [PubMed]
23. Park, J.H.; Park, H.Y. Association between muscular strength, abdominal obesity, and incident nonalcoholic fatty liver disease in a Korean population. *Sci. Rep.* **2025**, *15*, 17602. [CrossRef] [PubMed]
24. Park, M.J.; Choi, K.M. Interplay of skeletal muscle and adipose tissue: Sarcopenic obesity. *Metabolism* **2023**, *144*, 155577. [CrossRef]
25. Vaidya, S.M.; Nariya, D.M. Handgrip strength as a predictor of muscular strength and endurance: A cross-sectional study. *J. Clin. Diagn. Res.* **2021**, *15*, YC01–YC04. [CrossRef]
26. Rho, H.S.; Choi, W.S. Low muscle strength as risk factor for non-alcoholic fatty liver disease in different metabolic conditions. *Korean J. Fam. Med.* **2024**, *45*, 89–95. [CrossRef]
27. McLean, R.; Shardell, M.; Alley, D.; Cawthon, P.; Fragala, M.; Harris, T.; Kenny, A.M.; Pe-ters, K.W.; Luigi, F.; Guralnik, J.M. Criteria for clinically relevant weakness and low lean mass and their longitudinal association with incident mobility impairment and mortality: The foundation for the National Institutes of Health (FNIH) sarcopenia project. *J. Gerontol.* **2014**, *69*, 576–583. [CrossRef]
28. Shaik Mohamed Sayed, U.F.; Moshawih, S.; Goh, H.P.; Kifli, N.; Gupta, G.; Singh, S.K.; Chellappan, D.K.; Dua, K.; Hermansyah, A.; Ser, H.L.; et al. Natural products as novel anti-obesity agents: Insights into mechanisms of action and potential for therapeutic management. *Front. Pharmacol.* **2023**, *14*, 1182937. [CrossRef]
29. Martins, T.; Ferreira, T.; Nascimento-Gonçalves, E.; Castro-Ribeiro, C.; Lemos, S.; Rosa, E.; Antunes, L.M.; Oliveira, P.A. Obesity rodent models applied to research with food products and natural compounds. *Obesities* **2022**, *2*, 171–204. [CrossRef]
30. Chan, Y.; Ng, S.W.; Tan, J.Z.X.; Gupta, G.; Chellappan, D.K. Natural products in the management of obesity: Fundamental mechanisms and pharmacotherapy. *S. Afr. J. Bot.* **2021**, *143*, 176–197. [CrossRef]
31. Sharma, R.; Yadav, V.; Jha, S.; Dighe, S.; Jain, S. Unveiling the potential of ursolic acid modified hyaluronate nanoparticles for combination drug therapy in triple negative breast cancer. *Carbohydr. Polym.* **2024**, *338*, 122196. [CrossRef]
32. Parthiban, A.; Sachithanandam, V.; Lalitha, P.; Adhikari, P.; Prakash, S.; Ramasubburayan, R.; Dhillon, D.; Muthukumaran, J.; Vinithkumar, N.V.; Sridhar, R.; et al. Decoding the multi-functional potential of ursolic acid: Antioxidant, antiproliferative, molecular dynamics, and biodegradability evaluations of a mangrove-derived terpenoid. *J. Comput.-Aided Mol. Des.* **2025**, *39*, 22. [CrossRef] [PubMed]
33. Miatmoko, A.; Jauhari, A.A.; Faradisa, A.A.; Cahyani, D.M.; Hariawan, B.S.; Susanto, J.; Pa-rumasivam, T.; Hendradi, E.; Sari, R. Oral curative therapeutic study of N-nitrosodiethylamine-induced mouse liver damage of chitosan-coated ursolic acid nio-somes. *Sci. Rep.* **2025**, *15*, 19225. [CrossRef] [PubMed]

34. Zafar, S.; Khan, K.; Hafeez, A.; Irfan, M.; Armaghan, M.; Rahman, A.U.; Güreş, E.S.; Sharifi-Rad, J.; Butnariu, M.; Bagiu, I.C. Ursolic acid: A natural modulator of signaling networks in different cancers. *Cancer Cell Int.* **2022**, *22*, 399. [CrossRef] [PubMed]
35. Liu, G.; Qin, P.; Cheng, X.; Wu, L.; Wang, R.; Gao, W. Ursolic acid: Biological functions and application in animal husbandry. *Front. Vet. Sci.* **2023**, *10*, 1251248. [CrossRef]
36. Yang, B.; Zhu, Q.; Wang, X.; Mao, J.; Zhou, S. Using network pharmacology and molecular docking verification to explore the mechanism of ursolic acid in the treatment of osteoporosis. *Medicine* **2022**, *101*, e32222. [CrossRef]
37. Kunkel, S.D.; Elmore, C.J.; Bongers, K.S.; Ebert, S.M.; Fox, D.K.; Dyle, M.C.; Bullard, S.A.; Adams, C.M. Ursolic acid increases skeletal muscle and brown fat and decreases diet-induced obesity, glucose intolerance and fatty liver disease. *PLoS ONE* **2012**, *7*, e39332. [CrossRef]
38. Sun, A.; Hu, X.; Chen, H.; Ma, Y.; Yan, X.; Peng, D.; Ping, J.; Yan, Y. Ursolic acid induces white adipose tissue beiging in high-fat-diet obese male mice. *Food Funct.* **2021**, *12*, 6490–6501. [CrossRef]
39. Wang, T.Z.; Zuo, G.W.; Yao, L.; Yuan, C.L.; Li, H.F.; Lai, Y.; Chen, Z.W.; Zhang, J.; Jin, Y.Q.; Yamahara, J.; et al. Ursolic acid ameliorates adipose tissue insulin resistance in aged rats via activating the Akt-glucose transporter 4 signaling pathway and inhibiting inflammation. *Exp. Ther. Med.* **2021**, *22*, 10901. [CrossRef]
40. Li, S.; Liao, X.; Meng, F.; Wang, Y.; Sun, Z.; Guo, F.; Li, X.; Meng, M.; Li, Y.; Sun, C. Therapeutic role of ursolic Acid on ameliorating hepatic steatosis and improving metabolic disorders in high-fat diet-induced non-alcoholic fatty liver disease rats. *PLoS ONE* **2014**, *9*, e86724. [CrossRef]
41. Colla, A.R.; Rosa, J.M.; Cunha, M.P.; Rodrigues, A.L. Anxiolytic-like effects of ursolic acid in mice. *Eur. J. Pharmacol.* **2015**, *758*, 171–176. [CrossRef]
42. Kannampuzha, S.; Gopalakrishnan, A.V. Protective role of ursolic acid against cisplatin-induced oxidative stress and ferroptosis in the liver of Swiss albino mice. *Med. Oncol.* **2025**, *42*, 327. [CrossRef] [PubMed]
43. National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for the Care and Use of Laboratory Animals*, 8th ed.; National Academies Press: Washington, DC, USA, 2011.
44. Sakamoto, M.; Tsujikawa, H.; Effendi, K.; Ojima, H.; Harada, K.; Zen, Y.; Kondo, F.; Nakano, M.; Kage, M.; Sumida, Y.; et al. Pathological findings of nonalcoholic steatohepatitis and nonalcoholic fatty liver disease. *Pathol. Int.* **2017**, *67*, 1–7. [CrossRef] [PubMed]
45. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta C(T))} Method. *Methods* **2001**, *25*, 402–408. [CrossRef] [PubMed]
46. Harper, M.E.; Dent, R.R.M.; McPherson, R. High-quality weight loss in obesity: Importance of skeletal muscle. *Diabetes* **2025**, *74*, dbi250003. [CrossRef]
47. Lahiri, S.; Kim, H.; Garcia-Perez, I.; Reza, M.M.; Martin, K.A.; Kundu, P.; Cox, L.M.; Selkirk, J.; Posma, J.M.; Zhang, H.; et al. The gut microbiota influences skeletal muscle mass and function in mice. *Sci. Transl. Med.* **2019**, *1*, eaa5662. [CrossRef]
48. Castillo, Í.M.P.; Argilés, J.M.; Rueda, R.; Ramírez, M.; Pedrosa, J.M.L. Skeletal muscle atrophy and dysfunction in obesity and type-2 diabetes mellitus: Myocellular mechanisms involved. *Rev. Endocr. Metab. Dis.* **2025**. [CrossRef]
49. Yi, D.W.; Khang, A.R.; Lee, H.W.; Son, S.M.; Kang, Y.H. Relative handgrip strength as a marker of metabolic syndrome: The Korea National Health and Nutrition Examination Survey (KNHANES) VI (2014–2015). *Diabetes Metab. Syndr. Obes.* **2018**, *11*, 227–240. [CrossRef]
50. Zhu, J.; Wang, J.; Fan, C.; Wu, D.; Feng, Q. Handgrip strength and low muscle strength rate in chinese adults-China, 2020. *China CDC Wkly.* **2024**, *6*, 821–824. [CrossRef]
51. Porto, J.M.; Nakaishi, A.P.M.; Cangussu-Oliveira, L.M.; Freire Júnior, R.C.; Spilla, S.B.; Abreu, D.C.C. Relationship between grip strength and global muscle strength in community-dwelling older people. *Arch. Gerontol. Geriatr.* **2019**, *82*, 273–278. [CrossRef]
52. Bohannon, R.W. Muscle strength: Clinical and prognostic value of hand-grip dynamometry. *Curr. Opin. Clin. Nutr. Metab. Care* **2015**, *18*, 465–470. [CrossRef]
53. Momma, H.; Kato, K.; Sawada, S.S.; Gando, Y.; Kawakami, R.; Miyachi, M.; Nagatomi, R.; Tashiro, M.; Matsubayashi, Y.; Kodama, S.; et al. Physical fitness and dyslipidemia among japanese: A cohort study from the niigata wellness study. *J. Epidemiol.* **2021**, *31*, 287–296. [CrossRef]
54. Jo, J.; Gavrilova, O.; Pack, S.; Jou, W.; Mullen, S.; Sumner, A.E.; Cushman, S.W.; Periwai, V. Hypertrophy and/or hyperplasia: Dynamics of adipose tissue growth. *PLoS Comput. Biol.* **2009**, *5*, e1000324. [CrossRef]
55. Romieu, I.; Dossus, L.; Barquera, S.; Blotière, H.M.; Franks, P.W.; Gunter, M.; Hwalla, N.; Hursting, S.D.; Leitzmann, M.; Margetts, B.; et al. Energy balance and obesity: What are the main drivers? *Cancer Causes Control* **2017**, *28*, 247–258. [CrossRef] [PubMed]
56. Bays, H.E.; Toth, P.P.; Kris-Etherton, P.M.; Abate, N.; Aronne, L.J.; Brown, W.V.; Gonzalez-Campoy, J.M.; Jones, S.R.; Kumar, R.; La Forge, R.; et al. Obesity, adiposity, and dyslipidemia: A consensus statement from the national lipid association. *J. Clin. Lipidol.* **2013**, *7*, 304–383. [CrossRef] [PubMed]
57. Nussbaumerova, B.; Rosolova, H. Obesity and dyslipidemia. *Curr. Atheroscler. Rep.* **2023**, *25*, 947–955. [CrossRef] [PubMed]

58. Pijet, M.; Pijet, B.; Litwiniuk, A.; Pajak, B.; Gajkowska, B.; Orzechowski, A. Leptin impairs myogenesis in C2C12 cells through JAK/STAT and MEK signaling pathways. *Cytokine* **2013**, *61*, 445–454. [CrossRef]
59. Skubic, C.; Drakulić, Ž.; Rozman, D. Personalized therapy when tackling nonalcoholic fatty liver disease: A focus on sex, genes, and drugs. *Expert Opin. Drug Metab. Toxicol.* **2018**, *14*, 831–841. [CrossRef]
60. Adolph, T.E.; Grander, C.; Grabherr, F.; Tilg, H. Adipokines and non-alcoholic fatty liver disease: Multiple interactions. *Int. J. Mol. Sci.* **2017**, *18*, 1649. [CrossRef]
61. Fuentes, T.; Ara, I.; Guadalupe-Grau, A.; Larsen, S.; Stallknecht, B.; Olmedillas, H.; Santana, A.; Helge, J.W.; Calbet, J.A.; Guerra, B. Leptin receptor 170 kDa (OB-R170) protein expression is reduced in obese human skeletal muscle: A potential mechanism of leptin resistance. *Exp. Physiol.* **2010**, *95*, 160–171. [CrossRef]
62. Kohara, K.; Ochi, M.; Tabara, Y.; Nagai, T.; Igase, M.; Miki, T. Leptin in sarcopenic visceral obesity: Possible link between adipocytes and myocytes. *PLoS ONE* **2011**, *6*, e24633. [CrossRef]
63. Yavuz, S.; Salgado Nunez Del Prado, S.; Celi, F.S. Thyroid hormone action and energy expenditure. *J. Endocr. Soc.* **2019**, *3*, 1345–1356. [CrossRef] [PubMed]
64. Shao, S.S.; Zhao, Y.F.; Song, Y.F.; Xu, C.; Yang, J.M.; Xuan, S.M.; Yan, H.L.; Yu, C.X.; Zhao, M.; Xu, J.; et al. Dietary high-fat lard intake induces thyroid dysfunction and abnormal morphology in rats. *Acta Pharmacol. Sin.* **2014**, *35*, 1411–1420. [CrossRef] [PubMed]
65. Wolf, E.; Wanke, R.; Schenck, E.; Hermanns, W.; Brem, G. Effects of growth hormone overproduction on grip strength of transgenic mice. *Eur. J. Endocrinol.* **1995**, *133*, 735–740. [CrossRef] [PubMed]
66. Malpani, A.; Welch, L.; Plummer, D.; Churilla, J.; Benson, M.; Hossain, J.; Permuy, J.; Carakushansky, M.; Mauras, N. Impact of growth hormone on skeletal muscle strength, power, endurance, and agility in prepubertal boys with short stature. *J. Clin. Endocrinol. Metab.* **2025**, *2*, dgaf203. [CrossRef]
67. Jiang, H.; Ge, X. Meat science and muscle biology symposium—Mechanism of growth hormone stimulation of skeletal muscle growth in cattle. *J. Anim. Sci.* **2014**, *92*, 21–29. [CrossRef]
68. Rudman, D.; Feller, A.G.; Nagraj, H.S.; Gergans, G.A.; Lalitha, P.Y.; Goldberg, A.F.; Schlenker, R.A.; Cohn, L.; Rudman, I.W.; Mattson, D.E. Effects of human growth hormone in men over 60 years old. *N. Engl. J. Med.* **1990**, *323*, 1–6. [CrossRef]
69. Mathes, S.; Fahrner, A.; Luca, E.; Krützfeldt, J. Growth hormone/IGF-I-dependent signaling restores decreased expression of the myokine SPARC in aged skeletal muscle. *J. Mol. Med.* **2022**, *100*, 1647–1658. [CrossRef]
70. Green, H.; Morikawa, M.; Nixon, T. A dual effector theory of growth-hormone action. *Differentiation* **1985**, *29*, 195–198. [CrossRef]
71. Cappola, A.R.; Bandeen-Roche, K.; Wand, G.S.; Volpato, S.; Fried, L.P. Association of IGF-I levels with muscle strength and mobility in older women. *J. Clin. Endocrinol. Metab.* **2001**, *86*, 4139–4146. [CrossRef]
72. Theilade, S.; Christensen, M.B.; Vilsbøll, T.; Knop, F.K. An overview of obesity mechanisms in humans: Endocrine regulation of food intake, eating behaviour and common determinants of body weight. *Diabetes Obes. Metab.* **2021**, *23*, 17–35. [CrossRef]
73. Lumeng, C.N.; Saltiel, A.R. Inflammatory links between obesity and metabolic disease. *J. Clin. Invest.* **2011**, *121*, 2111–2117. [CrossRef] [PubMed]
74. Romacho, T.; Glosse, P.; Richter, I.; Elsen, M.; Schoemaker, M.H.; van Tol, E.A.; Eckel, J. Nutritional ingredients modulate adipokine secretion and inflammation in human primary adipocytes. *Nutrients* **2015**, *7*, 865–886. [CrossRef] [PubMed]
75. Saha, S.; Panigrahi, D.P.; Patil, S.; Bhutia, S.K. Autophagy in health and disease: A comprehensive review. *Biomed. Pharmacother.* **2018**, *104*, 485–495. [CrossRef] [PubMed]
76. Pedersen, B.K. Skeletal muscle as an endocrine organ: The role of myokines in muscle-fat cross-talk. *Inflamm. Res.* **2010**, *59*, S275–S276.
77. Hotamisligil, G.S.; Shargill, N.S.; Spiegelman, B.M. Adipose expression of tumor necrosis factor- α : Direct role in obesity-linked insulin resistance. *Science* **1993**, *259*, 87–91. [CrossRef]
78. Kern, P.A.; Ranganathan, S.; Li, C.; Wood, L.; Ranganathan, G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *Am. J. Physiol. Endocrinol. Metab.* **2001**, *280*, E745–E751. [CrossRef]
79. Luo, J.; Chen, M.; Ji, H.; Su, W.; Song, W.; Zhang, D.; Su, W.; Liu, S. Hypolipidemic and anti-obesity effect of anserine on mice orally administered with high-fat diet via regulating SREBP-1, NLRP3, and UCP-1. *Mol. Nutr. Food. Res.* **2024**, *68*, e2300471. [CrossRef]
80. Sahebkar, A. Potential efficacy of ginger as a natural supplement for nonalcoholic fatty liver disease. *World. J. Gastroenterol.* **2011**, *17*, 271–272. [CrossRef]
81. Pereira, R.M.; Rodrigues, K.C.D.C.; Anaruma, C.P.; Sant’Ana, M.R.; de Campos, T.D.P.; Gaspar, R.S.; Canciglieri, R.D.S.; de Melo, D.G.; Mekary, R.A.; da Silva, A.S.R.; et al. Short-term strength training reduces gluconeogenesis and NAFLD in obese mice. *J. Endocrinol.* **2019**, *241*, 59–70. [CrossRef]

82. Polyzos, S.A.; Perakakis, N.; Mantzoros, C.S. Fatty liver in lipodystrophy: A review with a focus on therapeutic perspectives of adiponectin and/or leptin replacement. *Metabolism* **2019**, *96*, 66–82. [CrossRef]
83. Duan, X.; Meng, Q.; Wang, C.; Liu, Z.; Sun, H.; Huo, X.; Sun, P.; Ma, X.; Peng, J.; Liu, K. Effects of calycosin against high-fat diet-induced nonalcoholic fatty liver disease in mice. *J. Gastroenterol. Hepatol.* **2018**, *33*, 533–542. [CrossRef]

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Article

Prevalence of Overweight, Obesity, and Abdominal Obesity in Polish Adults: Sociodemographic Analysis from the 2016–2020 National Health Program

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Abstract: Introduction: Excessive body weight, particularly the abdominal distribution of adipose tissue, has remained an important public health concern for years. Objectives: The study aimed to assess the prevalence of general overweight status and obesity, as well as abdominal overweight status and obesity in the adult Polish population. Material and methods: The results are based on the analysis of anthropometric data of 3735 people aged 19 and over who were surveyed under the 2016–2020 National Health Program. Results: Excessive body weight (BMI > 25 kg/m²) was reported in 56.6% of the respondents. Abdominal overweight was found in 20.8% of respondents and abdominal obesity in 31.7% of the respondents. Abdominal obesity was more common in women than in men (44.3% vs. 18.0%, $p < 0.001$). Over half of women over 55 (approx. 60%) had abdominal obesity. The odds of becoming overweight/obese as measured by the BMI depended on the age and sex of the respondents. It was confirmed that men were over 28% more likely to develop such a condition than women (OR = 1.288, $p < 0.001$). The odds increased with age (OR = 1.029, $p < 0.001$). The type of person with obesity/found to be overweight in Polish population was most often a rural resident, aged 65.0–74.9, assessing their financial situation as average, being married/in a partnership, and not declaring the occurrence of cardiovascular diseases. The person with an overweight status had secondary education, and the person with obesity had primary/lower secondary/vocational education. Conclusions: Being overweight and having obesity, both general and abdominal, are still a major epidemiological problem in Poland. The results obtained in this study suggest that the prevalence of being overweight and having obesity has decreased over the last 10 years, though this assumption requires further investigation. Rural residents with lower education should be covered by educational activities adapted to their needs and possibilities, considering that obesity in young women, especially when having abdominal obesity, may be associated with an increased risk of infertility caused by ovulation disorders. Further research and educational activities are necessary. Excessive body weight affected almost 42% of the women from the youngest age group, and abdominal obesity was found in 21% of them.

Keywords: adult Polish population; anthropometric measurements; obesity; overweight; prevalence

1. Introduction

Excessive body weight, especially obesity, exerts an enormous negative effect on the lives of people affected by the pathology, both in the social and health aspects. Obesity is a severe metabolic disease that may lead to disability and premature death by increasing the risk of developing numerous other serious diseases, including cardiovascular diseases, type 2 diabetes, hypertension, dyslipidemia, and some cancers [1–4]. Obesity is associated with a host of physical health issues [5]. Moreover, individuals living with obesity often face social exclusion and loneliness, which may contribute to mental health issues such as depression, although the two can co-occur [6]. The discussion about the excessive spread of being overweight and being obese in the world has been going on for many years [7]. According to the data published so far, the prevalence of being overweight and being obese has also increased in Poland. At the end of the 20th century, excessive body weight affected more than half of the adult population of Poland (men at 56.7% vs. women at 48.6%) [8]. In 2005, the values increased and amounted to 60.2% in men and 50% in women. A decade later, an increase in the number of overweight and people with obesity was noted again, i.e., in 2013–2014, 67.3% of men and 52.9% of women were affected, including obesity measured in 24.4% of men and 25.0% of women [9].

In the second decade of the twenty-first century, two large educational projects were carried out in Poland, the aim of which was to prevent becoming overweight and the spread of obesity in the adult Polish population: (Pol-Health) [10] and Keep the Balance [11]. It is difficult to determine to what extent these projects and many other smaller ones implemented in Poland have had in terms of impact on the current frequency of the discussed phenomenon in Poland. However, our research, being a part of the 2016–2019 NPZ project, indicates that the prevalence of being overweight and having obesity in the 19–64 age group is currently 51%. Although we confirmed that this phenomenon is still more common in the group of men than women (55 vs. 47%), it may suggest a decrease in the overall prevalence of excess body weight among adult residents of Poland [12]. The results of these recent studies prompted us to conduct an in-depth analysis of anthropometric data in the population of Polish adults aged 19 and over. The study aimed to assess the prevalence of general overweight and obesity diagnoses, as well as abdominal overweight and obesity diagnoses in the adult Polish population.

2. Material and Methods

2.1. Study Design and Patients Selection

The data analyzed in the present study were obtained from two representative cross-sectional studies of the dietary habits and nutritional status reports of the adult Polish population conducted in the years 2017–2020 within the National Health Program (NPZ 01 and NPZ 02) financed by the Ministry of Health. The NPZ 02 program included 2000 people aged 19–65, and the NPZ 01 program included 1735 people aged over 65. All analyses of anthropometric data concerned 3735 people. Age-standardized prevalence rates were calculated with the direct method using weights assessed for the population of Poland based on the data of Statistics Poland as of 31 December 2022 [13]. The weighted size of the study group was 2061, including 985 men aged 19+. Prior to the COVID-19 pandemic (October 2017–March 2020), all data were collected using the Computer-Assisted Personal Interviews (CAPIs) method. In the period from June to December 2020, the telephone interview method (CATI) was used. The number of respondents who completed the study prior to the COVID-19 pandemic was 90% of individuals.

2.2. Data Collection and Instruments

Anthropometric indicators were calculated on the basis of data obtained from measurements taken by interviewers, i.e., body weight, height, and waist circumference (WC). The measurements were performed according to established standards. Portable Omron electronic scales (HBF-212 model) were used to measure body weight (with an accuracy of 0.1 kg). Body height measurements (with an accuracy of 0.1 cm) were performed using a graduated anthropometric tape for linear tests and a set square. The same anthropometric tape was used to measure waist circumference. General overweight definition was defined as a BMI value from 25 to 29.9 kg/m², and general obesity as a BMI of 30 kg/m² or higher. In addition, for respondents aged 65 and older, the Lipschitz criteria were used, according to which excessive body weight in seniors is diagnosed with the BMI of >27 kg/m² [14]. According to European guidelines, waist circumference of 94 to 101.9 cm in men and of 80 to 87.9 cm in women was classified as abdominal overweight and that of 102 cm or higher in men and 88 cm or higher in women as abdominal obesity [15]. The crude prevalence rate of general and abdominal obesity and overweight status with 95% confidence interval (CI) was calculated for the following age groups: 19–34.9, 35–44.9, 45.0–54.9, 55–64.9, 65.0–74.9, and 75 years and older in men and women.

During the pandemic, when direct contact with the respondents was impossible, the necessary anthropometric data were obtained based on the respondents' declarations. A detailed description of the anthropometric measurements performed is provided in another publication [16].

2.3. Statistical Analysis

In this study, BMI and WC indices were analyzed using comprehensive descriptive statistical methods. The BMI data were articulated in terms of the mean, accompanied by a 95% confidence interval (CI) and standard deviation (SD), with stratification across sex and age groups. Additionally, specific percentile values were calculated. Frequency distributions for the BMI and WC categories were reported in numbers (*n*) and percentages (%), employing bootstrapping (10,000 iterations) for determining the 95% CI for each frequency.

The application of weights that corresponded to the actual frequencies of characteristics such as the sex, age, place of residence, and educational level in the Polish population was a crucial aspect of the analysis. These weights were adapted based on the data from the latest 2021 National Population and Housing Census conducted by the Statistics Poland [13], ensuring the representativeness of the sample.

For inferential statistical analysis, a null hypothesis testing approach was adopted. The comparison of mean BMI values across different groups was conducted using the Analysis of Variance (ANOVA) with the Games–Howell post hoc test. The Mann–Whitney U test with tie correction was utilized to examine differences in the frequency across various BMI or WC categories, with the eta-squared (η^2) measure providing the effect size.

Logistic regression modeling was applied to estimate the probability of developing excess weight or obesity, including abdominal obesity. The model parameters were estimated using the maximum likelihood method, providing ORs and 95% CIs for sex and age as independent variables. Furthermore, an adjusted OR was calculated in a multivariate regression model, considering additional variables like the place of residence, education, financial status, marital status, professional situation, and the presence of cardiovascular diseases. Predictors were selected for inclusion in the model based on their clinical relevance and prior evidence of association with being overweight and having obesity from the literature. All selected variables were entered into the logistic regression model simultaneously using the enter method (forced entry). To ensure the model's validity, we assessed multicollinearity among the independent variables using Variance Inflation Factors (VIFs). All VIF values were below 5, indicating no significant multicollinearity issues. The goodness-of-fit of the model was evaluated using the Akaike Information Criterion (AIC). Detailed diagnostic results are provided in the Supplementary Materials.

Data completeness was ensured in this study, as the target sample size ($n = 3735$) was achieved with complete responses to all questionnaire items from all participants. Therefore, there were no missing or erroneous data to handle in the analysis.

All statistical analyses were carried out using STATISTICA™ version 13.3 (TIBCO® Software Inc., Palo Alto, CA, USA). A 5% level of significance was set for rejecting the null hypothesis in all statistical tests.

2.4. Ethical Considerations

This study was approved by the Ethical Review Board at the Medical University of Warsaw (approval No. AKBE/163/17 and AKBE/164/17). The study was conducted in accordance with the General Data Protection Regulation [17].

3. Results

Sociodemographic sample characteristics by sex in Poland (2017–2020) are presented in Table 1. The respondents differed significantly in terms of sex depending on their place of residence, level of education, current financial situation of the family, marital status, selected professional situations, and the occurrence of cardiovascular diseases.

Table 1. Sociodemographic sample characteristics by sex in Poland (2017–2020) [$n = 3735$].

Variable	Women		Men		Total	χ^2	p -Value ^a
	n	%	n	%	n		
Age group							
19.0–34.9	241	22.4	245	24.9	486	14.830	0.011
35.0–44.9	204	19.0	231	23.5	435		
45.0–54.9	199	18.5	164	16.7	363		
55.0–64.9	131	12.2	107	10.9	238		
65.0–74.9	235	21.8	202	20.5	437		
≥75.0	66	6.1	36	3.7	102		
Place of residence							
Village	319	29.7	347	35.2	666	22.914	0.003
Towns to 10,000	89	8.3	76	7.7	165		
Towns 10–19 thousand	76	7.1	60	6.1	136		
Towns 20–49 thousand	130	12.1	112	11.4	242		
Towns 50–99 thousand	93	8.6	99	10.1	192		
Cities 100–199 thousand	93	8.6	68	6.9	161		
Cities 200–499 thousand	90	8.4	91	9.2	181		
Cities 500 thousand–1 million	95	8.8	89	9.0	184		
City > 1 million	91	8.5	43	4.4	134		
Education							
Primary/junior high school/vocational	342	31.8	424	43.1	766	43.337	<0.001
Secondary	518	48.1	450	45.7	968		
Tertiary	216	20.1	111	11.3	327		
Financial situation							
Good	205	19.1	292	29.6	497	32.631	<0.001
Average	763	70.9	619	62.8	1382		
Poor	108	10.0	74	7.5	182		
Marital status							
Unmarried	136	12.6	226	22.9	362	85.213	<0.001
Married/a cohabitation relationship	742	69.0	685	69.5	1427		
Divorced/separated	68	6.3	41	4.2	109		
Widow(er)	130	12.1	33	3.4	163		
Professional situation							
Retired/disability pensioner	379	35.2	279	28.3	658	11.259	0.001
Parental leave, unemployed, runs the house	86	8.0	27	2.7	113	27.366	<0.001
Casual employment	47	4.4	49	5.0	96	0.426	0.514
Permanent employment	551	51.2	620	62.9	1171	28.868	<0.001
Studies	27	2.5	22	2.2	49	0.169	0.681
Cardiovascular diseases ^b							
No	927	86.2	882	89.5	1809	5.509	0.019
Yes	149	13.9	103	10.5	252		

^a chi-squared test, ^b hypertension, a history of stroke, a history of myocardial infarction, coronary heart disease, heart failure, atherosclerosis of lower extremity arteries, lipid disorders.

There were no statistically significant differences between the mean BMI values in the group of men and women (M: 26.40 (± 5.17) vs. F: 26.26 (± 3.71), $t = 0.804$, $p = 0.421$) (Table 2). An increase in the average BMI value was observed in both the men and women groups (Table 3), but in the women's group, it was stronger ((F: 5.664), $t = 7.110$, $p < 0.001$) (Figure 1).

Table 2. BMI depending on the age and gender.

	Females ($n = 1076$)		Males ($n = 985$)		t	p -Value ^a	Cohen's d (95% CI)
	M (95% CI)	SD	M (95% CI)	SD			
BMI	26.56 (26.26; 26.86)	3.71	26.40 (26.18; 26.62)	5.17	0.804	0.421	0.034 (−0.048; 0.115)

BMI—body mass index; CI—confidence interval; M—mean; SD—standard deviation. ^a Student's t -test.

Table 3. BMI depending on the age and gender.

BMI Categories	Females ($n = 1076$)		Males ($n = 985$)		z	p -Value ^a	η^2
	n	% (95% CI)	n	% (95% CI)			
<18.5	30	2.8 (1.8; 3.8)	1	0.1 (0.0; 0.3)	1.866	0.062	0.001
18.5–24.9	468	43.5 (40.5; 46.5)	393	39.9 (36.8; 43.0)			
25.0–29.9	363	33.7 (30.9; 36.5)	469	47.6 (44.5; 50.7)			
≥ 30.0	215	20.0 (17.6; 22.4)	122	12.4 (10.3; 14.5)			

CI—confidence interval; η^2 —eta squared for effect size ^a Mann–Whitney U test with tie correction.

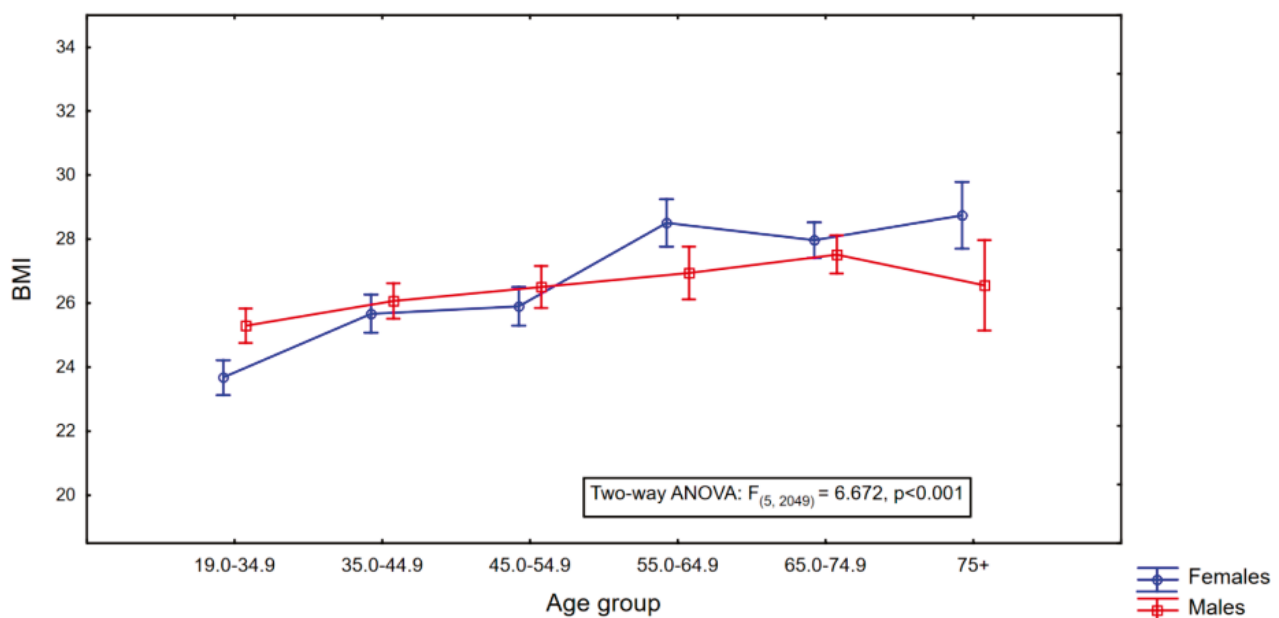


Figure 1. Sex- and age-related differences in mean body mass index (BMI) among a sample of Polish adults. The midpoints represent the mean BMI values for each age group, and the vertical lines indicate the 95% confidence intervals.

The healthy weight range was recorded in 41.8% of the study population. The overweight and obesity (BMI > 25 kg/m²) ranges were found in 56.6% of respondents, while

1.63% had too-low body weight. In the group of men overall, a higher percentage of people with excessive body weight was recorded than in the group of women (60.0 vs. 53.7%).

The analysis of the prevalence of being overweight and being obese measured by BMI categories in age groups showed that the prevalence of excess body weight increased with the age of the respondents until the age of 64.9, when it reached the value of 73.1% and decreased slightly in senior age. In all age groups, men were more likely than women to be overweight. However, women with obesity were significantly more likely than men (except for people aged 35.0–44.9 years) when the difference did not show statistical significance (Table 4). Analyzing the prevalence of excess body weight in seniors based on the Lipschitz criteria, the percentage of people with BMI > 27 kg/m² exceeded 50%, except for the oldest men, in whom excess body weight occurred less frequently and affected 44.4% of people.

Table 4. BMI categories by sex and age in Poland for 2017–2020.

Age Group	BMI Category	Women		Men		Total		z	p-Value ^a	η ²
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)			
19.0–34.9	<18.5	15	6.2 (3.2; 9.3)	0	0.0 (0.0; 1.5)	15	3.1 (1.4; 4.8)	−3.746	<0.001	0.014
	18.5–24.9	152	63.1 (57.0; 69.2)	132	53.9 (47.7; 60.1)	284	58.4 (54.0; 62.8)			
	25.0–29.9	53	22.0 (16.8; 27.2)	98	40.0 (33.9; 46.2)	151	31.1 (27.0; 35.2)			
	≥30.0	21	8.7 (5.1; 12.3)	15	6.1 (3.0; 9.2)	36	7.4 (5.2; 9.6)			
35.0–44.9	<18.5	7	3.4 (0.5; 6.3)	0	0.0 (0.0; 1.6)	7	1.6 (0.5; 3.7)	−1.270	0.204	0.003
	18.5–24.9	103	50.5 (43.9; 57.1)	104	45.0 (38.6; 51.4)	207	47.6 (42.9; 52.3)			
	25.0–29.9	67	32.8 (26.6; 39.0)	95	41.1 (34.8; 47.4)	162	37.2 (32.6; 41.8)			
	≥30.0	27	13.2 (8.5; 17.9)	32	13.9 (9.6; 18.2)	59	13.6 (10.4; 16.8)			
45.0–54.9	<18.5	3	1.5 (0.0; 3.2)	0	0.0 (0.0; 2.3)	3	0.8 (0.1; 2.5)	−2.323	0.020	0.009
	18.5–24.9	92	46.2 (39.3; 53.1)	60	36.6 (29.5; 43.7)	152	41.9 (36.9; 46.9)			
	25.0–29.9	76	38.2 (31.5; 44.9)	83	50.6 (43.2; 58.0)	159	43.8 (38.8; 48.8)			
	≥30.0	28	14.1 (9.5; 18.7)	21	12.8 (7.8; 17.8)	49	13.5 (10.2; 16.8)			
55.0–64.9	<18.5	3	2.3 (0.0; 5.4)	0	0.0 (0.0; 3.4)	3	1.3 (0.0; 3.1)	2.014	0.044	0.010
	18.5–24.9	29	22.1 (15.0; 29.2)	32	29.9 (21.2; 38.6)	61	25.6 (20.1; 31.1)			
	25.0–29.9	52	39.7 (31.4; 48.0)	59	55.1 (45.7; 64.5)	111	46.6 (40.2; 53.0)			
	≥30.0	47	35.9 (27.7; 44.1)	16	15.0 (8.4; 21.6)	63	26.5 (21.0; 32.0)			
65.0–74.9	<18.5	2	0.9 (0.0; 2.7)	1	0.5 (0.0; 2.6)	3	0.7 (0.0; 1.8)	2.209	0.027	0.004
	18.5–24.9	72	30.6 (24.7; 36.5)	55	27.2 (21.2; 33.2)	127	29.1 (24.8; 33.4)			
	25.0–29.9	93	39.6 (33.6; 45.6)	112	55.5 (48.6; 62.4)	205	46.9 (42.3; 51.5)			
	≥30.0	68	28.9 (23.2; 34.6)	34	16.8 (11.7; 21.9)	102	23.3 (19.3; 27.3)			
	<22.0	18	7.7 (4.5; 10.9)	9	4.5 (1.7; 7.3)	27	6.2 (4.0; 8.4)	0.894	0.371	0.001
	22.0–27.0	95	40.4 (34.2; 46.6)	91	45.1 (38.3; 51.9)	186	42.6 (38.0; 47.2)			
	>27.0	122	51.9 (45.6; 58.2)	102	50.5 (43.6; 57.4)	224	51.3 (46.7; 55.9)			

Table 4. Cont.

Age Group	BMI Category	Women		Men		Total		z	p-Value ^a	η^2
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)			
≥75.0	<18.5	0	0.0 (0.0; 5.5)	0	0.0 (0.0; 9.7)	0	0.0 (0.0; 3.6)	3.258	0.001	0.014
	18.5–24.9	20	30.3 (19.0; 41.6)	10	27.8 (13.3; 42.3)	30	29.4 (20.4; 38.4)			
	25.0–29.9	22	33.3 (21.7; 44.9)	22	61.1 (45.5; 76.7)	44	43.1 (33.5; 52.7)			
	≥30.0	24	36.4 (24.6; 48.2)	4	11.1 (1.0; 21.2)	28	27.5 (18.6; 36.4)			
	<22.0	2	3.0 (0.0; 8.8)	1	2.8 (0.0; 8.2)	3	2.9 (0.0; 7.0)	1.712	0.087	0.004
	22.0–27.0	28	42.4 (30.4; 54.4)	19	52.8 (36.5; 69.1)	47	46.1 (36.6; 55.6)			
	>27.0	36	54.6 (42.2; 67.0)	16	44.4 (28.3; 60.5)	52	51.0 (41.3; 60.7)			
Total	<18.5	30	2.8 (1.8; 3.8)	1	0.1 (0.0; 0.4)	31	1.5 (1.0; 2.0)	1.866	0.062	0.001
	18.5–24.9	468	43.5 (40.5; 46.5)	393	39.9 (36.9; 42.9)	861	41.8 (39.7; 43.9)			
	25.0–29.9	363	33.7 (30.9; 36.5)	469	47.6 (44.6; 50.6)	832	40.4 (38.3; 42.5)			
	≥30.0	215	20.0 (17.6; 22.4)	122	12.4 (10.4; 14.4)	337	16.4 (14.8; 18.0)			
	<22.0	20	6.6 (4.0; 9.2)	10	4.2 (1.7; 6.7)	30	5.6 (3.7; 7.5)	1.780	0.075	0.002
	22.0–27.0	123	40.9 (35.3; 46.5)	110	46.2 (40.0; 52.4)	233	43.2 (39.0; 47.4)			
	>27.0	158	52.5 (46.8; 58.2)	118	49.6 (43.4; 55.8)	276	51.2 (47.0; 55.4)			

CI—confidence interval; η^2 —eta squared for the effect size; ^a Mann–Whitney U test with tie correction.

Figure 2 presents the prevalence of being overweight and being obese in age groups. The data clearly show how the prevalence of being overweight and being obese in Poland changes with age depending on gender. The percentage of women with excessive body weight in the youngest analyzed age group was just over 30%; at the same age, the percentage of men with excessive body weight reached 46.1%, of which obesity occurred in 8.7% of women and 6.1% of men, respectively. In respondents 10 years and older (aged 35–44.9), the prevalence of obesity reached nearly 14% in men and 13.2% in women.

The percentage of adults with obesity in Poland up to 55 years of age in the analyzed age groups was at a maximum of 14.1% in the group of women aged 45–54.9 years and 13.9% in the group of men aged 35–44.9 years. In the following decades of life, in the group of men, the prevalence of obesity increased to 16.8% (age 65.0–74.9 years) and decreased to 11.1% in the oldest seniors. Women aged 55 and older struggled with obesity much more often than men; this was especially true for women aged 55–64.9 and seniors aged ≥75, where obesity exceeded 36%. The overweight status, in turn, dominated in the group of men. The highest percentage of overweight men (61.1%) was recorded in the group of the oldest seniors. In the group of women, the most overweight people (39.7%) were aged 55.0–64.9.

The analysis of the prevalence of abdominal overweight and abdominal obesity measured by waist circumference showed that they concerned 20.8% and 31.7% of the respondents, respectively. In the group of women, a significantly higher percentage of individuals with abdominal obesity was noted compared to the group of men (44.3% vs. 18.0%, $p < 0.001$). The percentages of women with abdominal overweight were similar across all age groups and ranged from 18.3% to 24.2%. An exception was noted in the group of women aged 45–54.9 years, in whom the prevalence of abdominal overweight cases was 29.7%. In the case of men, the lowest percentage of individuals with abdominal

overweight was found in the group over 75 years of age (13.9%), and the highest was found among men aged 55–64.9 years (24.3%).

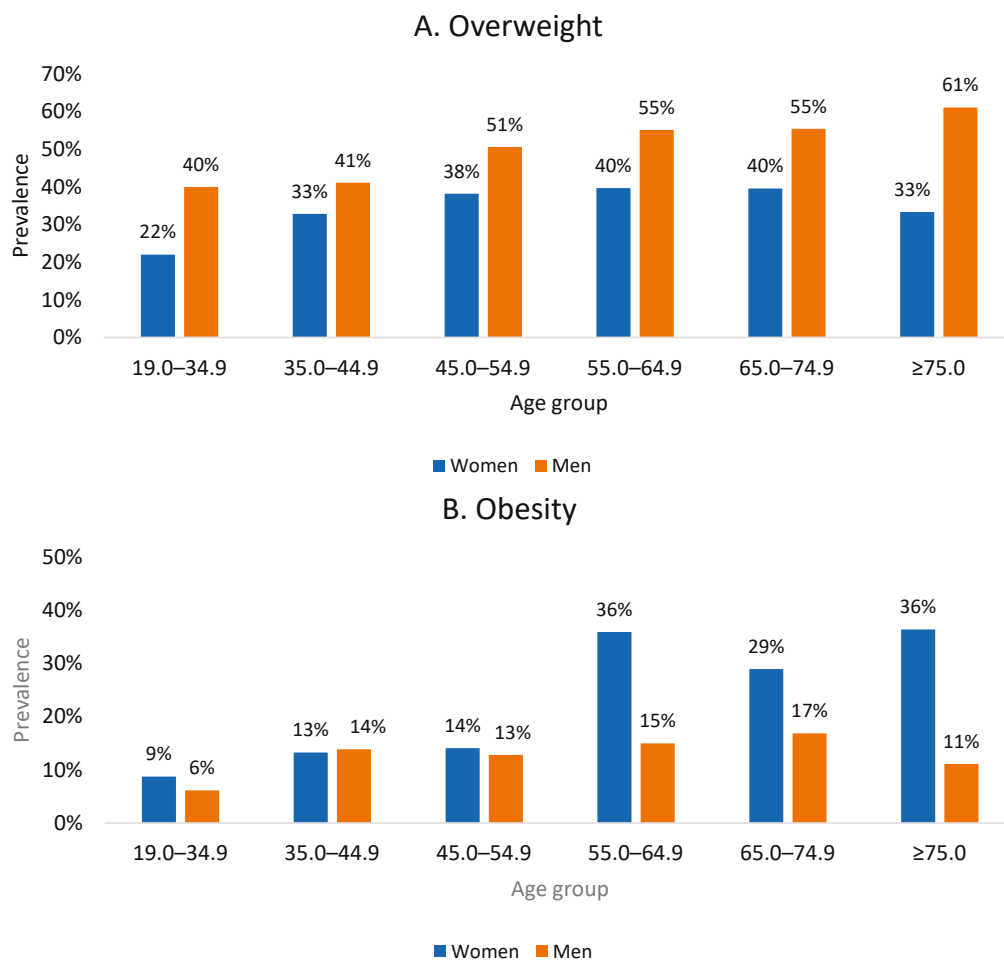


Figure 2. Prevalence of being overweight (A) and being obese (B) in the study group depending on age and gender.

The highest percentage of people with abdominal obesity was found in women over 55 years of age (approx. 60%). In this age group, abdominal obesity was significantly less common among men (20–30%) (Table 5).

Table 5. The prevalence of abdominal overweight status and abdominal obesity by sex and age in Poland, 2017–2020.

Age Group	WC Category	Women		Men		Total		z	p-Value ^a	η^2
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)			
19.0–34.9	W < 80.0 M < 94.0	146	60.6 (54.4; 66.8)	183	74.7 (69.1; 80.3)	329	67.7 (63.5; 71.9)	6.194	<0.001	0.035
	W 80–87.9 M 94–101.9	44	18.3 (13.4; 23.2)	41	16.7 (12.1; 21.3)	85	17.5 (14.0; 21.0)			
	W ≥ 88.0 M ≥ 102.0	51	21.2 (16.0; 26.4)	21	8.6 (5.2; 12.0)	72	14.8 (11.5; 18.1)			
35.0–44.9	W < 80.0 M < 94.0	80	39.2 (32.6; 45.8)	156	67.5 (61.4; 73.6)	236	54.3 (49.5; 59.1)	8.130	<0.001	0.103
	W 80–87.9 M 94–101.9	39	19.1 (13.6; 24.6)	47	20.4 (15.1; 25.7)	86	19.8 (16.1; 23.5)			
	W ≥ 88.0 M ≥ 102.0	85	41.7 (35.0; 48.4)	28	12.1 (7.8; 16.4)	113	26.0 (22.0; 30.0)			

Table 5. Cont.

Age Group	WC Category	Women		Men		Total		z	p-Value ^a	η^2
		n	% (95% CI)	n	% (95% CI)	n	% (95% CI)			
45.0–54.9	W < 80.0 M < 94.0	64	32.2 (25.8; 38.6)	93	56.7 (49.0; 64.4)	157	43.3 (38.2; 48.4)	5.712	<0.001	0.057
	W 80–87.9 M 94–101.9	59	29.7 (23.4; 36.0)	33	20.1 (13.9; 26.3)	92	25.3 (20.8; 29.8)			
	W ≥ 88.0 M ≥ 102.0	76	38.2 (31.5; 44.9)	38	23.2 (16.5; 29.9)	114	31.4 (26.8; 36.0)			
55.0–64.9	W < 80.0 M < 94.0	17	13.0 (7.2; 18.8)	59	55.1 (45.4; 64.8)	76	31.9 (25.9; 37.9)	7.500	<0.001	0.141
	W 80–87.9 M 94–101.9	31	23.7 (16.4; 31.0)	26	24.3 (16.1; 32.5)	57	24.0 (18.6; 29.4)			
	W ≥ 88.0 M ≥ 102.0	83	63.3 (55.0; 71.8)	22	20.6 (12.9; 28.3)	105	44.1 (37.9; 50.3)			
65.0–74.9	W < 80.0 M < 94.0	38	16.2 (11.5; 20.9)	110	54.5 (47.7; 61.3)	148	33.9 (29.5; 38.3)	15.331	<0.001	0.173
	W 80–87.9 M 94–101.9	54	23.0 (17.7; 28.3)	33	16.3 (11.3; 21.3)	87	19.9 (16.2; 23.6)			
	W ≥ 88.0 M ≥ 102.0	143	60.9 (54.8; 67.0)	59	29.2 (22.9; 35.5)	202	46.2 (41.5; 50.9)			
≥75.0	W < 80.0 M < 94.0	11	16.7 (8.2; 25.2)	22	61.1 (44.5; 77.7)	33	32.4 (23.2; 41.6)	8.966	<0.001	0.097
	W 80–87.9 M 94–101.9	16	24.2 (14.0; 34.4)	5	13.9 (1.8; 26.0)	21	20.6 (12.8; 28.4)			
	W ≥ 88.0 M ≥ 102.0	39	59.1 (47.0; 71.2)	9	25.0 (10.5; 39.5)	48	47.0 (37.3; 56.9)			
Total:	W < 80.0 M < 94.0	356	33.1 (30.3; 35.9)	623	63.2 (60.2; 66.4)	979	47.5 (45.4; 49.6)	23.071	<0.001	0.115
	W 80–87.9 M 94–101.9	243	22.6 (20.1; 25.1)	185	18.8 (16.4; 21.2)	428	20.8 (19.1; 22.5)			
	W ≥ 88.0 M ≥ 102.0	477	44.3 (41.3; 47.3)	177	18.0 (15.6; 20.4)	654	31.7 (29.8; 33.6)			

Abdominal overweight: Man WC 94–101.9 cm; Woman WC 80–87.9 cm. Abdominal obesity: Man WC ≥ 102; Woman WC ≥ 88 cm. CI—confidence interval, η^2 —eta squared for effect size. ^a Mann–Whitney U test with tie correction.

Chances of Developing Overweight or Obesity

Analysis with the logistic regression model indicated that the odds of developing excess weight/obesity measured by the BMI index were dependent on the age and sex of the respondents. It was confirmed that men were over 28% more likely to develop such a condition than women (OR = 1.288, $p < 0.001$). In addition, the odds also increased with age (OR = 1.029, $p < 0.001$) (Table 6).

Table 6. Odds of becoming overweight/obesity (BMI ≥ 25.00 [19–64 yo] or BMI > 27.00 [65+ yo]) in Poland, 2017–2020.

	OR	−95% CI	+95% CI	Wald Stat.	p-Value
Sex (W vs. M) Age	0.263	0.209	0.332	127.175	<0.001
	1.288	1.126	1.473	13.620	<0.001
	1.029	1.025	1.033	228.830	<0.001
Sex (W vs. M) Age	OR ^a	−95% CI	+95% CI	Wald stat.	p-value
	0.166	0.077	0.358	20.988	0.000
	1.278	1.101	1.484	10.341	0.001
	1.020	1.011	1.028	21.407	0.000

CI—confidence interval; OR—odds ratio; ^a adjusted odds ratio: region, place of residence, education, economic situation, marital status of respondents, professional situation, and the presence of cardiovascular disease.

The analysis of the prevalence of being overweight comprising sociodemographic variables showed that an overweight person was most often a rural resident, aged 65.0–74.9, with secondary education, assessing their financial situation as average, remaining in a marriage/partnership, and not declaring the occurrence of cardiovascular diseases (Table 7). The analysis of obesity prevalence comprising sociodemographic variables revealed that a person with obesity was most often a person living in rural areas, aged 65.0–74.9, with primary/lower secondary/vocational education, assessing their financial situation as average, being married/in a partnership, and not declaring the occurrence of cardiovascular diseases (Table 7).

Table 7. Profile of an overweight woman/man and a woman/man with obesity.

Profile of an Overweight Woman/Man: BMI 25.00–29.90 [<i>n</i> = 863, 41.9% of the Population]							
Variable	Women		Men		Total	χ^2	<i>p</i> -Value ^a
	<i>n</i>	%	<i>n</i>	%	<i>n</i>		
Place of residence							
Village	99	27.1	176	35.4	275	37.428	<0.001
Towns to 10,000	20	5.5	25	5.0	45		
Towns 10–19 thousand	29	7.9	32	6.4	61		
Towns 20–49 thousand	48	13.1	50	10.1	98		
Towns 50–99 thousand	25	6.8	57	11.5	82		
Cities 100–199 thousand	42	11.5	22	4.4	64		
Cities 200–499 thousand	29	7.9	48	9.7	77		
Cities 500 thousand–1 million	33	9.0	60	12.1	93		
City > 1 million	41	11.2	27	5.4	68		
Age group						9.082	0.106
19.0–34.9	53	14.5	107	21.5	160		
35.0–44.9	67	18.3	100	20.1	167		
45.0–54.9	77	21.0	85	17.1	162		
55.0–64.9	53	14.5	66	13.3	119		
65.0–74.9	94	25.7	115	23.1	209		
≥75.0	22	6.0	24	4.8	46		
Education						20.593	<0.001
Primary/junior high school/vocational	126	34.4	213	42.9	339		
Secondary	164	44.8	234	47.1	398		
Tertiary	76	20.8	50	10.1	126		
Financial situation						26.232	<0.001
Good	60	16.4	153	30.8	213		
Average	263	71.9	310	62.4	573		
Poor	43	11.8	34	6.8	77		
Marital status						32.003	<0.001
Unmarried	32	8.7	89	17.9	121		
Married/a cohabitation relationship	263	71.9	365	73.4	628		
Divorced/separated	25	6.8	19	3.8	44		
Widow(er)	46	12.6	24	4.8	70		
Professional situation						4.429	0.035
Retired/disability pensioner	147	40.2	165	33.2	312		
Parental leave, unemployed, runs the house	22	6.0	10	2.0	32	9.440	0.002
Casual employment	14	3.8	13	2.6	27	1.017	0.313
Permanent employment	183	50.0	308	62.0	491	12.318	<0.001
Studies	4	1.1	5	1.0	9	0.015	0.901
Cardiovascular diseases ^b						2.264	0.132
No	308	84.2	436	87.7	744		
Yes	58	15.9	61	12.3	119		
Profile of a woman/man with obesity bmi ≥30 [<i>n</i> = 350, 17.0% of the population]							
Variable	Women		Men		Total	χ^2	<i>p</i> -Value ^a
	<i>n</i>	%	<i>n</i>	%	<i>n</i>		
Place of residence						17.799	0.023
Village	62	28.31	43	32.8	105		
Towns to 10,000	16	7.31	11	8.4	27		
Towns 10–19 thousand	13	5.94	3	2.3	16		
Towns 20–49 thousand	17	7.76	12	9.2	29		
Towns 50–99 thousand	23	10.50	13	9.9	36		
Cities 100–199 thousand	20	9.13	9	6.9	29		
Cities 200–499 thousand	16	7.31	17	13.0	33		
Cities 500 thousand–1 million	27	12.33	21	16.0	48		
City > 1 million	25	11.42	2	1.5	27		

Table 7. Cont.

Profile of a woman/man with obesity bmi ≥ 30 [$n = 350$, 17.0% of the population]							
Variable	Women		Men		Total	χ^2	p -Value ^a
	<i>n</i>	%	<i>n</i>	%	<i>n</i>		
Age group							
19.0–34.9	21	9.59	17	13.0	38	20.046	0.001
35.0–44.9	27	12.33	34	26.0	61		
45.0–54.9	31	14.16	23	17.6	54		
55.0–64.9	48	21.92	18	13.7	66		
65.0–74.9	68	31.05	35	26.7	103		
≥ 75.0	24	10.96	4	3.1	28		
Education							
Primary/junior high school/vocational	104	47.49	63	48.1	167	2.112	0.348
Secondary	92	42.01	48	36.6	140		
Tertiary	23	10.50	20	15.3	43		
Financial situation							
Good	28	12.79	42	32.1	70	19.447	<0.001
Average	164	74.89	79	60.3	243		
Poor	27	12.33	10	7.6	37		
Marital status							
Unmarried	12	5.48	22	16.8	34	27.385	<0.001
Married/a cohabitation relationship	144	65.75	96	73.3	240		
Divorced/separated	16	7.31	7	5.3	23		
Widow(er)	47	21.46	6	4.6	53		
Professional situation							
Retired/disability pensioner	122	55.71	43	32.8	165	17.225	<0.001
Parental leave, unemployed, runs the house	19	8.68	5	3.8	24	3.030	0.082
Casual employment	7	3.20	4	3.1	11	0.005	0.941
Permanent employment	72	32.88	82	62.6	154	29.381	<0.001
Studies	1	0.46	0	0.0	1	0.600	0.439
Cardiovascular diseases ^b							
No	163	74.43	100	76.3	263	0.160	0.690
Yes	56	25.57	31	23.7	87		

^a chi-squared test. ^b hypertension, a history of stroke, a history of myocardial infarction, coronary heart disease, heart failure, atherosclerosis of lower extremity arteries, lipid disorders.

4. Discussion

Overweight conditions and obesity still remain a serious problem in Poland. Our research showed that excessive body weight affected 56.8% of people aged 19 and over, including obesity in 16.4%. Excessive body weight was more prevalent in men than in women (60.2 vs. 53.1%), while obesity was much more common in women (20% vs. 12.4%). The percentage of individuals with excessive body weight exceeded the average for the European Union countries, which was 52.7% in 2019 [18] and lower than the value (58%) provided in the quoted Eurostat report. The neighboring countries also struggle with a high prevalence of overweight diagnoses and obesity. In the Czech Republic, this phenomenon affects 60% of the population: 59% in Slovakia, 54% in Germany, and 57% in Lithuania. The percentage of people who are overweight and people suffering from obesity was below 50% in Italy (46%) and France (47%). The analysis of research conducted worldwide indicates that being overweight and being obese still remain an important public health problem in the United States (70%) [19], Australia (67%) [20], and Canada (65%) [21]. Chinese research published in 2023 indicated that almost half of adult residents (48.9%) were characterized by excessive body weight [22]. Despite the high prevalence of overweight cases and obesity cases in the contemporary world, this does not mean that it cannot be otherwise. This was highlighted by Japanese data, according to which excessive body weight occurred in 24% of the population, including obesity in 4% [23].

In Poland, in the years 2003–2014, the indices of excessive body weight increased. In 2003–2005, obesity was noted in 20.8% of men and 22.4% of women [24], and several years later (2013–2014), it was reported in 23.2% of men and 23.1% of women [9]. Excessive body weight in 2013–2014 occurred in 67.6% of men and 55.5% of women. Seemingly, the lower increase in the prevalence of overweight cases and obesity nowadays may be due to the growing interest in a healthy lifestyle, being a result of the educational programs

mentioned earlier, as well as the improvement of the financial situation of the inhabitants of Poland [25]. However, it is too early to talk about a downward trend, although even stopping the increase in this phenomenon should be considered beneficial. As regards further preventive measures, attention should be paid to the problem of the increase in the frequency of excessive body weight with age. The lowest percentage of overweight and people with obesity (38.5%) occurred in the group of respondents aged 19–34.9 years, and the highest percentage (73.1%) in those aged 55–64.9 years. It decreased only slightly until late old age (70.6% of people had a BMI ≥ 25 kg/m²). Data on the body mass index in the oldest respondents were also analyzed in relation to the Lipschitz classification [14], according to which BMI ranging from 22.0 to 27.0 kg/m² in senior age is considered normal. Values above 27.0 kg/m² indicate excessive body weight. In the study group, 51.3% of people aged 65.0–74.9 and 51.0% of people aged ≥ 75 years met the criteria of excessive body weight according to the Lipschitz classification. Research showed that the low body weight of seniors was related to the occurrence of frailty syndrome, and a high BMI would be unfavorable due to the increased risk of functional disability and coexisting factors of weakness [26]. The comparison of the frequency of underweight cases in seniors according to the WHO and Lipschitz criteria indicates slight differences, i.e., in people aged 65–74.9 years, it was 0.7% and 6.2%, and in people ≥ 75 years, it was 0.0% and 2.9%, respectively, which clearly indicates that excessive body weight is a more common problem among Polish seniors. However, it does not exclude the problem of frailty syndrome, because a significant percentage of older people have sarcopenic obesity. Depending on the diagnostic criteria, the prevalence, of sarcopenia in people over 60 years of age was 10–27% [27], and for sarcopenic obesity, it was 11% [28].

Research showed that the prevalence of excessive body weight varied according to sex. In Poland, being overweight is more common in men, while obesity dominates in women, and the differences increase with age. In the youngest age group, obesity affected 8.7% of women and 6.1% of men, and at the age of 55.0–64.9, the difference was already marked (35.9% vs. 15.0%). The occurrence of differences in excessive body weight depending on sex and age was confirmed with logistic regression analysis. Men were found to have 28% higher odds of becoming overweight than women. Regarding age as a differentiating criterion, it was shown that the OR (odds ratio) was 1.029. The prevalence of excessive body weight in Poland also depends on sociodemographic variables. Further analysis is required according to the type of work performed by the respondents and the physical activity undertaken, which may affect the body weight of the respondents, especially since low physical activity and a sedentary lifestyle resulting from professional activity are the risk factors for the development of obesity [29]. Simultaneously, being overweight and having obesity were found in nearly one in three rural residents, while the activity rate in rural and urban areas in Poland was 58.5% in 2022 [30]. The present study also showed that almost every second overweight person had secondary education, which might be considered in relation to the percentage of the professionally active population of Poland. In 2022, the group of people with secondary education included 55% of professionally active people, while the group with tertiary education included 81% of professionally active individuals [30]. At the same time, the results obtained in our study are consistent with the conceptual model of the “obesity transition” by Jaacks LM, et al. (2020). According to the concept, European countries are at “Stage 3” of the model that assumes that obesity is more common in people with lower socioeconomic status, e.g., they are more often in the group with a lower level of education compared to people with higher education [31]. Our study revealed a very high percentage of overweight people or people with obesity in the group declaring an average financial situation, which was also confirmed by the results by Stival Ch, et al. (2022). They demonstrated that the prevalence of obesity was associated with a lower level of education and socioeconomic status in European countries [32].

When discussing the issue of excessive body weight, it is worth noting the distribution of body fat. It is known that the accumulation of fat within the abdominal cavity is accompanied by an increased secretion of inflammatory cytokines by macrophages infiltrating the

adipose tissue. It leads to the development of chronic low-grade inflammatory state in the body. Moreover, this type of obesity contributes to the reduction in tissue insulin sensitivity, the development of insulin resistance, and the risk of developing type 2 diabetes [33–35]. The obtained results indicated that every fifth adult resident of Poland had abdominal overweight, and nearly every third had abdominal obesity. The excessive accumulation of the adipose tissue in the visceral region is significantly more common in women. Based on the measurement of waist circumference, 22.6% of them had visceral overweight, and 44.3% had visceral obesity. Visceral overweight affected 18.8% of men and visceral obesity affected 18.0%.

It is worth paying attention to the incidence of abdominal obesity in women of menopausal age, which usually occurs between 45–55 years of age [36]. In this age range, over 38% of Polish women have been characterized by abdominal obesity, and the percentage of women with this disorder has been found to increase until the age of 75. It should be emphasized that excess visceral adipose tissue was found to have a stronger adverse effect on cardiovascular risk in women than in men [37]. Therefore, the high prevalence of the visceral distribution of the adipose tissue that we observed in women is a reason for concern.

The accumulation of the adipose tissue in the visceral area changes with the age of the respondents. It occurred in 32.5% of the respondents in the youngest group (19–34.9 years), and in the group of people aged 55–64.9 years, it was reported in 68.1% of the respondents. As regards the oldest age groups, waist circumference values were still too high in about 67% of people. In the years of 2013–2014, the prevalence of the excessive accumulation of the adipose tissue in the visceral region was higher than at present. At the beginning of the previous decade, abdominal overweight occurred in 21.7% of women and 27.2% of men, and abdominal obesity occurred in 48.7% of women and 32.2% of men. Moreover, waist circumference was too high in 91.8% of women (≥ 80 cm) and 77.2% of men (≥ 94 cm) at the age of 65–74.9. The excessive accumulation of the adipose tissue in the visceral region was reported even in the youngest age group (20–34.9 years), with 41.9% of women being affected. It is worth emphasizing that obesity in young women was associated with an increased risk of infertility caused by ovulation disorders [38], and the abdominal distribution of the adipose tissue was found to be linked with polycystic ovary syndrome [39–41]. Therefore, it may be assumed that obesity, especially abdominal, is at least partly responsible for such widespread fertility disorders in women nowadays. The problem of excessive body weight also affects a large percentage of young men. In our study, it occurred in 46.1% of men in the youngest age group and in 55% of those aged 35–45. Notably, it is also an important risk factor for infertility in the male population [42]. Thus, overweight/obesity in the partner should also be taken into account in the prevention and treatment of infertility in couples.

The results of the study suggest a reduction in the prevalence of general and abdominal obesity in the Polish population compared to research from 2013, which may indicate the effectiveness of population-scale preventive efforts. However, this observation needs to be confirmed through performing an additional analysis consisting in the standardization of the obtained results by age according to the demographic structure of the Polish population from 2013. Further analysis is required in terms of the diet and other lifestyle elements such as physical activity and length and quality of sleep, as these may help explain this beneficial change.

Our study also has some limitations. One of them was related to the change in the methods of collecting nutritional data—from the direct (in person) to the remote method (via telephone). During the pandemic period, the current anthropometric measurements were declared by the respondents (10% study group). However, the respondents were not asked about the exact measurement time. The rates of medical conditions were based on respondent declarations. The shift from in-person to telephone interviews might introduce inconsistencies in data quality. In addition, the respondents' place of residence (city, village, etc.) and their financial situation require more detailed analyses.

5. Conclusions

Overweight cases and obesity, both general and abdominal, are still a major epidemiological problem in Poland.

The person with obesity/excess weight in the Polish population was most often a rural resident, aged 65.0–74.9, assessing their financial situation as average, being married/in a partnership, and not declaring the occurrence of cardiovascular diseases. The person with excess weight had a secondary education, and the person with obesity had primary/lower secondary/vocational education. The obtained results indicate that rural residents with lower education should be covered by educational activities adapted to their needs and possibilities.

Excessive body weight affected almost 42% of the examined women from the youngest age group, and abdominal obesity was found in 21% of women of this age. Further research and educational activities are necessary within the aspect of fertility, considering that obesity in young women, especially abdominal obesity, may be associated with an increased risk of infertility caused by ovulation disorders.

The results obtained in this study, when compared to previously published ones, suggest that the prevalence of being overweight and being obese has decreased over the last 10 years. However, this assumption requires further investigation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu16234248/s1>, File S1: Detailed Diagnostics of Logistic Regression Models.

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References

1. Safaei, M.; Sundararajan, E.A.; Driss, M.; Boulila, W.; Shapi'i, A. A systematic literature review on obesity: Understanding the causes & consequences of obesity and reviewing various machine learning approaches used to predict obesity. *Comput. Biol. Med.* **2021**, *136*, 104754.
2. Mohammadian Khonsari, N.; Khashayar, P.; Shahrestanaki, E.; Kelishadi, R.; Mohammadpoor Nami, S.; Heidari-Beni, M.; Esmaeili Abdar, Z.; Tabatabaei-Malazy, O.; Qorbani, M. Normal Weight Obesity and Cardiometabolic Risk Factors: A Systematic Review and Meta-Analysis. *Front. Endocrinol.* **2022**, *13*, 857930. [CrossRef] [PubMed]
3. Iyengar, N.M.; Gucalp, A.; Dannenberg, A.J.; Hudis, C.A. Obesity and Cancer Mechanisms: Tumor Microenvironment and Inflammation. *J. Clin. Oncol.* **2016**, *34*, 4270–4276. [CrossRef] [PubMed]
4. Rosano, G.M.C.; Rodriguez-Martinez, M.A.; Spoletini, I.; Regidor, P.A. Obesity and contraceptive use: Impact on cardiovascular risk. *ESC Heart Fail.* **2022**, *9*, 3761–3767, Erratum in *ESC Heart Fail.* **2023**, *10*, 1497. [CrossRef]
5. Yau, C.E.; Lim, G.S.J.; Ang, A.Y.H.; Lim, Y.L.; Goh, O.Q.M.; Siah, K.T.H.; Ng, Q.X. Examining the Association Between Overweight, Obesity, and Irritable Bowel Syndrome: A Systematic Review and Meta-Analysis. *Nutrients* **2024**, *16*, 3984. [CrossRef]
6. Abiri, B.; Hosseini, F.; Banihashem, S.; Madinehzad, S.A.; Valizadeh, M. Mental health and quality of life in different obesity phenotypes: A systematic review. *Health Qual. Life Outcomes* **2022**, *20*, 63. [CrossRef]
7. Salem, V.; AlHusseini, N.; Abdul Razack, H.I.; Naoum, A.; Sims, O.T.; Alqahtani, S.A. Prevalence, risk factors, and interventions for obesity in Saudi Arabia: A systematic review. *Obes. Rev.* **2022**, *23*, e13448. [CrossRef]

8. Szponar, L.; Sekuła, W.; Rychlik, E.; Ołtarzewski, M.; Figurska, K. *Research on Individual Food Consumption and Nutritional Status in Households*; Institute of Food and Nutrition, Works of IŻŻ 101: Warsaw, Poland, 2003. (In Polish)
9. Stepaniak, U.; Micek, A.; Waśkiewicz, A.; Bielecki, W.; Drygas, W.; Janion, M.; Kozakiewicz, K.; Niklas, A.; Puch-Walczak, A.; Pająk, A. Prevalence of general and abdominal obesity and overweight among adults in Poland. Results of the WOBASZ II study (2013–2014) and comparison with the WOBASZ study (2003–2005). *Pol. Arch. Med. Wewn.* **2016**, *126*, 662–671. [CrossRef]
10. Jarosz, M. (Ed.) *POL-HEALTH Narodowy Program Zapobiegania Nadwadze i Otyłości Oraz Przewlekłym Chorobom Niezakaźnym Poprzez Poprawę Żywności i Aktywności Fizycznej 2007–2016 (POL-HEALTH, 2007-2016 National Program to Prevent Overweight, Obesity and Chronic Non-Communicable Diseases by the Improvement of Nutrition and Physical Activity)*; Instytut Żywności i Żywienia (Warsaw, Food and Nutrition Institute): Warszawa, Poland, 2006. (In Polish)
11. Zapobieganie Nadwadze i Otyłości Oraz Chorobom Przewlekłym Poprzez Edukację Społeczeństwa w Zakresie Żywności i Aktywności Fizycznej, Projekt KIK/34 w Szwajcarsko-Polskim Programie Współpracy Zachowaj Równowagę (Prevention of Overweight, Obesity and Chronic Diseases by the Education of the Society on Nutrition and Physical Activity. KIK/34 Project in a Swiss and Polish “Maintain Balance” Cooperation Program. Available online: <https://www.gov.pl/web/zdrowie/projekt-kik/34-zapobieganie-nadwadze-i-otylosci-oraz-chorobom-przewleklym-poprzez-edukacje-spoleczenstwa-w-zakresie-zywnosci-i-aktywnosci-fizycznej-szwajcarsko-polski-program-wspolpracy> (accessed on 18 December 2023). (In Polish)
12. Traczyk, I.; Kucharska, A.; Sinska, B.I.; Panczyk, M.; Wronka, L.; Raciborski, F.; Szostak-Wegierek, D.; Samolinski, B. Every second adult inhabitant of Poland (aged 18–64) is overweight—Results of representative cross-sectional studies conducted in 2017–2020. *Ann. Agric. Environ. Med.* **2023**, *30*, 322–330. [CrossRef]
13. National Census of Population and Housing 2022. *Report on Preliminary Results*; Statistics Poland: Warsaw, Poland, 2022. (In Polish)
14. Lipschitz, D.A. Screening for nutritional status in the elderly. *Prim Care.* **1994**, *21*, 55–67. [CrossRef]
15. The IDF Consensus Worldwide Definition of the Metabolic Syndrome. Available online: <https://sites.pitt.edu/~super1/Metabolic/IDF1.pdf> (accessed on 18 December 2023).
16. Traczyk, I.; Raciborski, F.; Kucharska, A.; Sińska, B.I.; Milewska, M.; Samoliński, B.; Szostak-Węgierek, D. A National Study of Nutrition and Nutritional Status of the Adult Polish Population in the Years 2017–2020 before and during the COVID-19 Pandemic—Design and Methods. *Nutrients* **2021**, *13*, 2568. [CrossRef] [PubMed]
17. Regulation (EU) 2016/679 of the European Parliament and of the Council of 27 April 2016 on the Protection of Individuals with Regard to the Processing of Personal Data and on the Free Movement of Such Data, and Repealing Directive 95/46/EC (General Regulation on the Protection of Data). Available online: <https://www.uodo.gov.pl/pl/131/224> (accessed on 18 December 2023). (In Polish)
18. Eurostat. Overweight and Obesity—BMI Statistics. Available online: https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Overweight_and_obesity_-_BMI_statistics (accessed on 18 December 2023).
19. USAFacts. US Obesity Rates Have Tripled over the Last 60 Years. Available online: <https://usafacts.org/articles/obesity-rate-nearly-triples-united-states-over-last-50-years/> (accessed on 18 December 2023).
20. Overweight and Obesity Web Report: Overweight & Obesity. Australian Institute of Health and Welfare 2023. Available online: <https://www.aihw.gov.au/getmedia/3b5e2302-15d2-411f-8867-b5a50867e87c/overweight-and-obesity.pdf?v=20231208181324&inline=true> (accessed on 18 December 2023).
21. Overweight and Obesity in Canada. Statistics Report on Overweight and Obesity in Canada. Available online: <https://www.statista.com/study/115167/overweight-and-obesity-in-canada/> (accessed on 18 December 2023).
22. Chen, K.; Shen, Z.; Gu, W.; Lyu, Z.; Qi, X.; Mu, Y.; Ning, Y.; Meinian Investigator Group. Prevalence of obesity and associated complications in China: A cross-sectional, real-world study in 15.8 million adults. *Diabetes Obes. Metab.* **2023**, *25*, 3390–3399. [CrossRef] [PubMed]
23. Body Weight Status Among Japanese. National Health and Nutrition Survey 2016. National Institute of Health and Nutrition. Available online: https://www.nibiohn.go.jp/eiken/kenkounippon21/download_files/other/document_en_02.pdf (accessed on 18 December 2023).
24. Biela, U.; Pająk, A.; Kaczmarczyk-Chałas, K.; Głuszek, J.; Tendera, M.; Wawrzyńska, M.; Kurjata, P.; Wyrzykowski, B. Częstość występowania nadwagi i otyłości u kobiet i mężczyzn w wieku 20–74 lat. Wyniki program u WOBASZ. *Kardiologia Pol.* **2005**, *63*, 632–635. (In Polish)
25. Household Budget Survey in 2019. Statistical Information. Warsaw, Poland. 2020. Available online: https://stat.gov.pl/files/gfx/portalinformacyjny/pl/defaultaktualnosci/5486/9/14/1/budzety_gospodarstw_domowych_w_2019.pdf (accessed on 18 December 2023). (In Polish)
26. Pessanha, F.; Lustosa, L.P.; Carneiro, J.A.; Pfrimer, K.; Fassini, P.G.; Alves, N.M.; Pontes, T.L.; Moriguti, J.C.; Lima, N.K.; Ferrioli, E. Body Mass Index and Its Relationship with Disability, Chronic Diseases and Frailty in Older People: A Comparison of the Lipschitz and Who Classifications. *J. Frailty Aging.* **2017**, *6*, 24–28. [PubMed]
27. Petermann-Rocha, F.; Balntzi, V.; Gray, S.R.; Lara, J.; Ho, F.K.; Pell, J.P.; Celis-Morales, C. Global prevalence of sarcopenia and severe sarcopenia: A systematic review and meta-analysis. *J. Cachexia Sarcopenia Muscle.* **2022**, *13*, 86–99. [CrossRef]
28. Gao, Q.; Mei, F.; Shang, Y.; Hu, K.; Chen, F.; Zhao, L.; Ma, B. Global prevalence of sarcopenic obesity in older adults: A systematic review and meta-analysis. *Clin. Nutr.* **2021**, *40*, 4633–4641. [CrossRef]

29. Silveira, E.A.; Mendonça, C.R.; Delpino, F.M.; Souza, G.V.E.; de Souza Rosa, L.P.; de Oliveira, C.; Noll, M. Sedentary behavior, physical inactivity, abdominal obesity and obesity in adults and older adults: A systematic review and meta-analysis. *Clin. Nutr. ESPEN* **2022**, *50*, 63–73. [CrossRef]
30. Główny Urząd Statystyczny. Rocznik Statystyczny Pracy 2023 (Statistics Poland. 2023 Labour Statistical Yearbook). 2023. Available online: <https://stat.gov.pl/obszary-tematyczne/roczniki-statystyczne/roczniki-statystyczne/rocznik-statystyczny-pracy-2023,7,8.html> (accessed on 5 January 2024). (In Polish)
31. Jaacks, L.M.; Vandevijvere, S.; Pan, A.; McGowan, C.J.; Wallace, C.; Imamura, F.; Mozaffarian, D.; Swinburn, B.; Ezzati, M. The Obesity Transition: Stages of the global epidemic. *Lancet Diabetes Endocrinol.* **2019**, *7*, 231–240. [CrossRef]
32. Stival, C.; Lugo, A.; Odone, A.; Van den Brandt, P.A.; Fernandez, E.; Tigova, O.; Soriano, J.B.; José López, M.; Scaglioni, S.; Gallus, S.; et al. Prevalence and Correlates of Overweight and Obesity in 12 European Countries in 2017–2018. *Obes. Facts.* **2022**, *15*, 655–665. [CrossRef]
33. Ghaben, A.L.; Scherer, P.E. Adipogenesis and metabolic health. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 242–258. [CrossRef] [PubMed]
34. Frank, A.P.; de Souza Santos, R.; Palmer, B.F.; Clegg, D.J. Determinants of body fat distribution in humans may provide insight about obesity-related health risks. *J. Lipid Res.* **2019**, *60*, 1710–1719. [CrossRef] [PubMed]
35. Kinlen, D.; Cody, D.; O’shea, D. Complications of obesity. *QJM* **2018**, *111*, 437–443. [CrossRef] [PubMed]
36. Ambikairajah, A.; Walsh, E.; Cherbuin, N. A review of menopause nomenclature. *Reprod Health* **2022**, *19*, 29. [CrossRef]
37. Li, H.; Konja, D.; Wang, L.; Wang, Y. Sex Differences in Adiposity and Cardiovascular Diseases. *Int. J. Mol. Sci.* **2022**, *23*, 9338. [CrossRef]
38. Silvestris, E.; de Pergola, G.; Rosania, R.; Loverro, G. Obesity as disruptor of the female fertility. *Reprod. Biol. Endocrinol.* **2018**, *16*, 22. [CrossRef]
39. Lim, S.S.; Davies, M.J.; Norman, R.J.; Moran, L.J. Overweight, obesity and central obesity in women with polycystic ovarysyn-drome: A systematic review and meta-analysis. *Hum. Reprod. Update.* **2012**, *18*, 618–637. [CrossRef]
40. Jurczewska, J.; Ostrowska, J.; Chelchowska, M.; Panczyk, M.; Rudnicka, E.; Kucharski, M.; Smolarczyk, R.; Szostak-Węgierek, D. Physical Activity, Rather Than Diet, Is Linked to Lower Insulin Resistance in PCOS Women-A Case-Control Study. *Nutrients* **2023**, *15*, 2111. [CrossRef]
41. Jurczewska, J.; Ostrowska, J.; Chelchowska, M.; Panczyk, M.; Rudnicka, E.; Kucharski, M.; Smolarczyk, R.; Szostak-Węgierek, D. Abdominal Obesity in Women with Polycystic Ovary Syndrome and Its Relationship with Diet, Physical Activity and Insulin Resistance: A Pilot Study. *Nutrients* **2023**, *15*, 3652. [CrossRef]
42. Ameratunga, D.; Gebeh, A.; Amoako, A. Obesity and male infertility. *Best. Pract. Res. Clin. Obstet. Gynaecol.* **2023**, *90*, 102393. [CrossRef]

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Article

Body Composition Trend in Slovene Adults: A Two-Year Follow-Up

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Abstract: This research re-evaluates the prevalence of obesity in a cohort of 432 Slovenian adults two years after an initial population-based cross-sectional examination, utilizing the World Health Organization's body mass index (BMI) and total body fat percentage (FAT%) classifications. Herein, a medically approved electrical bioimpedance monitor was used to analyse body composition, and the results revealed a significant increase in the percentage of individuals classified as having overweight or obesity on the basis of BMI, from 40.7% initially to 45.2% at follow-up (FU); however, these percentages were notably higher in males than in females. The increases in body mass, BMI, fat mass, and FAT% were observed mainly in adult females, whereas in adult males, the increases in BM and BMI were attributed to fat-free mass (FFM) rather than fat mass. In this study, BMI was also compared with the FAT% obesity classification, and the BMI classification was shown to result in significantly fewer participants with obesity in both measures. However, the proportion of participants with obesity based on the two classifications did not differ substantially between the initial and FU measurements. In this study, mixed linear models were used to analyse overall trends and subgroup variations and highlight the importance of considering sex, age, and time of measurement when assessing body composition metrics. These findings emphasize the need for personalized health assessments and the importance of measuring body composition to evaluate adult obesity more accurately for both clinical assessments and public health policies. The state, in collaboration with social initiatives and industrial stakeholders, should prioritize these data and advance public health through innovative educational and awareness initiatives that are founded on robust scientific principles and that empower and promote the adoption of a healthy, active lifestyle.

Keywords: adults; population-based; body mass index; body composition; body fat; fat-free mass; obesity classification; follow-up

1. Introduction

Obesity is a major public health challenge, and its global incidence has been increasing for decades [1,2]. This increase has been driven by urbanization, sedentary lifestyles, inadequate sleep, and high-calorie processed foods [3]. Overweight and obesity are associated with numerous chronic diseases. In the next 30 years, individuals with overweight are expected to account for 60% of patients newly diagnosed with diabetes, 18% of patients with cardiovascular disease, 11% of patients with dementia, and 8% of patients with cancer [4,5]. Among individuals aged 30–70 years, overweight is predicted to cause approximately 3 million premature deaths annually [6]. Obesity also leads to significant healthcare costs and a loss of productivity [7,8]. Compared with individuals who are overweight, individuals with obesity have 32% higher healthcare expenses and 68% higher indirect costs [7].

Preventable chronic diseases related to obesity are predicted to impose a global financial burden of 44 trillion euros as of 2023 [9].

According to the World Health Organization (WHO), approximately 59% of adults in Europe are classified as having overweight or obesity, highlighting a concerning trend [10]. If current trends continue, it is projected that by 2025, 33 of 52 Organization for Economic Cooperation and Development (OECD) countries will have an obesity prevalence of 20% or more [6]. Importantly, Slovenia has the highest prevalence of adult obesity (20.8%) among 20 European countries [10]. The Slovenian National Dietary Survey (SI.Menu) was conducted from 2017 to 2018 and included a sample size of 780; the results revealed that 59% of adults and 74% of elderly individuals had overweight or obesity [5]. Additionally, the initial study revealed that 42% of adults and 64% of elderly individuals had overweight or obesity [11]. Accordingly, between 1989 and the late 2000s, the percentage of Slovene children classified as having obesity tripled for both sexes, whereas the percentage of children with overweight doubled. Additionally, between 1989 and 2019, subcutaneous fat (rather than muscle mass) increased in children of all ages and sexes [12,13]. These high prevalence rates of overweight and obesity likely occur because fewer than 5% of adult Slovenes are healthy [14], and only approximately 10% of adult and adolescent Slovenes maintain a recommended diet with respect to the intake of fibre, vitamin D, and folate [15–17], as well as various plant-based food groups, such as vegetables, whole grains, potatoes, legumes, nuts, and seeds [18]. These statistics underscore the urgent need for effective interventions against obesity [19,20].

Body mass index (BMI) categorizes individuals on the basis of body height (BH) and body mass (BM), but BMI is not a reliable measure of total body fat percentage (FAT%). In addition, BMI does not distinguish between lean body mass (LBM) and fat mass (FATM), thus leading to possible inaccuracies [11,21–23]. Since BMI does not account for the FATM distribution [23], health authorities advise against its use alone for assessing overweight or obesity [24,25]. The use of BMI as a measure for estimating the prevalence of obesity may also be inaccurate, thereby hindering prevention and control efforts [26]. BMI should not be the only factor considered when assessing eligibility for bariatric surgery or semaglutide use [27]. Low muscle mass may present a greater health risk than high FATM [28]. Research in the U.S. has shown that BMI is not a dependable predictor of cardiometabolic disease and that it leads to the misclassification of approximately 30% of adults [29]. Thus, focusing only on BMI and BM reduction might not provide substantial health benefits and could contribute to weight-related discrimination and other harms [28].

Given the limitations of BMI, integrating BMI with body composition measurements is valuable, especially for studies that are smaller and/or focus on populations with a higher prevalence of sarcopenic obesity [21,30]. In our initial analysis of data used in this study, with a sample size of 844, proportions of obesity were also compared via BMI and FAT%, revealing that BMI underestimated the prevalence of obesity in females (13%) compared with the use of FAT% (17%) [11]. The secondary analysis explored leg FAT% and its relationship with FAT% and trunk FAT% according to the WHO classification's BMI obesity cut-off values (Table 1), sex, and age. Additionally, linear regression revealed that variations in FAT%, sex, and age explained 82.5% of the variation in leg FAT% [31].

Table 1. Obesity classification according to BMI and FAT% [32,33].

BMI Categories		FAT% Categories	
Status	BMI (kg/m ²)	Status	FAT%
Underweight	<18.5	Female—normal	<35
Normal body mass	18.5–24.9	Female—obesity	>35
Pre-obesity/overweight	25.0–29.9	Male—normal	<25
Obesity class I	30.0–34.9	Male—obesity	>25
Obesity class II	35.0–39.9		
Obesity class III	Above ≥ 40		

BMI = body mass index and FAT% = total body fat percentage.

More than thirty years ago, the U.S. National Institutes of Health classified males with a BMI of 27.8 kg/m^2 and females with a BMI of 27.3 kg/m^2 as overweight. Individuals with BMIs below these values were considered 'normal' on the basis of an 85% cut-off from the National Health and Nutrition Examination Study II. In 1997, the International Obesity Task Force introduced 'pre-obesity' and a detailed BMI system: class I obesity ($30\text{--}34.9 \text{ kg/m}^2$), class II obesity ($35\text{--}39.9 \text{ kg/m}^2$), and class III obesity ($\geq 40 \text{ kg/m}^2$) [23]. In 1998, the U.S. Obesity Task Force set the normal-overweight BMI threshold to 25 kg/m^2 and eliminated obesity subdivisions [34]. Importantly, different ethnic groups, such as Asians, may have lower BMI cut-off values. Furthermore, various guidelines caution against the universal application of BMI as a metric [27]. Therefore, some scholars have proposed focusing on fat-free mass (FFM) and body composition, asserting that the sole use of BMI and FAT% is insufficient to assess obesity-related health risks [28].

The primary objective of this two-year follow-up (FU) study was to assess trends in BM/BMI, body composition, and obesity prevalence on the basis of the WHO's BMI and FAT% classifications among participants from the initial study [11]. The hypothesis that rates of overweight and obesity increase, particularly among adult females, was tested. As a secondary objective, obesity prevalence was compared between the initial and FU studies via BMI and FAT% cut-off values, and linear mixed models were employed to account for trends and subgroup variations.

2. Materials and Methods

2.1. Study Design and Eligibility

This cross-sectional study was approved by the Slovenian Ethical Committee in the Field of Sports on 26 June 2022 (approval no. 033-41/2022-5), and it was registered on 30 June 2022 at <https://clinicaltrials.gov> (document no. NCT05438966) [35]. To initially recruit participants, representatives from hotels, local governmental agencies, and fire stations that facilitate events on a national scale (such as conferences and meetings) were contacted. Additionally, social media platforms were utilized to ascertain the locations, dates, and types of complementary public programs (including seminars, workshops, training sessions, lectures, and courses) that did not require registration fees. In exchange for the collection and analysis of anonymized data from these measurements, organizers were provided with a complimentary body composition analysis, including interpretation and electronic printouts distributed to participants via email. Initial measurements occurred from 1 July to 31 August 2022 [11,35], with FU measurements taken in June 2024. Feedback on body composition was emailed as PDF printouts during the initial study, and completed questionnaires pertaining to dietary habits were collected [11]. The participants received email invitations for the FU and completed a brief demographic and lifestyle questionnaire via email. The FU initially included randomly recruited participants. No monetary incentives were offered in exchange for participation. The study was conducted in Slovenia, which has a population of 2.1 million. All participants were thoroughly briefed and asked to provide renewed consent.

2.2. Subjects

Healthy adults were invited to participate. There were no restrictions regarding the BMI of the participants. However, athletes, pregnant or lactating females, individuals with active chronic diseases, and individuals taking medication for chronic conditions (such as hypertension, hyperlipidaemia, diabetes, and thyroid disorders) were excluded. Those who recently changed their physical activity and diet were not excluded from the FU, as was the case during the initial study, aiming to minimize baseline measurement variability. Two pregnant females, two lactating females, and seven males who recently began taking hypertension and hyperlipidaemia medication were excluded from the FU study, which was conducted in six Slovenian cities/towns: Kranjska Gora, Kranj, Ljubljana, Novo Mesto, Maribor, and Šentilj. Therefore, the FU study included 432 adult Slovenes, accounting for 51% of the original participants ($n = 844$) [11].

2.3. Outcomes

2.3.1. Body Mass Index and Body Composition Status

This study aimed to compare adult Slovene participants' BM/BMI and body composition at two time points and aimed primarily to identify any significant demographic changes [11]. A calibrated 8-electrode bioelectrical impedance analysis (BIA) monitor (Tanita 780 S MA, Tokyo, Japan) [36] was used for all the measurements. Body height (BH) was measured with a floor scale (Kern, MPE 250K100HM, Kern & Sohn, Balingen, Germany) only during the initial study. Body mass index was calculated by dividing the BM by the square of the BH in metres. The body composition monitoring data included BM, BMI, FAT%, FATM, FATM per unit area of BH (FATM/BH) in kg/cm², FFM in percentage and kg, FFM per unit area of BH (FFM/BH) in kg/cm², total body water (TBW), and phase angle (PhA).

Body composition indices were compared with the WHO's BMI and FAT% obesity targets. Obesity rates were examined on the basis of BMI (≥ 30 kg/m²) and FAT% (>35% for females, >25% for males) criteria [32,33]. The data were compared with the initial study results [11].

The findings were also delineated on the basis of age categories. The definition of 'adult' differs across sources and is frequently subject to inconsistent discourse [37–39]. In this analysis, the categorization of age was undertaken by delineating three distinct cohorts: (1) young adults, comprising individuals aged 18–39 years; (2) middle-aged adults, consisting of those aged 40–64 years; and (3) older adults, including persons aged 65 years and above.

In addition, linear mixed models were used to capture overall trends and subgroup variations, which are useful for data collected in hierarchical or nested structures, such as repeated measurements from the same participants. Due to the limited sample size of older adults, the focus of the study was predominantly on reporting findings for an 'adult' cohort (encompassing the young and middle-aged adult cohorts in our analysis). Nevertheless, the Supplemental Tables and Charts present comprehensive data encompassing the entire sample, including data from older adults. Additionally, analyses of BMI and body composition changes were stratified into age and sex categories on the basis of the 'adult' and 'older adult' cohorts and age and sex categories, incorporating further distinctions on the basis of age classifications relevant to the discourse on 'normal BMI'.

2.3.2. Participant Characteristics

A concise sociodemographic and lifestyle questionnaire devised by the authors was used to gather essential participant data, including date of birth, residential region, education level (ranging from elementary to postgraduate), smoking status (categorized as current, never, or former), and frequency of physical activity (classified as fewer than three times per week or three or more times per week). Due to constraints in terms of human resources and time, it was not feasible to associate the basic demographic questionnaire directly with individual participants. Consequently, the data were automatically exported from the BIA via the manufacturer's software. This limitation precludes the determination of whether increased physical activity correlates with improvements in body composition, thereby obstructing the establishment of meaningful correlations and comprehension of the impact of physical activity on health outcomes.

2.4. Statistical Analysis

Data preparation: All the statistical analyses were conducted via R version 4.3.1 and RStudio version 2024.04.1 Build 748. RStudio, an integrated development environment for R, provides a comprehensive suite of tools and packages for robust and efficient data handling and analysis, thereby enhancing the analysis. The tidyverse package [40] was utilized for data manipulation and visualization, as it has a cohesive framework for an efficient workflow. The janitor package [41] facilitates data cleaning, generating tidy datasets that are ready for analysis.

Descriptive statistics: The Arsenal package was used for detailed key study variable summaries and statistics [42]. The data were comprehensively described with means, medians, standard deviations, and CIs for continuous variables and with percentages and CIs for categorical variables. Additionally, the ggstatsplot package [43] was used to perform statistical analyses, such as the Mann–Whitney U and Kruskal–Wallis tests.

Analysis of differences in obesity: To assess differences in obesity proportions between BMI and FAT%, a two-sample test with continuity correction was used for large sample sizes. The p values were adjusted with the Benjamini–Hochberg method to control the false discovery rate and reduce type I errors.

Parametric and nonparametric tests: Continuous data normality was assessed via the Shapiro–Wilk test. If the data followed a normal distribution, paired t tests were used to compare means. For non-normal data, nonparametric methods such as the Wilcoxon signed-rank test for paired samples and the Mann–Whitney U test for independent samples were used. The Kruskal–Wallis test was applied for data involving more than two groups. This approach ensures the use of suitable statistical tests on the basis of data distribution.

Hierarchical models: For hierarchical data, the lme4 package [44] was employed to generate linear mixed models, accounting for both fixed and random effects. This approach enabled us to account for the hierarchical structure of the data and mitigate the influence of potential confounding variables, thereby yielding results that are more precise and generalizable.

Presentation of results: The data are summarized as the means and standard deviations for continuous variables and as percentages for categorical variables. Statistical significance was set at $p < 0.05$, with all analyses conducted without missing data.

3. Results

3.1. Demographic Characteristics and Lifestyle

This study examined the body composition of a cohort of 432 Slovenian adults, of whom 287 were females (66%) and 145 were males (34%). Measurements were administered twice over the course of two years, culminating in a FU investigation comprising 389 adult participants, including 43 older adults. Within the female subgroup, 255 were categorized as adult females, and 32 were categorized as older adult females. In the male subgroup, 133 were designated young or middle-aged adult males, and 11 were categorized as older adult males. The geographical distribution of the participants was as follows: 26% from the capital city Ljubljana (including its environs), 25% from Upper Carniola, 17% from Styria, 16% from Lower Carniola, 9% from Inner Carinthia, and 3% each from Prekmurje and the Littoral Region (Figure 1). Furthermore, the education levels of the participants were categorized as follows: four years of secondary school (39%), higher education (24%), vocational education (16%), higher education professional (Bologna level 1) (12%), university programs (Bologna level 1) (7%), professional master’s degrees (Bologna level 2), doctorate degrees (Bologna level 3) (1%), and primary school (1%). With respect to smoking status, 16% were current smokers, 53% were ex-smokers, and 31% were nonsmokers. More than half (51%) of the participants engaged in physical activity at least three times per week, with a significantly greater proportion of males than females ($p < 0.001$).

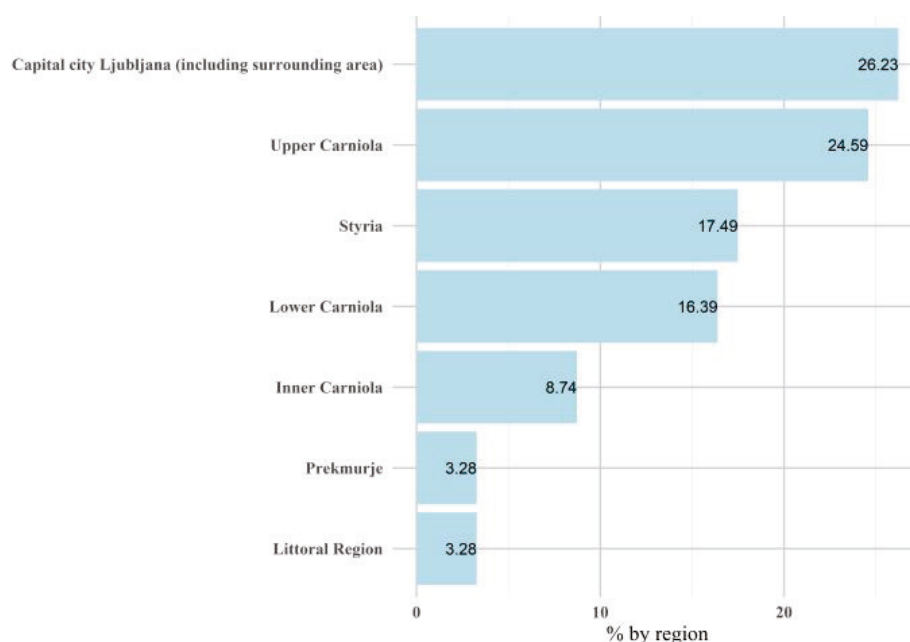


Figure 1. Regional distribution of participants.

3.2. Body Mass Index and Body Composition Status by Sex and Age

The mean BH values of the females and males were $165.9 \text{ cm} \pm 6.5 \text{ cm}$ and $179.2 \text{ cm} \pm 7.5 \text{ cm}$ ($p < 0.001$), respectively. Both adult females and males were taller than older adults were ($166.3 \text{ cm} \pm 6.4 \text{ cm}$ vs. $162.6 \text{ cm} \pm 7.0 \text{ cm}$ and $179.4 \text{ cm} \pm 7.7 \text{ cm}$ vs. $177.2 \text{ cm} \pm 4.8 \text{ cm}$, respectively; $p < 0.001$). Adult females had higher BM, BMI, FAT%, and FATM values and lower FFM% due to increased FATM. In addition, adult males had higher BM and BMI values, primarily due to higher FFM rather than FATM. The details are presented in Table 2.

3.3. Body Mass Index and FAT% Status According to BMI and FAT% Obesity Classification

Initially, 40.7% of individuals were classified as having overweight or obesity by BMI, increasing to 45.2% at the FU ($p < 0.001$). Notably, the percentage was significantly greater in males than in females (58.9% vs. 31.7% and 61.6% vs. 36.9%, respectively; both $p < 0.001$). The BMI classification revealed significantly fewer participants with obesity than the FAT% did ($p = 0.013$). Body mass index classification initially identified 13.4% of the participants as having obesity, increasing to 16% at the FU ($p = 0.333$), whereas FAT% initially identified 20%, increasing to 21.6% at the FU ($p = 0.612$). The BMI and FAT% status data for both classifications (initial vs. FU) for the entire sample (adults and older adults), including 95% confidence intervals, are presented in Table S1.

Among adults, 38.2% were in the overweight or obese classifications by BMI initially, increasing to 42.4% at FU ($p < 0.001$), with higher rates in males than in females (55.7% vs. 29.2% and 59.0% vs. 33.9%, respectively; $p < 0.001$). Initially, 11.6% were classified as having obesity according to BMI, increasing to 14.2% at the FU ($p = 0.007$); FAT% classified 17.6% as having obesity initially, increasing to 19.5% at the FU ($p < 0.810$). The initial vs. FU BMI and FAT% data are shown in Table 3.

Table 2. Body mass index and body composition status by sex and age.

Age (Years)	All 18–64			All 65+			Females 18–64			Females 65+			Males 18–64			Males 65+		
	IN	FU	p Value	IN	FU	p Value	IN	FU	p Value	IN	FU	p Value	IN	FU	p Value	IN	FU	p Value
BM (kg)	72.6 ± 16.77	73.7 ± 16.7	<0.001	75.4 ± 13.07	76.3 ± 12.90	0.170	66.2 ± 12.46	74.4 ± 12.5	<0.001	71.7 ± 12.27	72.4 ± 11.80	0.464	85.1 ± 17.08	86.1 ± 17.0	0.032	85.9 ± 9.3	87.1 ± 9.3	0.999
BMI (kg/m ²)	24.8 ± 4.7	25.1 ± 4.6	<0.001	27.2 ± 3.9	27.5 ± 3.8	0.120	23.9 ± 4.4	24.3 ± 4.3	<0.001	27.1 ± 4.4	27.4 ± 4.3	0.261	26.4 ± 4.8	26.7 ± 4.9	0.039	27.3 ± 2.0	27.6 ± 2.1	0.999
FATM (kg)	18.7 ± 9.2	18.8 ± 9.1	<0.001	23.5 ± 7.8	23.9 ± 7.6	0.999	18.6 ± 8.5	19.2 ± 8.4	<0.001	24.8 ± 8.0	25.2 ± 7.8	0.999	17.6 ± 10.3	17.9 ± 10.2	0.999	19.7 ± 5.7	20.3 ± 6.0	0.999
FAT%	24.5 ± 8.1	24.9 ± 8.0	0.053	30.9 ± 7.5	31.1 ± 7.3	0.999	27.0 ± 7.5	27.5 ± 7.4	0.048	33.9 ± 5.7	34.0 ± 5.5	0.999	19.7 ± 6.8	19.8 ± 6.8	0.999	22.6 ± 5.3	23.0 ± 5.2	0.999
FATM/BH (kg/cm)	10.7 ± 5.4	11.0 ± 5.3	<0.001	14.1 ± 4.8	14.4 ± 4.7	0.885	11.2 ± 5.1	11.6 ± 5.0	<0.001	15.3 ± 4.8	15.5 ± 4.7	0.999	9.8 ± 5.7	10.0 ± 5.7	0.999	10.9 ± 3.0	11.4 ± 3.2	0.999
FFM (kg)	54.3 ± 11.65	54.9 ± 11.7	<0.001	51.9 ± 10.15	52.4 ± 10.10	0.972	47.6 ± 5.5	48.2 ± 5.6	<0.001	46.9 ± 5.3	47.3 ± 5.1	0.999	67.5 ± 8.7	68.1 ± 8.8	0.012	66.3 ± 5.8	66.8 ± 5.4	0.863
FFM (%)	75.5 ± 8.1	75.1 ± 8.0	0.059	69.1 ± 7.5	68.8 ± 7.3	0.999	73.0 ± 7.5	72.5 ± 7.4	0.046	66.1 ± 5.7	65.9 ± 5.5	0.999	80.3 ± 6.8	80.2 ± 6.8	0.999	77.4 ± 5.3	77.0 ± 5.2	0.999
FFM/BH (kg/cm)	31.6 ± 5.4	32.0 ± 5.4	<0.001	31.0 ± 4.7	31.3 ± 4.7	0.999	28.6 ± 2.9	28.9 ± 2.9	<0.001	28.8 ± 2.9	29.0 ± 2.7	0.999	37.5 ± 4.0	37.9 ± 4.0	0.025	37.4 ± 2.6	37.6 ± 2.5	0.999
TBW (kg)	38.8 ± 8.3	39.2 ± 8.4	<0.001	36.3 ± 7.0	36.7 ± 6.9	0.999	34.0 ± 4.0	34.4 ± 4.0	0.002	33.0 ± 3.8	33.3 ± 3.6	0.935	48.0 ± 6.4	48.6 ± 6.7	0.018	45.9 ± 4.6	46.3 ± 4.3	0.999
PhA (°)	5.9 ± 0.7	5.9 ± 0.7	0.999	5.2 ± 0.6	5.2 ± 0.6	0.999	5.6 ± 0.5	5.6 ± 0.6	0.999	5.2 ± 0.5	5.2 ± 0.6	0.999	6.5 ± 0.6	6.5 ± 0.6	0.999	5.3 ± 0.7	5.3 ± 0.7	0.863

The data are presented as the means ± standard deviations. Statistically significant values are shown in bold. IN = initial, FU = follow-up, BH = body height, BM = body mass, BMI = body mass index, FAT% = total body fat percentage, FATM = total body fat mass, FFM = fat-free mass, TBW = total body water, and PhA = whole-body phase angle.

Table 3. Body mass index and FAT% status of adults according to BMI and FAT% obesity classification.

		Initial (<i>n</i> = 389)			FU (<i>n</i> = 389)		
Parameter		All	Females	Males	All	Females	Males
According to BMI classification (%) [†]							
Normal (BMI 18.5–24.9 kg/m ²)		61.8	70.9	44.2	57.6	66.1	41.1
Overweight (BMI 25–29.9 kg/m ²)		26.6	19.9	39.5	28.2	23.1	38.0
Obesity 1 class (BMI 30–34.9 kg/m ²)		8.7	7.2	11.6	11.1	8.0	17.1
Obesity 2 class (BMI 35–39.9 kg/m ²)		1.6	1.2	2.3	1.8	2.0	1.6
Obesity 3 class (BMI >40 kg/m ²)		1.3	0.8	2.3	1.3	0.8	2.3
<i>p</i> value			<0.001			<0.001	
According to BMI obesity classification (%) [†]							
Normal		88.4	90.8	83.7	85.8	89.2	79.1
With obesity		11.6	9.2	16.3	14.2	10.8	20.9
<i>p</i> value			0.040			0.009	
According to FAT% obesity classification (%) [†]							
Females	<35% (normal)		84.1			80.9	
	>35% (obesity)		15.9			19.1	
Males	<25% (normal)			79.1			79.8
	>25% (obesity)			20.9			20.2
All	Normal	82.4			80.5		
	Obesity	17.6			19.5		
<i>p</i> value			0.226			0.891	

Statistically significant values are shown in bold. [†] Body mass index (BMI) and FAT% obesity classifications by the WHO [33,45]. BMI = body mass index and FAT% = total body fat percentage.

In the older adult cohort, 62.2% were initially in the overweight or obese classifications according to BMI, increasing to 68.4% at FU ($p < 0.001$), and this was higher in males (91.7%) than in females (51.5% or 60.5%). At FU, the rate of obesity according to BMI was 36.4% in females and 16.7% in males, although nonsignificantly different, and that of obesity according to FAT% was 39.4% in females and 41.7% in males, also nonsignificantly different.

Notably, 23% of females, 38% of males, and 28% of the adult cohorts were in the overweight category, whereas 24% of females, 75% of males, and 40% of older adults were in the overweight category.

3.4. Body Mass Index and FAT% According to Age

The age structure report illustrates a progressive distribution of BMI and FAT%, manifesting a notable increase in each age group. Specifically, young adults presented a lower BMI and FAT%. However, within each category, males had a higher BMI but a lower FAT% (Table 4). The median BMI and FAT% according to age, sex, and time of measurement (initial and FU assessments) are presented in Figure S1.

Table 4. Body mass index and FAT% according to age structure.

Initial					Follow-Up			
					<i>Females</i>			
Age category (years)	18–39 (<i>n</i> = 99)	40–64 (<i>n</i> = 156)	≥65 (<i>n</i> = 32)	<i>p</i> value	18–39 (<i>n</i> = 99)	40–64 (<i>n</i> = 156)	≥65 (<i>n</i> = 32)	<i>p</i> value
BMI (kg/m ²)	23.0 ± 4.0	24.5 ± 4.5	26.9 ± 4.4	<0.001	23.4 ± 4.1	24.9 ± 4.4	27.2 ± 4.2	<0.001
FAT%	24.4 ± 7.5	28.5 ± 7.1	33.9 ± 5.5		24.6 ± 7.5	29.2 ± 6.8	33.9 ± 5.4	
					<i>Males</i>			
Age category (years)	18–39 (<i>n</i> = 56)	40–64 (<i>n</i> = 78)	≥65 (<i>n</i> = 11)	<i>p</i> value	18–39 (<i>n</i> = 56)	40–64 (<i>n</i> = 78)	≥65 (<i>n</i> = 11)	<i>p</i> value
BMI (kg/m ²)	25.3 ± 4.7	27.1 ± 4.8	27.6 ± 2.2	<0.001	25.6 ± 4.7	27.4 ± 4.8	28.0 ± 2.3	0.002
FAT%	17.5 ± 6.7	21.0 ± 6.5	23.2 ± 5.5		17.9 ± 6.7	21.0 ± 6.5	23.7 ± 5.5	

BMI = body mass index and FAT% = total body fat percentage.

3.5. Trends and Variations Within Groups for Adults

Using linear mixed models, the study included 389 adults with 778 observations. Ageing led to a 0.21-unit annual weight gain; males weighed 18.83 units more than females did. The time of measurement increased the BM by 1.14 units. The random effects model showed a residual variance of 6.28 and an intercept variance of 188.70, with a 0.97 intraclass correlation. Fixed factors (age, sex, and time of measurement) explained 30.4% of the weight variability, whereas combined fixed and random effects explained 97.8%, highlighting individual differences. Each year of age increased BMI by 0.09 units, with the BMI of males being 2.46 units higher than that of females. The time of measurement increased BMI by 0.36 units, with 97% variance due to individual differences. Fixed factors accounted for 10.8% of BMI variability, with fixed and random effects accounting for 97.0%. Age increased the FAT% by 0.20% annually; males had a 7.41% lower FAT% than females did. The time of measurement increased the FAT% by 0.38%. Interindividual differences accounted for 93% of the FAT% variability, and fixed factors accounted for 27.7%, with both effects explaining 95%. Age did not significantly affect FFM; males had 19.88 units more FFM than females did, and each year of age increased FFM by 0.60. Interindividual differences explained 95% of the FFM variability, and fixed factors explained 65.5%, with both effects combined accounting for 98.1%. Each year of age, PhA decreased by 0.01 degrees; the PhA of males was 0.88 degrees higher than that of females. The time of measurement did not significantly affect PhA. Individual differences accounted for 83% of the PhA variance, and fixed factors accounted for 31.8%, with both accounting for 89.6%. The trends for the adults and the older adults are shown in Table 5 and Table S2, respectively. The BMI, FAT%, FFM, and PhA distributions are shown in Figure S2.

Table 5. Trends and variations within groups for adults.

Predictors	BM		BMI		FAT%		FFM		PhA	
	Estimates	p Value	Estimates	p Value	Estimates	p Value	Estimates	p Value	Estimates	p Value
Intercept	55.83	<0.001	19.81	<0.001	18.07	<0.001	46.30	<0.001	6.18	<0.001
Age	0.21	<0.001	0.09	<0.001	0.20	<0.001	0.02	0.593	−0.01	<0.001
Sex (male)	18.83	<0.001	2.46	<0.001	−7.41	<0.001	19.88	<0.001	0.88	<0.001
Time of measurement	1.14	<0.001	0.36	<0.001	0.38	0.004	0.60	<0.001	0.01	0.450

Statistically significant values are indicated in bold. BM = body mass, BMI = body mass index, FAT% = total body fat percentage, FFM = fat-free mass, and PhA = whole-body phase angle.

4. Discussion

4.1. Main Findings

In this cohort study, trends in BMI and body composition among adult Slovenes were examined over a two-year period. The initial findings revealed that 40.7% of the participants were classified as having either overweight or obesity according to their BMI, and this percentage increased to 45.2% at the FU assessment. A comparable pattern was observed exclusively among adults. Importantly, this percentage was markedly greater in males than in females. The BMI classification estimated fewer participants with obesity than

did the FAT%; however, the proportion of participants with obesity in both classifications remained similar between the initial and FU measurements. The hypothesis suggested an overall increase in overweight and obesity rates on the basis of both BMI and FAT% classifications, with the most significant increase observed among adult females. In contrast, adult males presented an increase in BM at the expense of FFM rather than FATM. Older adult females and males did not experience any major adverse changes. The linear mixed models highlighted the influence of sex, age, and time of measurement, emphasizing sex differences, the effects of ageing, and the importance of personalized health assessments.

4.2. Obesity Rates and BMI

Global obesity has overtaken underweight in the past four decades, shifting the overall health status of the human population [46]. The incidence of obesity in most European countries is increasing, being near 20% or higher, and healthcare costs and economic burdens are increasing [47,48]. Slovenia has the highest obesity rate among the 20 European countries studied recently, according to BMI classification (20.8%) [49].

Initially, 40.7% of the participants (adults and older adults) were in the overweight or obesity classifications on the basis of BMI; the prevalence rate significantly increased to 45.2% at the FU, with 61.6% for males and 36.9% for females. At the initial BMI measurements, 13.4% of the participants were in the obesity ranges; this prevalence rate increased nonsignificantly to 16% at the FU. Initially, 20.0% of the participants were categorized as in the obesity ranges according to the FAT%, increasing to 21.6% at the FU. Among adults, 38.2% were within overweight or obese categories according to BMI, increasing to 42.4% at FU, with 59.0% for males and 33.9% for females. Initially, 11.6% of the participants had obesity according to BMI, which increased to 14.2% at the FU. FAT% initially classified 17.6% as having obesity, increasing to 19.5% at the FU.

The results revealed a significant decline in adult females within two years of the initial measurement. Notably, 54% were aged 40 to 64 years. This review highlights how menopause affects body composition and fat distribution. Research shows that the menopausal transition impacts BMI and fat distribution, particularly during the perimenopausal and postmenopausal stages [50,51]. It is possible that increased health metrics result in part from midlife hormonal changes, such as fluctuating hormones and lower oestrogen levels in females. However, body composition data alone cannot provide definitive conclusions.

A previous study involving 151 individuals who switched to a plant-based diet yielded initial results similar to those of the current study. Specifically, 50% of the participants had overweight or obesity before adopting a plant-based diet [52]. Like the FU cohort study, the plant-based study sample included individuals from all Slovenian regions, with more females than males and more adults than older adults. However, these estimates regarding overweight or obesity rates slightly differ from the data gathered by the Slovenian national dietary survey (SI.Menu 2017/2018) on 780 adults and older adults, where 67% of the respondents were within the overweight or obesity ranges, with 59% being adults (68% males and 50% females) and 74% being older adults (79% males and 73% females) [17,53]. In Serbia, 2013 data showed that 60.5% of adults had overweight or obesity [54], aligning with the SI.Menu survey's results [17,53]. A 2010 pan-European survey across 16 countries, including Croatia, Italy, Austria, and Hungary, revealed that 47.6% of European adults had overweight or obesity, with rates of 58.2% in Croatia, 38.5% in Italy, 44.5% in Austria, and 65.4% in Hungary [54]. These data, which are nearly 15 years old, are based on self-reported height and weight. Both the SI.Menu and pan-European surveys use BMI classification instead of direct FAT% measurements to calculate obesity rates.

4.3. Body Composition Assessment

Nutritional epidemiology faces challenges in the role of BMI in initial assessments, such as eligibility for various treatment modalities, e.g., bariatric surgery or semaglutide. Further evidence-based consensus is needed for guidelines and thresholds for different populations [27]. As the understanding of health and body composition evolves, the

limitations of BMI for assessing health risks become more evident [55]. The calculation of BMI involves a quick assessment of height and weight but does not distinguish between FATM and FFM/LBM, risking health misclassification, particularly for individuals with high muscle mass or low muscle density [27,56]. The validity of BMI data is challenging, particularly in large samples, when height and weight are self-reported [54,57]. Relying solely on BMI and weight loss may offer limited health benefits and promote weight-related discrimination [28]. A U.S. study suggested that BMI may inaccurately reflect cardiometabolic disease, misclassifying approximately 75 million adults [29].

Dual-energy X-ray absorptiometry (DXA) is the gold standard for determining body composition because of its accuracy in differentiating bone mineral content, LBM, and FATM, outperforming BIA. However, the high cost, limited portability, and use of radiation of DXA limit its use. BIA is more practical, portable, cost-effective, and radiation-free, with good reproducibility and a reasonable correlation with DXA for overall FAT and FFM/LBM [58,59]. BIA accuracy can be affected by hydration and disease, leading to potential misestimation in different BMI groups [60–63]. A study of 35,000 UK Biobank participants revealed a strong correlation between BIA and DXA. BIA slightly underestimates the FATM and overestimates the FFM, with individual disparities linked to anthropometric measures [60]. Advancements in imaging have enhanced body fat measurements, including visceral adipose tissue (VAT) and ectopic fat in the heart and liver. DXA is practical, but MRI is the gold standard for precise VAT assessment, although it is more expensive and time-consuming [64,65]. Compared with BMI, advanced technologies such as BIA and DXA provide better insights into FAT distribution and composition.

4.4. Age and ‘Normal BM’

In this analysis, adults were compared, and older adults were highlighted despite the limited sample size. Overweight was more common in older adults than in non-older adults (40% vs. 28%). This was observed mostly among males, with 75% of older adult males and 38% of adult males having overweight. This is due to the age distribution; most females aged 40–64 years were below the overweight threshold (24.9 kg/m²), whereas most males of the same age were within the overweight range (27.4 kg/m²).

Research indicates that a slightly elevated BMI may provide protective benefits for older adults. The “obesity paradox” suggests that individuals with an overweight BMI might have lower mortality risks and better functional and cognitive health than individuals with a lower BMI, especially those with an underweight BMI [66]. Older adults with a higher BMI often show better cognitive ability, physical independence, fewer depressive symptoms, and improved survival rates, although this does not always apply to individuals with obesity. Age-related factors such as obesity degree, muscle mass loss, fat distribution changes, and comorbidities can influence this pattern, making a slightly higher BMI potentially more beneficial than a normal BMI [66–68]. Lifestyle choices and pre-existing health conditions significantly impact outcomes in older adults, emphasizing the need for age-specific BMI criteria. Therefore, analyses of BMI in older adults should focus mainly on trends rather than health risks or benefits. Current BMI thresholds may not accurately reflect the health risks of excess fat in older adults, necessitating revised cut-off values. The inclusion of body composition metrics could improve health risk assessments for obesity in older age groups [66,69,70].

4.5. Linear Mixed Models

The general conclusions from the linear mixed models are as follows: (i) males tended to have higher BMI, FFM, and PhA but lower FAT%; (ii) in general, BMI and FAT% were higher in older adults, and PhA was lower, with no significant age effect on BM or FFM; and (iii) over a two-year period, BM, FFM, and FAT% decreased, but PhA remained unchanged. Overall, weight, BMI, FAT%, and FFM slightly increased with age and time of measurement, and PhA slightly decreased with age. The high intraclass correlation coefficient (ICC) values

suggest that variability is due mainly to interindividual differences, indicating consistency within individuals.

The models emphasize the need for sex-specific health and fitness assessments, the varying impacts of ageing on body composition, and the necessity for regular monitoring. Individual variability underscores the importance of personalized health programs. In the cohort study, obesity was more prevalent in males than in females, which contrasts with global trends [2,71] and that reported in the SI.Menu study [53]. Interestingly, in the FU cohort and SI.Menu study, a higher proportion of adult males were classified as having overweight. Additionally, in the SI.Menu study, older adults typically had higher BMIs [53], which aligns with the findings of the present study. BMI increases with age due to changes in body composition, necessitating various health approaches [72,73].

4.6. Physical Activity and the BM

The initial study began shortly after the COVID-19 pandemic, during which lockdowns were implemented. The responses varied, with Slovenia expanding from regional to municipal partial lockdowns and advising low to moderate physical activity without specifying types. While the gyms were closed for several months, people's activity levels fluctuated due to anxiety and uncertainty. Two years later, FU measurements revealed that BMI and body composition declined, raising questions about current activity levels as people returned to their routines. Half of the participants reported exercising at least three times weekly in the previous two months, but self-reported data are limited with regard to the definitive conclusions that can be drawn about overall activity trends.

A spring 2021 online survey in Slovenia with 1161 participants revealed that 80% of the participants maintained or increased their physical activity, mainly in natural settings [74]. It is likely that the physical activity was mainly low to moderate intensity instead of vigorous exercise, which is crucial for stimulating skeletal muscle. Additionally, in a recent review of 40 studies from 22 countries, excluding Slovenia but including Italy, physical inactivity, sedentary lifestyle, and poor eating habits were identified as common risk factors for obesity during the COVID-19 pandemic [75].

The degree of physical inactivity is increasing globally [76]. Managing obesity and improving body composition require a holistic approach that combines dietary changes with resistance and endurance exercises [77–81]. Choosing healthier foods can increase physical activity, and regular exercise can lead to better eating habits [82,83]. The synergy between physical activity and nutrition enhances a healthy lifestyle.

4.7. Possible Public Health Strategies

The FU study of Slovene adults revealed an imbalanced diet with low intake of complex carbohydrates and high consumption of ultra-processed foods. The intake of essential macronutrients such as carbohydrates and dietary fibre is insufficient, whereas the intake of total fat and saturated fatty acid is excessive. Micronutrient deficiencies were found for vitamins C, D, and E and calcium, and the sodium levels were above the recommended guidelines [11]. A national SI.Menu highlighted poor dietary quality with deficiencies in vitamin D, dietary fibre, and folate. Notably, 32% of adults and 46% of older adults, especially females aged 18 to 50 years, lack sufficient iron and vitamin B12 intake [15–17,84].

In terms of consumption according to food group, assessments indicate that the Slovenian diet is unbalanced, with excessive intake of meat, sugar, fat, and salt and insufficient intake of fruits, vegetables, and dairy products [85]. Research indicates that Slovenian meat consumption is approximately four times greater than the recommended level and six times greater than the Planetary Health Diet standard [85–87]. While some young adults are interested in reducing meat consumption, many of them continue to follow meat-heavy diets due to societal norms. This high meat intake, along with an overall unbalanced diet, poses serious health risks. Additionally, many young adults are unaware of the health and environmental impacts of excessive meat consumption, reducing their motivation to

change their diets [88]. Research shows that individuals often lack sustainable dietary habits and are concerned with health, environmental issues, animal welfare, and dietary diversity [89]. Older adults are more proactive than younger individuals in reducing meat consumption [90]. Comprehensive strategies with cross-departmental collaboration and public engagement are urgently needed to address these dietary challenges.

In the FU study, 51% of the participants exercised at least three times weekly. In 2016, 56% of Slovenians met the WHO's physical activity guidelines, with higher rates in males (59%) than in females (52%). Higher education correlated slightly with increased physical activity [91]. In terms of smoking status, 16% were current smokers, 53% were former smokers, and 31% were nonsmokers. In the national SI.Menu study, 55% of adults were nonsmokers [92]. These insights, along with poor dietary habits, highlight the urgent need for better lifestyle choices.

The widespread availability and marketing of a wide variety of inexpensive, convenient, energy-dense ultra-processed foods whose portion sizes are large and are high in fat, salt, and sugar and low in protein and fibre are major dietary drivers of increased obesity and common chronic diseases [93–95]. Additionally, low or irregular physical activity exacerbates hedonic behaviours and promotes overconsumption [93,96], and a firm government policy is essential to establish a healthy, profitable, fair, and sustainable food system that benefits everyone [19,20,97]. Nearly 1.8 billion adults worldwide are at risk of various diseases due to insufficient physical activity [76]. Engaging in regular physical activity and maintaining a healthy diet are typical methods to reduce excess BM/FATM and maintain FFM during BM loss [79,98–100], but this was not observed in the cohort of the present study.

The Slovenian Strategic Council for Nutrition has set 2023/24 activities to improve health and promote sustainable food. The government is encouraging healthier diets to benefit public health and the environment. A 2023–2025 action plan is being created to support national nutrition and physical activity programs, which are aligned with the Council's initiatives and open for public review. The Council endorses the "Planetary Health Diet", which consists mainly of plant-based products but includes some animal products and is linked to lower risks of diabetes, cardiovascular disease, cancer, and mortality, as shown by a recent systematic review [19,87,101].

4.8. Strengths and Limitations

This FU cohort study was the first to investigate body composition trends in the same group of Slovenian adults (two years after the COVID-19 pandemic). It extends the initial research, which was the only study on this topic at the population level in Slovenia. The measurements were consistent, taken within a month and at the same time of year as the initial study, thus minimizing seasonal influence. The initial sample was randomly selected, ensuring regional representation. The limitations of this study were the reduced FU adult sample ($n = 432$; 51%) compared with the initial large sample ($n = 844$) of the Slovene population (2,100,000 citizens in total). This sample included fewer older adults; therefore, despite random assignment and FU completion, the findings should be generalized with caution. BIA was used in this study; it is a cost-effective, radiation-free and portable method that is ideal for large ethnic groups [58,59] and is widely used in Slovenia at universities, medical institutions, rehabilitation centres, sports clubs and fitness centres [102] to assess body composition. In addition, compared with DXA, the 8-electrode BIA device offers accurate FAT% measurements [60,103,104], even when habitual physical activity is considered [105,106]. However, in the present study, a validity study of the specific BIA model versus DXA in various BMI categories is lacking [107,108]. While initial BIA measurements were used, gathering individuals for the same DXA device measurements was impractical and incomparable. Dietary intake was assessed in the initial study [11] but not during the FU, representing a limitation for interpretation and requiring reliance on speculation and literature. Although random sampling was used and all regions of Slovenia were covered, uneven representation limits the generalizability of the findings.

5. Conclusions

This is the first nationwide study on adult body composition in Slovenia. This representative cohort study revealed that from the initial study to the two-year FU, the proportion of adults with overweight and obesity increased from 40.7% to 45.2%, notably among females. Other groups, such as older females and males, presented no significant changes. Interestingly, the BM among adult males increased primarily because of FFM, not FAT. The study also revealed that BMI classification resulted in fewer participants with obesity than FAT% classification did, indicating the need for body composition analysis to determine obesity. The study highlighted the influence of sex, age, and time of measurement when assessing body composition, emphasizing the importance of personalized health assessments. This study highlights the need for joint efforts by governments, social organizations, and the food industry to encourage lifestyle changes through education and a supportive environment for healthy living.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu16234123/s1>, Table S1: Body mass index and FAT% status of adults and older adults according to BMI and FAT% obesity classification; Table S2: Trends and variations among adult and older adult groups; Figure S1: BMI and FAT% medians according to age categories at the initial and FU; Figure S2: Distribution of BMI (a), BF% (b), FFM (c), and PhA (d).

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References

1. Koliaki, C.; Dalamaga, M.; Liatis, S. Update on the Obesity Epidemic: After the Sudden Rise, Is the Upward Trajectory Beginning to Flatten? *Curr. Obes. Rep.* **2023**, *12*, 514–527. [CrossRef]
2. Phelps, N.H.; Singleton, R.K.; Zhou, B.; Heap, R.A.; Mishra, A.; Bennett, J.E.; Paciorek, C.J.; Lhoste, V.P.; Carrillo-Larco, R.M.; Stevens, G.A.; et al. Worldwide trends in underweight and obesity from 1990 to 2022: A pooled analysis of 3663 population-representative studies with 222 million children, adolescents, and adults. *Lancet* **2024**, *403*, 1027–1050. [CrossRef] [PubMed]
3. Masood, B.; Moorthy, M. Causes of obesity: A review. *Clin. Med.* **2023**, *23*, 284–291. [CrossRef]
4. Kivimäki, M.; Strandberg, T.; Pentti, J.; Nyberg, S.T.; Frank, P.; Jokela, M.; Ervasti, J.; Suominen, S.B.; Vahtera, J.; Sipilä, P.N.; et al. Body-mass index and risk of obesity-related complex multimorbidity: An observational multicohort study. *Lancet Diabetes Endocrinol.* **2022**, *10*, 253–263. [CrossRef] [PubMed]
5. Leyden, E.; Hanson, P.; Halder, L.; Rout, L.; Cherry, I.; Shuttlewood, E.; Poole, D.; Loveder, M.; Abraham, J.; Kyrou, I.; et al. Older age does not influence the success of weight loss through the implementation of lifestyle modification. *Clin. Endocrinol.* **2020**, *94*, 204–209. [CrossRef]
6. Organisation for Economic Cooperation and Development (OECD). The Heavy Burden of Obesity: The Economics of Prevention. Available online: <https://www.oecd-ilibrary.org/sites/6cc2aacc-en/index.html?itemId=/content/component/6cc2aacc-en> (accessed on 26 March 2024).
7. Yusefzadeh, H.; Rashidi, A.; Rahimi, B. Economic burden of obesity: A systematic review. *Soc. Health Behav.* **2024**, *2*, 7–12. [CrossRef]
8. Hoogendoorn, M.; Galekop, M.; van Baal, P. The lifetime health and economic burden of obesity in five European countries: What is the potential impact of prevention? *Diabetes Obes. Metab.* **2023**, *25*, 2351–2361. [CrossRef] [PubMed]

9. Gambert, S.R. The Burden of Chronic Disease. *Mayo Clin. Proc. Innov. Qual. Outcomes* **2024**, *8*, 112–119. [CrossRef]
10. World Health Organization. Regional Office for Europe WHO European Regional Obesity Report 2022. Available online: <https://iris.who.int/bitstream/handle/10665/353747/9789289057738-eng.pdf> (accessed on 26 March 2024).
11. Jakše, B.; Godnov, U.; Pinter, S. Nutritional Status of Slovene Adults in the Post-COVID-19 Epidemic Period. *Eur. J. Investig. Health Psychol. Educ.* **2022**, *12*, 1729–1742. [CrossRef] [PubMed]
12. Sorić, M.; Jurak, G.; Đurić, S.; Kovač, M.; Strel, J.; Starc, G. Increasing trends in childhood overweight have mostly reversed: 30 years of continuous surveillance of Slovenian youth. *Sci. Rep.* **2020**, *10*, 11022. [CrossRef] [PubMed]
13. Radulović, A.; Jurak, G.; Leskošek, B.; Starc, G.; Blagus, R. Secular trends in physical fitness of Slovenian boys and girls aged 7 to 15 years from 1989 to 2019: A population-based study. *Sci. Rep.* **2022**, *12*, 10495. [CrossRef]
14. Marques, A.; Peralta, M.; Martins, J.; Loureiro, V.; Almanzar, P.C.; de Matos, M.G. Few European Adults are Living a Healthy Lifestyle. *Am. J. Health Promot.* **2019**, *33*, 391–398. [CrossRef]
15. Seljak, B.K.; Valenčič, E.; Hristov, H.; Hribar, M.; Lavriša, Ž.; Kušar, A.; Žmitek, K.; Krušič, S.; Gregorič, M.; Blaznik, U.; et al. Inadequate Intake of Dietary Fibre in Adolescents, Adults, and Elderlies: Results of Slovenian Representative SI. Menu Study. *Nutrients* **2021**, *13*, 3826. [CrossRef] [PubMed]
16. Pravst, I.; Lavriša, Ž.; Hribar, M.; Hristov, H.; Kvarantan, N.; Seljak, B.K.; Gregorič, M.; Blaznik, U.; Gregorič, N.; Zaletel, K.; et al. Dietary Intake of Folate and Assessment of the Folate Deficiency Prevalence in Slovenia Using Serum Biomarkers. *Nutrients* **2021**, *13*, 3860. [CrossRef] [PubMed]
17. Hribar, M.; Hristov, H.; Lavriša, Ž.; Seljak, B.K.; Gregorič, M.; Blaznik, U.; Žmitek, K.; Pravst, I. Vitamin D Intake in Slovenian Adolescents, Adults, and the Elderly Population. *Nutrients* **2021**, *13*, 3528. [CrossRef]
18. Poličnik, R.; Hristov, H.; Lavriša, Ž.; Farkaš, J.; Smole Možina, S.; Koroušić Seljak, B.; Blaznik, U.; Gregorič, M.; Pravst, I. Dietary Intake of Adolescents and Alignment with Recommendations for Healthy and Sustainable Diets: Results of the SI.Menu Study. *Nutrients* **2024**, *16*, 1912. [CrossRef] [PubMed]
19. Fras, Z.; Jakše, B.; Kreft, S.; Malek, Ž.; Kamin, T.; Tavčar, N.; Fidler Mis, N. The Activities of the Slovenian Strategic Council for Nutrition 2023/24 to Improve the Health of the Slovenian Population and the Sustainability of Food: A Narrative Review. *Nutrients* **2023**, *15*, 4390. [CrossRef] [PubMed]
20. Fras, Z.; Jug, B.; Jakše, B.; Kreft, S.; Mikec, N.; Malek, Ž.; Bavec, M.; Vovk, A.; Frelih-Larsen, A.; Mis, N.F. Slovenia's Food-Based Dietary Guidelines 2024: Eating for Health and the Planet. *Foods* **2024**, *13*, 3026. [CrossRef]
21. Chrysant, S.G.; Chrysant, G.S. The single use of body mass index for the obesity paradox is misleading and should be used in conjunction with other obesity indices. *Postgrad. Med.* **2019**, *131*, 96–102. [CrossRef] [PubMed]
22. Chooi, Y.C.; Ding, C.; Magkos, F. The epidemiology of obesity. *Metabolism* **2019**, *92*, 6–10. [CrossRef]
23. Nuttall, F.Q. Body Mass Index: Obesity, BMI, and Health: A Critical Review. *Nutr. Today* **2015**, *50*, 117–128. [CrossRef]
24. Rubino, F.; Batterham, R.L.; Koch, M.; Mingrone, G.; le Roux, C.W.; Farooqi, I.S.; Farpour-Lambert, N.; Gregg, E.W.; Cummings, D.E. Lancet Diabetes & Endocrinology Commission on the Definition and Diagnosis of Clinical Obesity. *Lancet Diabetes Endocrinol.* **2023**, *11*, 226–228. [CrossRef] [PubMed]
25. Tanne, J.H. Obesity: Avoid using BMI alone when evaluating patients, say US doctors' leaders. *BMJ* **2023**, *381*, 1400. [CrossRef] [PubMed]
26. Okorodudu, D.O.; Jumeau, M.F.; Montori, V.M.; Romero-Corral, A.; Somers, V.K.; Erwin, P.J.; Lopez-Jimenez, F. Diagnostic performance of body mass index to identify obesity as defined by body adiposity: A systematic review and meta-analysis. *Int. J. Obes.* **2010**, *34*, 791–799. [CrossRef]
27. Wu, Y.; Li, D.; Vermund, S.H. Advantages and Limitations of the Body Mass Index (BMI) to Assess Adult Obesity. *Int. J. Environ. Res. Public Health* **2024**, *21*, 757. [CrossRef]
28. Flegal, K.M. Use and Misuse of BMI Categories. *AMA J. Ethics* **2023**, *25*, E550–E558. [CrossRef] [PubMed]
29. Tomiyama, A.J.; Hunger, J.M.; Nguyen-Cuu, J.; Wells, C. Misclassification of cardiometabolic health when using body mass index categories in NHANES 2005–2012. *Int. J. Obes.* **2016**, *40*, 883–886. [CrossRef] [PubMed]
30. Ghesmaty Sangachin, M.; Cavuoto, L.A.; Wang, Y. Use of various obesity measurement and classification methods in occupational safety and health research: A systematic review of the literature. *BMC Obes.* **2018**, *5*, 28. [CrossRef] [PubMed]
31. Jakše, B.; Pinter, S.; Godnov, U. Increased legs-to-total fat percentage ratio in females with a normal body mass index: A change in lifestyle or the adaptation process of the body. *Exerc. Qual. Life J.* **2023**, *15*, 13–24. [CrossRef]
32. World Health Organization. A Healthy Lifestyle—WHO Recommendations. Available online: <https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle---who-recommendations> (accessed on 31 March 2024).
33. World Health Organization. Physical status: The Use and Interpretation of Anthropometry. Report of a WHO Expert Committee—PubMed. *World Health Organ. Tech. Rep. Ser.* **1995**, *854*, 1–452.
34. National Institutes of Health. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults-The Evidence Report. *Obes. Res.* **1998**, *6*, 51S–209S.
35. Jakše, B. Nutritional Status of Adult Slovenes After the Covid-19 Epidemic (SloFitCovid). Available online: <https://clinicaltrials.gov/study/NCT05438966> (accessed on 25 March 2024).
36. Tanita, C. Body Composition Analyzer. Available online: <https://tanita.eu/media/wysiwyg/manuals/medical-approved-body-composition-monitors/mc-780-portable-instruction-manual.pdf> (accessed on 7 May 2020).

37. Bauman, A.; Ainsworth, B.E.; Sallis, J.F.; Hagströmer, M.; Craig, C.L.; Bull, F.C.; Pratt, M.; Venugopal, K.; Chau, J.; Sjöström, M. The descriptive epidemiology of sitting. A 20-country comparison using the International Physical Activity Questionnaire (IPAQ). *Am. J. Prev. Med.* **2011**, *41*, 228–235. [CrossRef]
38. Statistical Office of the Republic of Slovenia. Population Number and Structure. Available online: <https://www.stat.si/statweb/en/Field/Index/17/104> (accessed on 8 November 2024).
39. Andersen, N.H.; Christiansen, J.A.; la Cour, K.; Aagesen, M.; Tang, L.H.; Joergensen, D.S.; Pilegaard, M.S. Differences in functioning between young adults with cancer and older age groups: A cross-sectional study. *Eur. J. Cancer Care* **2022**, *31*, e13660. [CrossRef] [PubMed]
40. Wickham, H.; Averick, M.; Bryan, J.; Chang, W.; D’L.; McGowan, A.; François, R.; Grolemund, G.; Hayes, A.; Henry, L.; et al. Welcome to the Tidyverse. *J. Open Source Softw.* **2019**, *4*, 1686. [CrossRef]
41. Firke, S. janitor: Simple Tools for Examining and Cleaning Dirty Data [R Package Janitor Version 2.2.0]. Available online: <https://cran.r-project.org/web/packages/janitor/janitor.pdf> (accessed on 19 July 2024).
42. Heinzen, E.; Sinnwell, J.; Atkinson, E.; Gunderson, T.; Dougherty, G.; Votruba, P.; Lennon, R.; Lundt, E.; Broderick, B.; McCullough, M. *An Arsenal of “R” Functions for Large-Scale Statistical Summaries* [R Package Arsenal Version 3.6.3]; Comprehensive R Archive Network (CRAN): Online, 2021.
43. Patil, I. Visualizations with statistical details: The “ggstatsplot” approach. *J. Open Source Softw.* **2021**, *6*, 3167. [CrossRef]
44. Bates, D.; Mächler, M.; Bolker, B.M.; Walker, S.C. Fitting Linear Mixed-Effects Models Using lme4. *J. Stat. Softw.* **2015**, *67*, 1–48. [CrossRef]
45. World Health Organization. Body Mass Index—BMI. Available online: <https://www.who.int/data/gho/data/themes/topics/topic-details/GHO/body-mass-index> (accessed on 30 September 2022).
46. Di Cesare, M.; Bentham, J.; Stevens, G.A.; Zhou, B.; Danaei, G.; Lu, Y.; Bixby, H.; Cowan, M.J.; Riley, L.M.; Hajifathalian, K.; et al. Trends in adult body-mass index in 200 countries from 1975 to 2014: A pooled analysis of 1698 population-based measurement studies with 19.2 million participants. *Lancet* **2016**, *387*, 1377–1396. [CrossRef]
47. Pineda, E.; Sanchez-Romero, L.M.; Brown, M.; Jaccard, A.; Jewell, J.; Galea, G.; Webber, L.; Breda, J. Forecasting Future Trends in Obesity across Europe: The Value of Improving Surveillance. *Obes. Facts* **2018**, *11*, 360–371. [CrossRef]
48. Stival, C.; Lugo, A.; Odone, A.; Van Den Brandt, P.A.; Fernandez, E.; Tigova, O.; Soriano, J.B.; José López, M.; Scaglioni, S.; Gallus, S. Prevalence and Correlates of Overweight and Obesity in 12 European Countries in 2017–2018. *Obes. Facts* **2022**, *15*, 655–665. [CrossRef]
49. Marques, A.; Peralta, M.; Naia, A.; Loureiro, N.; De Matos, M.G. Prevalence of adult overweight and obesity in 20 European countries, 2014. *Eur. J. Public Health* **2018**, *28*, 295–300. [CrossRef] [PubMed]
50. Fenton, A. Weight, Shape, and Body Composition Changes at Menopause. *J. Midlife Health* **2021**, *12*, 187–192. [CrossRef]
51. Karvonen-Gutierrez, C.; Kim, C. Association of Mid-Life Changes in Body Size, Body Composition and Obesity Status with the Menopausal Transition. *Healthcare* **2016**, *4*, 42. [CrossRef] [PubMed]
52. Jakše, B.; Jakše, B.; Pinter, S.; Pajek, J.; Fidler Mis, N. Whole-Food Plant-Based Lifestyle Program and Decreased Obesity. *Am. J. Lifestyle Med.* **2022**, *16*, 260–270. [CrossRef] [PubMed]
53. Gregorič, M.; Blaznik, U.; Fajdiga Turk, V.; Delfar, N.; Korošec, A.; Lavtar, D.; Zaletel, M.; Koroušić Seljak, B.; Golja, P.; Zdešar Kotnik, K.; et al. Various Aspects of the Diet of the Inhabitants of Slovenia: Aged from 3 Months to 74 Years. Available online: https://nijz.si/wp-content/uploads/2022/07/razlicni_vidiki_prehranjevanja_prebivalcev_slovenije.pdf (accessed on 6 May 2020).
54. Gallus, S.; Lugo, A.; Murisic, B.; Bosetti, C.; Boffetta, P.; La Vecchia, C. Overweight and obesity in 16 European countries. *Eur. J. Nutr.* **2015**, *54*, 679–689. [CrossRef]
55. Muscogiuri, G.; Verde, L.; Colao, A. Body Mass Index (BMI): Still be used? *Eur. J. Intern. Med.* **2023**, *117*, 50–51. [CrossRef]
56. Zhu, Q.; Huang, B.; Li, Q.; Huang, L.; Shu, W.; Xu, L.; Deng, Q.; Ye, Z.; Li, C.; Liu, P. Body mass index and waist-to-hip ratio misclassification of overweight and obesity in Chinese military personnel. *J. Physiol. Anthropol.* **2020**, *39*, 24. [CrossRef]
57. Hodge, J.M.; Shah, R.; McCullough, M.L.; Gapstur, S.M.; Patel, A.V. Validation of self-reported height and weight in a large, nationwide cohort of U.S. adults. *PLoS ONE* **2020**, *15*, e0231229. [CrossRef]
58. Böhm, A.; Heitmann, B.L. The use of bioelectrical impedance analysis for body composition in epidemiological studies. *Eur. J. Clin. Nutr.* **2013**, *67*, S79–S85. [CrossRef] [PubMed]
59. Yamada, Y.; Nishizawa, M.; Uchiyama, T.; Kasahara, Y.; Shindo, M.; Miyachi, M.; Tanaka, S. Developing and Validating an Age-Independent Equation Using Multi-Frequency Bioelectrical Impedance Analysis for Estimation of Appendicular Skeletal Muscle Mass and Establishing a Cutoff for Sarcopenia. *Int. J. Environ. Res. Public Health* **2017**, *14*, 809. [CrossRef] [PubMed]
60. Feng, Q.; Bešević, J.; Conroy, M.; Omiyale, W.; Lacey, B.; Allen, N. Comparison of body composition measures assessed by bioelectrical impedance analysis versus dual-energy X-ray absorptiometry in the United Kingdom Biobank. *Clin. Nutr. ESPEN* **2024**, *63*, 214–225. [CrossRef]
61. Achamrah, N.; Colange, G.; Delay, J.; Rimbart, A.; Folope, V.; Petit, A.; Grigioni, S.; Déchelotte, P.; Coëffier, M. Comparison of body composition assessment by DXA and BIA according to the body mass index: A retrospective study on 3655 measures. *PLoS ONE* **2018**, *13*, e0200465. [CrossRef]
62. Branco, M.G.; Mateus, C.; Capelas, M.L.; Pimenta, N.; Santos, T.; Mäkitie, A.; Ganhão-Arranhado, S.; Trabulo, C.; Ravasco, P. Bioelectrical Impedance Analysis (BIA) for the Assessment of Body Composition in Oncology: A Scoping Review. *Nutrients* **2023**, *15*, 4792. [CrossRef]

63. Wang, W.; Feng, Y.; Long, Q.; Chen, F.; Chen, Y.; Ma, M.; Mao, S. A comparative analysis of body composition assessment by BIA and DXA in children with type II and III spinal muscular atrophy. *Front. Neurol.* **2022**, *13*, 1034894. [CrossRef] [PubMed]
64. Murata, H.; Yagi, T.; Midorikawa, T.; Torii, S.; Takai, E.; Taguchi, M. Comparison between DXA and MRI for the Visceral Fat Assessment in Athletes. *Int. J. Sports Med.* **2022**, *43*, 625. [CrossRef]
65. Mouchti, S.; Orliacq, J.; Reeves, G.; Chen, Z. Assessment of correlation between conventional anthropometric and imaging-derived measures of body fat composition: A systematic literature review and meta-analysis of observational studies. *BMC Med. Imaging* **2023**, *23*, 127. [CrossRef] [PubMed]
66. Dramé, M.; Godaert, L. The Obesity Paradox and Mortality in Older Adults: A Systematic Review. *Nutrients* **2023**, *15*, 1780. [CrossRef] [PubMed]
67. Jayanama, K.; Theou, O.; Godin, J.; Mayo, A.; Cahill, L.; Rockwood, K. Relationship of body mass index with frailty and all-cause mortality among middle-aged and older adults. *BMC Med.* **2022**, *20*, 404. [CrossRef] [PubMed]
68. Jia, H.; Lubetkin, E.I. Association between self-reported body mass index and active life expectancy in a large community-dwelling sample of older U.S. adults. *BMC Geriatr.* **2022**, *22*, 310. [CrossRef] [PubMed]
69. Bosello, O.; Vanzo, A. Obesity paradox and aging. *Eat. Weight Disord.* **2021**, *26*, 27–35. [CrossRef] [PubMed]
70. Javed, A.A.; Aljied, R.; Allison, D.J.; Anderson, L.N.; Ma, J.; Raina, P. Body mass index and all-cause mortality in older adults: A scoping review of observational studies. *Obes. Rev.* **2020**, *21*, e13035. [CrossRef] [PubMed]
71. Marino, M.; Puppo, F.; Del Bo', C.; Vinelli, V.; Riso, P.; Porrini, M.; Martini, D. A Systematic Review of Worldwide Consumption of Ultra-Processed Foods: Findings and Criticisms. *Nutrients* **2021**, *13*, 2778. [CrossRef] [PubMed]
72. Ler, P.; Ploner, A.; Finkel, D.; Reynolds, C.A.; Zhan, Y.; Jylhävä, J.; Dahl Aslan, A.K.; Karlsson, I.K. Interplay of body mass index and metabolic syndrome: Association with physiological age from midlife to late-life. *GeroScience* **2024**, *46*, 2605–2617. [CrossRef] [PubMed]
73. Woolley, C.; Thompson, C.; Hakendorf, P.; Horwood, C. The Effect of Age upon the Interrelationship of BMI and Inpatient Health Outcomes. *J. Nutr. Health Aging* **2019**, *23*, 558–563. [CrossRef] [PubMed]
74. Žlender, V.; Gemin, S. Different Environments and Physical Activity before and during the COVID-19 Lockdown: Data from Slovenia. *Land* **2023**, *12*, 282. [CrossRef]
75. Nour, T.Y.; Altıntaş, K.H. Effect of the COVID-19 pandemic on obesity and its risk factors: A systematic review. *BMC Public Health* **2023**, *23*, 1018. [CrossRef]
76. Strain, T.; Flaxman, S.; Guthold, R.; Semenova, E.; Cowan, M.; Riley, L.M.; Bull, F.C.; Stevens, G.A.; Raheem, R.A.; Agoudavi, K.; et al. National, regional, and global trends in insufficient physical activity among adults from 2000 to 2022: A pooled analysis of 507 population-based surveys with 5–7 million participants. *Lancet Glob. Health* **2024**, *12*, e1232–e1243. [CrossRef]
77. Cheng, C.C.; Hsu, C.Y.; Liu, J.F. Effects of dietary and exercise intervention on weight loss and body composition in obese postmenopausal women: A systematic review and meta-analysis. *Menopause* **2018**, *25*, 772–782. [CrossRef] [PubMed]
78. Olateju, I.V.; Opaleye-Enakhimion, T.; Udeogu, J.E.; Asuquo, J.; Olaleye, K.T.; Osa, E.; Oladunjoye, A.F. A systematic review on the effectiveness of diet and exercise in the management of obesity. *Diabetes Metab. Syndr.* **2023**, *17*, 102759. [CrossRef] [PubMed]
79. Johns, D.J.; Hartmann-Boyce, J.; Jebb, S.A.; Aveyard, P. Diet or Exercise Interventions vs Combined Behavioral Weight Management Programs: A Systematic Review and Meta-Analysis of Direct Comparisons. *J. Acad. Nutr. Diet.* **2014**, *114*, 1557–1568. [CrossRef] [PubMed]
80. Cava, E.; Yeat, N.C.; Mittendorfer, B. Preserving Healthy Muscle during Weight Loss. *Adv. Nutr.* **2017**, *8*, 511–519. [CrossRef] [PubMed]
81. McCarthy, D.; Berg, A. Weight Loss Strategies and the Risk of Skeletal Muscle Mass Loss. *Nutrients* **2021**, *13*, 2473. [CrossRef] [PubMed]
82. Aryannezhad, S.; Mok, A.; Imamura, F.; Wareham, N.J.; Brage, S.; Forouhi, N.G. Combined associations of physical activity, diet quality and their changes over time with mortality: Findings from the EPIC-Norfolk study, United Kingdom. *BMC Med.* **2024**, *22*, 464. [CrossRef]
83. Hershey, M.S.; Martínez-González, M.Á.; Álvarez-Álvarez, I.; Martínez Hernández, J.A.; Ruiz-Canela, M. The Mediterranean diet and physical activity: Better together than apart for the prevention of premature mortality. *Br. J. Nutr.* **2022**, *128*, 1413–1424. [CrossRef] [PubMed]
84. Lavriša, Ž.; Hristov, H.; Hribar, M.; Koroušić Seljak, B.; Gregorič, M.; Blaznik, U.; Zaletel, K.; Oblak, A.; Osredkar, J.; Kušar, A.; et al. Dietary Iron Intake and Biomarkers of Iron Status in Slovenian Population: Results of SI.Menu/Nutrihealth Study. *Nutrients* **2022**, *14*, 5144. [CrossRef] [PubMed]
85. Gregorič, M.; Hristov, H.; Blaznik, U.; Koroušić Seljak, B.; Delfar, N.; Pravst, I. Dietary Intakes of Slovenian Adults and Elderly: Design and Results of the National Dietary Study SI.Menu 2017/18. *Nutrients* **2022**, *14*, 3618. [CrossRef] [PubMed]
86. Oberritter, H.; Schäbethal, K.; Von Ruesten, A.; Boeing, H. Der DGE-Ernährungskreis—Darstellung und Basis der lebensmittelbezogenen Empfehlungen der DGE. *Ernähr. Umsch.* **2013**, *60*, 24–29. [CrossRef]
87. Willett, W.; Rockström, J.; Loken, B.; Springmann, M.; Lang, T.; Vermeulen, S.; Garnett, T.; Tilman, D.; DeClerck, F.; Wood, A.; et al. Food in the Anthropocene: The EAT–Lancet Commission on healthy diets from sustainable food systems. *Lancet* **2019**, *393*, 447–492. [CrossRef]
88. Kamin, T.; Vezovnik, A.; Bolko, I. Changing Our Food Habits One Bite at a Time: Exploring Young Flexitarians in a Country with a High Meat Intake. *Foods* **2024**, *13*, 3215. [CrossRef]

89. Kirbiš, A.; Lamot, M.; Javornik, M. The Role of Education in Sustainable Dietary Patterns in Slovenia. *Sustainability* **2021**, *13*, 13036. [CrossRef]
90. Kirbiš, A.; Korže, V.; Lubej, M. Predictors of Meat Reduction: The Case of Slovenia. *Foods* **2024**, *13*, 2346. [CrossRef] [PubMed]
91. National Institute of Public Health of Slovenia. 3 Determinante Zdravja—Dejavniki Tveganja. Telesna Dejavnost. Available online: https://www.nijz.si/sites/www.nijz.si/files/uploaded/publikacije/letopisi/2017/3.3_telesna_dejavnost_2017_dv4_koncna_4.pdf (accessed on 24 April 2021).
92. Marija, S.; Dragan, V.; Svetlana, R.; Nela, D. Socioeconomic inequalities in overweight and obesity in Serbia: Data from 2013 national health survey. *Front. Pharmacol.* **2018**, *8*, 967. [CrossRef] [PubMed]
93. Magkos, F.; Sørensen, T.I.A.; Raubenheimer, D.; Dhurandhar, N.V.; Loos, R.J.F.; Bosy-Westphal, A.; Clemmensen, C.; Hjorth, M.F.; Allison, D.B.; Taubes, G.; et al. On the pathogenesis of obesity: Causal models and missing pieces of the puzzle. *Nat. Metab.* **2024**, *6*, 1856–1865. [CrossRef] [PubMed]
94. Peeters, A. Obesity and the future of food policies that promote healthy diets. *Nat. Rev. Endocrinol.* **2018**, *14*, 430–437. [CrossRef] [PubMed]
95. Ikram, M.; Hill, K.A.; Williams, K.A. Marketing Cardiovascular Mortality? Healthy vs. Unhealthy Food in Television Advertising. *Int. J. Dis. Reversal Prev.* **2021**, *3*, 97–103. [CrossRef]
96. Dorling, J.; Broom, D.R.; Burns, S.F.; Clayton, D.J.; Deighton, K.; James, L.J.; King, J.A.; Miyashita, M.; Thackray, A.E.; Batterham, R.L.; et al. Acute and Chronic Effects of Exercise on Appetite, Energy Intake, and Appetite-Related Hormones: The Modulating Effect of Adiposity, Sex, and Habitual Physical Activity. *Nutrients* **2018**, *10*, 1140. [CrossRef]
97. Mozaffarian, D.; Angell, S.Y.; Lang, T.; Rivera, J.A. Role of government policy in nutrition—Barriers to and opportunities for healthier eating. *BMJ* **2018**, *361*, k2426. [CrossRef] [PubMed]
98. Mcleod, J.C.; Currier, B.S.; Lowisz, C.V.; Phillips, S.M. The influence of resistance exercise training prescription variables on skeletal muscle mass, strength, and physical function in healthy adults: An umbrella review. *J. Sport Health Sci.* **2024**, *13*, 47–60. [CrossRef] [PubMed]
99. Bellicha, A.; van Baak, M.A.; Battista, F.; Beaulieu, K.; Blundell, J.E.; Busetto, L.; Carraça, E.V.; Dicker, D.; Encantado, J.; Ermolao, A.; et al. Effect of exercise training on weight loss, body composition changes, and weight maintenance in adults with overweight or obesity: An overview of 12 systematic reviews and 149 studies. *Obes. Rev.* **2021**, *22*, e13256. [CrossRef]
100. Jakše, B.; Jakše, B.; Godnov, U.; Pinter, S. Ongoing Community-Based Whole-Food, Plant-Based Lifestyle Effectively Preserves Muscle Mass during Body Mass Loss. *Obesities* **2022**, *2*, 157–170. [CrossRef]
101. Liu, J.; Shen, Q.; Wang, X. Emerging EAT-Lancet planetary health diet is associated with major cardiovascular diseases and all-cause mortality: A global systematic review and meta-analysis. *Clin. Nutr.* **2024**, *43*, 167–179. [CrossRef]
102. Mind Trade, d.o.o. Reference TANITA. Available online: <https://www.tanita.si/reference> (accessed on 4 October 2020).
103. Ballesteros-Pomar, M.D.; González-Arnáiz, E.; Pintor-de-la Maza, B.; Barajas-Galindo, D.; Ariadel-Cobo, D.; González-Roza, L.; Cano-Rodríguez, I. Bioelectrical impedance analysis as an alternative to dual-energy x-ray absorptiometry in the assessment of fat mass and appendicular lean mass in patients with obesity. *Nutrition* **2022**, *93*, 111442. [CrossRef]
104. Liu, W.T.; Hu, W.P.; Lai, C.L.; Chen, Y.Y.; Hsieh, K.C. Validation of three bioelectrical impedance analysis for the assessment of appendicular lean tissue in older community-dwelling adults. *Int. J. Gerontol.* **2021**, *15*, 377–381. [CrossRef]
105. Verney, J.; Schwartz, C.; Amiche, S.; Pereira, B.; Thivel, D. Comparisons of a Multi-Frequency Bioelectrical Impedance Analysis to the Dual-Energy X-Ray Absorptiometry Scan in Healthy Young Adults Depending on their Physical Activity Level. *J. Hum. Kinet.* **2015**, *47*, 73–80. [CrossRef]
106. Miller, R.M.; Chambers, T.L.; Burns, S.P. Validating InBody® 570 multi-frequency bioelectrical impedance analyzer versus DXA for body fat percentage analysis. *J. Exerc. Physiol. Online* **2016**, *19*, 71–79. [CrossRef]
107. Faria, S.L.; Faria, O.P.; Cardeal, M.D.A.; Ito, M.K. Validation study of multi-frequency bioelectrical impedance with dual-energy X-ray absorptiometry among obese patients. *Obes. Surg.* **2014**, *24*, 1476–1480. [CrossRef] [PubMed]
108. Wang, J.; Song, A.; Tang, M.; Xiang, Y.; Zhou, Y.; Chen, Z.; Heber, D.; Tang, Q.; Xu, R. The applicability of a commercial 3DO body scanner in measuring body composition in Chinese adults with overweight and obesity: A secondary analysis based on a weight-loss clinical trial. *J. Int. Soc. Sports Nutr.* **2024**, *21*, 2307963. [CrossRef] [PubMed]

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Article

Application of Bioelectrical Impedance Analysis in Weight Management of Children with Spina Bifida

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Abstract: Background: Children with spina bifida (SB) face an elevated risk of obesity, which necessitates precise methods for assessing body composition to ensure effective weight management. Conventional measures like BMI are inadequate for this population because of variations in growth patterns and skeletal structure. Bioelectrical impedance analysis (BIA) is a method that offers a clearer picture of body composition, yet its use in children with SB remains underexplored. Methods: Conducted on 57 children with SB and 28 healthy controls, with a median age of 11 years, this study evaluated anthropometrics, including BMI and BIA-derived metrics. The Hoffer's scale to assess physical activity was applied in SB children. Results: Results showed that while 32% of SB patients were classified as overweight or obese based on BMI, 62% exhibited high body fat percentage via BIA. Fat-free mass, muscle and fat mass, and fat-to-muscle ratio (FMR) differed significantly compared to the reference group. Non-ambulators showed a higher median body fat mass percentage (25.9% vs. 17.8%, $p = 0.01$) and FMR (0.92 vs. 0.44, $p = 0.003$) in comparison to the community walkers. Conclusions: In SB children, BIA-measured fat mass is a better obesity indicator than BMI. Non-ambulatory, SB patients with obesity had the highest FMR values, indicating a higher risk for metabolic syndrome.

Keywords: weight management; spina bifida; body mass index; bioelectrical impedance analysis

1. Introduction

Congenital spina bifida (SB) is a neural tube defect that arises when the spine and spinal cord fail to develop properly during the early stages of pregnancy. This condition can lead to varying degrees of physical and neurological impairments, affecting mobility. Beyond these motor function impairments, children with SB are prone to various health problems affecting their neurological and musculoskeletal systems [1]. Accurate evaluation of body composition parameters is crucial for monitoring children with SB, as body composition changes may further complicate their health, increasing the risk of obesity, cardiovascular diseases, and metabolic disorders [2].

Common anthropometric measurements, such as body mass index (BMI) or weight-for-height ratios, are frequently utilized to estimate body fat and nutritional status in pediatrics. Due to altered growth patterns and body posture in children with SB, these metrics are poor predictors of body fat and have limited utility in guiding nutritional interventions. The presence of scoliosis or joint contractures often complicates obtaining precise height measurements, thereby diminishing the accuracy of BMI. Moreover, BMI fails to distinguish between lean and fat mass, further limiting its effectiveness [3]. While all methods for assessing body composition have inherent limitations, dual-energy X-ray absorptiometry (DXA) is widely regarded as the gold standard for body composition measurement. However, DXA has its own constraints, including its lack of portability, high cost, and the low-level radiation exposure involved. In contrast, bioelectrical impedance analysis (BIA) is relatively simple, fast, and non-invasive, and unlike DXA, it involves no

radiation exposure. This makes BIA particularly suitable for repeated measurements over time when tracking changes in body composition is required. It has been widely used in various clinical fields, offering high accuracy and efficiency at a relatively low cost [4–6]. Studies have shown that BIA is particularly useful in assessing body composition in infants and young children, providing important insights into early childhood development and growth. In pediatric populations with specific health concerns, such as obesity, BIA helps clinicians better understand fat distribution and muscle mass, offering more detailed information than BMI alone. This has led to a more refined approach in managing childhood obesity, especially in cases where traditional methods may overlook nuances like hydration status, as noted in recent findings [7,8]. BIA devices measure the resistance of body tissues to a small, safe electrical signal, allowing for the estimation of, for example, fat-free mass, body fat, and muscle mass. Despite widespread use of BIA, changes in body composition in children with SB are not well characterized. This gap in knowledge highlights the need for further research.

This study aims to investigate body composition in pediatric patients with SB, with a focus on the limitations of traditional BMI measurements and the potential benefits of alternative method: BIA. The specific objectives are to

1. Assess the prevalence of obesity in children with SB using both BMI and BIA.
2. Explore the limitations of BMI in accurately reflecting the total body mass of SB patients.
3. Evaluate the effectiveness of BIA as a more accurate, non-invasive method for assessing body composition in children with SB.
4. Compare the body composition of pediatric patients with SB to the reference group.

2. Materials and Methods

2.1. Patients

Cross-sectional data from a prospective cohort study were analyzed. This study involved 85 participants: 57 children diagnosed with congenital SB and 28 healthy volunteers who visited a pediatrician for balance tests aged 1–18 years and were without any known chronic health conditions. We measured body composition using two indirect methods, anthropometry and BIA, correlating these measurements with BMI to evaluate the concordance between BMI and fat mass in children with SB. The lesion level of SB patients was reported intraoperatively and radiologically and scored from 1 to 3 (1—thoracolumbar, 2—lumbosacral, and 3—sacral lesion). The ambulatory function of the SB patients was classified using the Hoffer’s scale (HS) into the categories of non-ambulators, exercise walkers, household walkers, and community walkers [9]. Exercise walkers, who walked only during therapy, and household walkers, who used braces or crutches for indoors and wheelchair outdoors, were grouped with non-ambulators, who were entirely wheelchair-dependent, due to their similarly low physical activity in daily life.

2.2. Anthropometrics

The weight and height of each participant were recorded. For participants with SB who depended on a wheelchair, weight was measured using an electronic scale. The participant, seated in the wheelchair, was wheeled onto the scale, and the weight of the wheelchair was subtracted from the total to determine the individual’s weight. Height for these participants was measured in a lying-down position. Body surface area (BSA) was calculated with the Dubois formula: $BSA = \text{height}^{0.725} \times \text{weight}^{0.425} \times 0.007184$.

BMI was calculated using the standard formula: the body mass divided by the square of the body height, expressed in kg/m^2 . The corresponding BMI-for-age percentiles based on Centers for Disease Control and Prevention growth charts for children ages 2–19 years were obtained [10]. BMI percentile categories were as follows: underweight < 5th percentile, normal weight from 5th–85th percentiles, overweight from 85th–95th percentiles, and obesity equal to or greater than the 95th percentile. Additionally, we converted BMI to BMI standard deviation scores (Z-BMI) and classified the results according to the World Health Organization age- and gender-specific growth standards [11]. Underweight was defined as

a BMI z-score < -2 standard deviations (SD), normal weight as a z-score between -2 SD and $+1$ SD, overweight as a z-score $> +1$ SD, and obesity as a z-score $> +2$ SD.

2.3. BIA

Whole-body BIA measurements were performed using the BioScan 916S device (Maltron International, Essex, UK) according to the manufacturer's instructions. This device delivers a current of 0.7 mA with an impedance of 100 to 1000 Ω , precision of $\pm 3 \Omega$, and a frequency of 50 KHz. The measurement was applied on the right side of the body with the participant lying supine. Body composition measurements of fat-free mass (FFM, kg and %), muscle mass (kg and %), total body water (TBW, lt and %), intracellular water (ICW, lt and %), extracellular water (ECW, lt and %), protein mass (kg), mineral mass (kg), total body calcium (g), and total body potassium (g) were obtained from BIA data, resistance, and reactance. The BIA-derived body fat percentage was applied to body fat percentile reference curves for children aged 3 to 16 years [12]. Due to these age limitations, the reference curves did not encompass the entire study sample, resulting in the absence of fat mass percentiles for 4 children younger than 3 years of age. For participants older than 16 years of age, we used the adults' cut-offs for high body fat percentage proposed by Macek et al. [13], established as 25.8% for men and 37.1% for women. We used 3 body fat mass categories, as follows: low < 10 th percentile, normal between 10th–90th, and overweight and obese above 90th and 97th body fat percentiles, respectively. The fat-to-muscle ratio (FMR) was determined by dividing body fat mass (kg) by muscle mass (kg) [14].

2.4. Statistics

The data were collected in a Microsoft Excel database. Statistical analysis was performed using Statistica 13.0. (StatSoft Inc., Tulsa, OK, USA). Continuous variables were expressed as the median and range, unless stated otherwise. All studied parameters were analyzed using nonparametric tests: Mann–Whitney, Kruskal–Wallis, and Chi2 analysis. Correlations were assessed with the Spearman test. Values of $p < 0.05$ were considered significant.

2.5. Ethical Issues

This study was approved by the Ethics Committee of the Medical University of Bialystok, which complies with the World Medical Association Declaration of Helsinki regarding the ethical conduct of research involving human subjects and/or animals. Patients and their caregivers were enrolled into the study after obtaining informed consent.

3. Results

The cohort consisted of 47/85 (55%) girls and 38/85 (45%) boys, with a median age of 11 years (2–18 years). There was no statistically significant difference between SB children and healthy control groups as regards to age, gender, body weight, BMI, and BSA except height measurements. This was due to the shorter vertebral dimension and malformations of the bone structure due to SB [15]. In the SB patient group, FFM, muscle mass, percentage of fat mass, FMR, TBW, ICW, protein, and mineral mass, including total body potassium, had significant changes when compared to the control group ($p < 0.05$). Statistically significant positive correlations were recorded between mineral mass and BMI ($r = 0.571$), fat mass in kg ($r = 0.509$), muscle mass in kg ($r = 0.89$) and % ($r = 0.62$), and total body calcium ($r = 0.861$). Table 1 shows the anthropometric and bioimpedance characteristics of both studied groups.

Table 1. Anthropometric and bioimpedance traits for participants.

Variables	SB Patients <i>n</i> = 57	Reference Group <i>n</i> = 28	<i>p</i> Value
Anthropometric parameters (median, min.–max.)			
Height (cm)	132 (70–174)	154 (108–181)	0.02 *
Weight (kg)	33 (8.5–95)	42.6 (17.5–77.5)	0.19
BMI (kg/m ²)	17.8 (10.4–35.3)	17.9 (13.1–23.6)	0.68
BMI (centiles)	52.6 (0–100)	52.7 (1.6–76.7)	0.79
Z-BMI	0.06 (−8–3.5)	0.06 (−2.1–0.7)	0.85
BSA (m ²)	1.1 (0.3–2)	1.36 (0.72–2)	0.1
BIA and body composition (median, min.–max.)			
FFM (kg)	22.3 (6.7–50.7)	35.6 (14.2–64.8)	0.03 *
(a) body cell mass (kg)	11.4 (2.6–32.5)	18.4 (6.5–33.7)	0.04 *
(b) extracellular solids (kg)	10.3 (4.1–25.9)	15.7 (7.5–31.1)	<0.001 *
FFM (%)	76.7 (47.6–96.9)	80.9 (69–95.9)	0.01 *
Fat mass (kg)	8 (0.69–45.6)	5.9 (1.4–17.6)	0.65
Fat mass (%)	23.7 (3–52.4)	19.1 (4–30.9)	<0.001 *
Fat mass (percentiles)	50 (3–97)	25 (3–75)	<0.001 *
Muscle mass (kg)	8.8 (1.9–25.4)	15.1 (4.8–32.3)	0.04 *
Muscle mass (%)	29.2 (21.2–42)	32.5 (27–41.7)	<0.001 *
FMR	0.81 (0.1–2.4)	0.55 (0.1–1.03)	0.002 *
TBW (lt)	17.9 (5.3–42.6)	25.6 (10.9–52.2)	0.04 *
(a) ICW (lt)	9.4 (2.1–28.9)	15.1 (5.9–27.3)	0.02 *
(b) ECW (lt)	7.4 (3.2–18.4)	10.2 (4.7–24.8)	0.12
TBW (%)	57.3 (39.1–79.7)	59.7 (48–82)	0.22
(a) ICW (%)	55.7 (21.3–69.5)	57.9 (52.4–66.4)	0.07
(b) ECW (%)	44.5 (30.5–78.7)	42.1 (33.6–47.6)	0.06
Protein mass (kg)	3.2 (0.91–13.5)	6.2 (2.3–16.6)	<0.001 *
Mineral mass (kg)	1.2 (0.31–4.7)	2.4 (0.9–5.8)	<0.001 *
Total body calcium (g)	363 (107–1116)	598 (231–1220)	0.06
Total body potassium (g)	54.3 (12.5–154.4)	85 (30.6–160.7)	0.04 *

SB, spina bifida; BMI, body mass index; Z-BMI, body mass index z-score; BSA, body surface area; FFM, fat-free mass; FMR, fat-to-muscle ratio; TBW, total body water; ICW, intracellular body water; ECW, extracellular body water; * *p* < 0.05.

3.1. The Agreement between BMI and Body Fat Percentage

The median BMI was similar in both groups (17.8 kg/m² in SB vs. 17.9 kg/m² in the reference group), but the median body fat mass percentage differed significantly (23.7% in SB vs. 19.1% in the reference group, *p* < 0.001). According to BMI measurements, 29 out of 53 (55%) of the children with SB were categorized as “unhealthy”, having either excessively high or low BMI. When evaluated by fat mass percentage, 41 out of 53 (77%) were found to have abnormal fat mass percentages, either excessively high or low. Although 18 out of 57 (32%) SB patients were classified as overweight or obese based on traditional BMI criteria, 30 out of 53 (62%) exhibited body fat percentages, measured via BIA, exceeding the 90th percentile. Table 2 illustrates the concordance between BMI categories and body fat percentage categories in both studied groups, detailing the number and percentage of children falling into each BMI and fat mass category.

Table 2. Agreement between BMI and fat mass categories in both studied groups.

Body Fat Mass Category				
<i>n</i> (%)	Low <10 pc	Normal 10–90 pc	High >90 pc	Total
SB patients				
BMI category				
underweight < 5 pc	3 (6)	5 (9)	3 (6)	11 (21)
normal weight 5–85 pc	7 (13)	5 (9)	12 (23)	24 (45)
overweight and obese > 85 pc	1 (2)	2(4)	15 (28)	18 (34)
Total	11 (21)	12 (23)	30 (56)	53 (100)
Reference group				
BMI category				
underweight < 5 pc	0	2 (7)	0	2 (7)
normal weight 5–85 pc	8 (29)	18 (64)	0	26 (93)
overweight and obese > 85 pc	0	0	0	0
Total	8 (29)	20 (71)	0	28 (100)

The body fat mass in SB patients exhibited significant variability, even among children with comparable BMIs, demonstrating considerable inter-individual differences. There was a significant positive correlation between BMI percentiles and fat mass in kg and % ($r = 0.625$, $r = 0.584$; respectively) in the SB group.

3.2. Body Composition and Daily Physical Activity in SB Children

The majority of SB patients, 34/57 (60%), had lumbosacral spinal lesions, 14/57 (25%) thoracolumbar, and 9/57 (15%) sacral lesions. Among all the study subjects, 28/57 (49%) were classified as non-ambulators, 5/57 (9%) as exercise walkers, 11/57 (19%) as household walkers, and 13/57 (23%) as community walkers. When comparing anthropometric parameters between non-ambulatory and ambulatory SB children, no differences were found concerning age, height, weight, and BMI ($p = 0.96$, $p = 0.49$, $p = 0.89$, $p = 0.21$ respectively, $p < 0.05$), but they significantly differed with BIA parameters. Non-ambulators showed a higher median body fat mass percentage in body composition in comparison to the community walkers (25.9% vs. 17.8%, $p = 0.01$). Consequently, they had statistically significantly higher median values of FMR (0.92 vs. 0.44, $p = 0.003$). Regarding constituents of FFM, differences were observed in the percentage of muscle mass and TBW. As expected, non-ambulators had a lower median muscle mass (8.6 kg vs. 12.4 kg, $p = 0.57$) and percentage (28.2% vs. 32%, $p < 0.001$), as well as lower TBW in Lt and % (17.4 Lt vs. 22.7 Lt, $p = 0.64$; 56% vs. 60%, $p = 0.03$), than the community walkers. More details are presented in Figure 1.

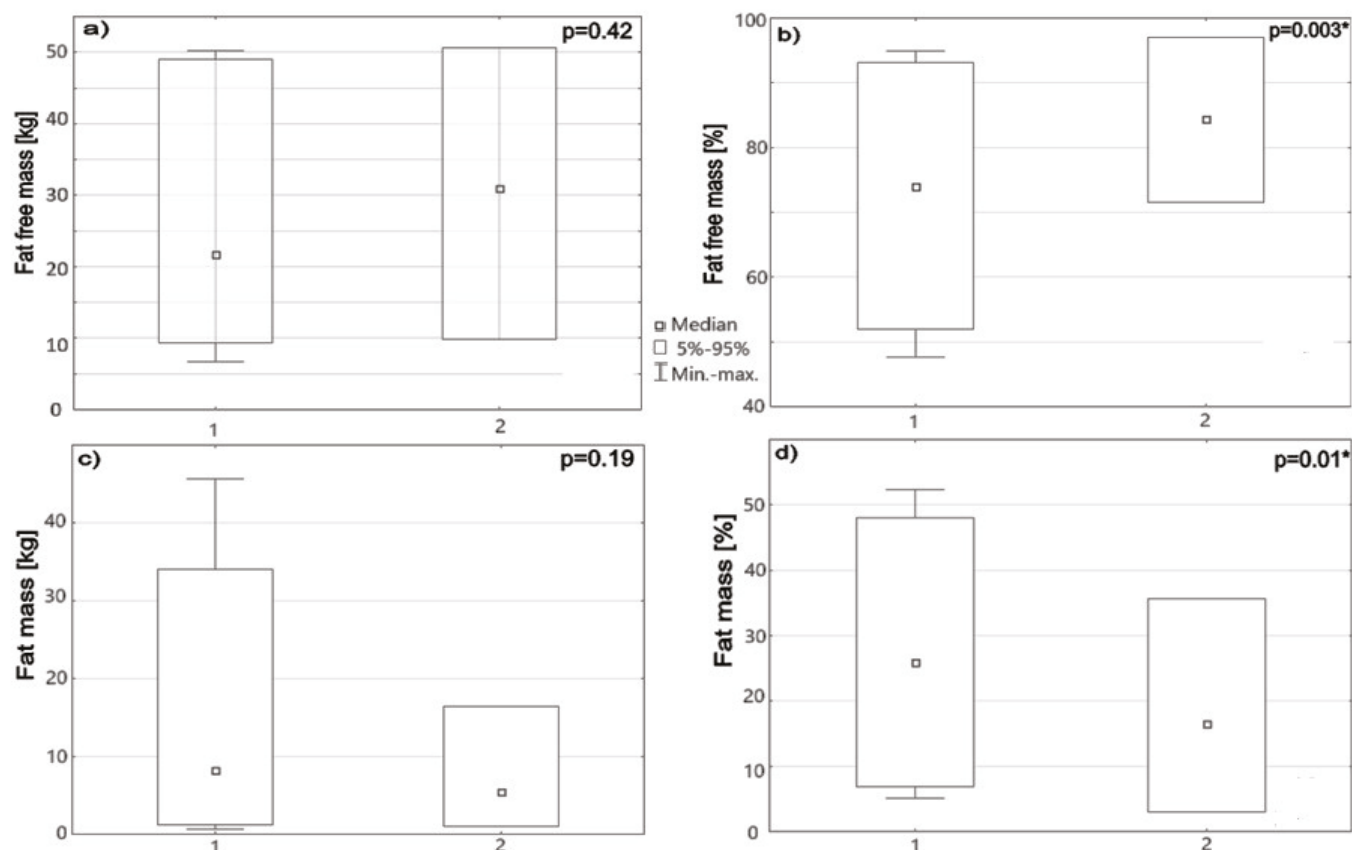


Figure 1. Box -whisker bars show (a) FFM (kg), (b) FFM (%), (c) fat mass (kg), and (d) fat mass (%) in SB patients divided into 2 groups: 1—low physical activity, 2—community walkers. * $p < 0.05$.

4. Discussion

The global rise in childhood obesity has been described as a pandemic and is considered one of the most pressing health issues of the twenty-first century. Focusing on children and adolescents is crucial, because those who become overweight or obese early in life are about five times more likely to remain so into adulthood [16]. Children with disabilities, including SB, are in a high-risk group, being two to three times more likely to become overweight or obese than their typically developing peers [17]. Hence, effective weight management is essential for providing high-quality health care to SB children.

Several studies have attempted to determine the obesity rates among children with SB, finding that its prevalence ranges from 18% to 50% [18–20]. A major concern is that these studies define obesity in children based on a certain BMI percentile for age and gender. However, using BMI in this patient population is controversial and there is currently no accepted standard to identify obesity [21]. First, height measurements in children with SB differ significantly from those in non-disabled children, which makes the use of BMI problematic. Factors such as lower limb and trunk hypoplasia, vertebral anomalies, and paralytic musculoskeletal deformities cause short stature, which was also observed in our study sample. Secondly, BMI does not directly measure body fat and its regional body distribution [3,22]. In our study, both cohorts exhibited median BMI percentiles within the normal range. However, traditional BMI criteria classified one-third of SB patients as overweight or obese. This proportion nearly doubled when body fat mass, assessed through BIA, was applied. Such discrepancies were not present in the reference group, where a strong concordance between BMI and fat mass categories was observed. The study by Liu J.S. et al. [23] expands the debate on the problems related to BMI as a marker of excess adiposity in SB. The investigation was conducted in small cohort of SB patients, including 18 adults, who underwent obesity classification using BMI by length and arm

span, abdominal girth, and percent trunk fat on DXA. Using BMI categories, 43.8% of subjects were classified as obese. However, alternative measures, such as anthropometric assessments focusing on body composition and fat distribution, revealed a higher obesity rate. Trunk fat percentage by DXA categorized 83.3% of subjects as obese. In our study, we utilized different method to assess body composition, BIA, yet the conclusion remains unchanged. Employing methods that more accurately evaluate true body composition in the SB population raises the number of individuals classified as obese. This suggests that a significant portion of subjects deemed to have a normal BMI by conventional standards may, in fact, be obese.

The life expectancy of individuals with SB has significantly improved, rendering it a life-long condition. From a clinical perspective, it is crucial for healthcare providers to monitor and manage long-term complications. Obesity has been identified as a primary factor contributing to the onset of metabolic syndrome. In a study analyzing 34 adolescents with SB, the prevalence of metabolic syndrome was found to be 32.4%, with a higher incidence observed among obese individuals [24]. In this paper, we applied the FMR, an alternative approach for assessing metabolic syndrome, which has been validated in adults. The higher the FMR levels, the greater risk of developing conditions like type 2 diabetes, cardiovascular diseases, and increased mortality. The recent study by Zhang JX et al. [25] showed a positive correlation between dyslipidemia and high FMR values in transitional-age youth. Our findings indicated that children with SB had higher FMRs than the reference group. Those who were non-ambulatory and obese had the highest FMR values. This suggests that these specific subgroups of SB patients are at a heightened risk for metabolic syndrome and its long-term health implications. While little is currently known about FMRs in children, our study aims to explore its potential use, though additional studies are needed to confirm our findings.

Both obesity and osteoporosis are complications associated with SB, putting these patients at risk of bone disease [26]. Our study found that children with SB had significantly lower mineral mass compared to healthy controls. Interestingly, we observed a positive correlation between mineral mass and BMI, fat mass, and muscle mass, suggesting that obesity might have a protective effect on mineral mass. This finding aligns with a study by Trinh A. et al. [27], which reported a positive correlation between fat mass and bone mineral density obtained by DXA in adult SB patients. They concluded that higher bone mineral density in those with a higher BMI could be due to increased lean tissue or fat mass. The main takeaway is that adults with SB are at high risk for both low bone density and obesity, along with related comorbidities. Our findings suggest that this process may begin as early as childhood.

The present study has several limitations. First, the sample size of 57 children with SB may be considered relatively small. However, it is important to acknowledge that SB is a rare congenital condition, and there is a global trend toward pregnancy termination following prenatal diagnosis, further reducing the available population for study. Multi-center collaborations would help mitigate that issue, enabling researchers to pool data from different regions. Second, as the data were obtained from a single, large tertiary referral center, the findings may not be fully generalizable and require external validation. Additionally, body composition was assessed using two indirect methods: anthropometry and BIA. While these methods are widely used, more direct methods, such as DXA and magnetic resonance imaging (MRI), exist. DXA is regarded as the gold standard for detailed body composition assessment; however, its high cost, limited availability, and exposure to radiation limit its practicality. MRI provides detailed imaging of soft tissues, including fat and muscle, but it also has limitations, especially in pediatric populations. MRI is expensive, time-consuming, and often restricted to specialized clinical or research environments, making it less feasible for use in large-scale studies. Moreover, younger children may struggle to remain still during the scan, leading to motion artifacts that compromise the accuracy of the measurements.

Future research could incorporate direct techniques to offer a more comprehensive understanding of body composition in children with SB. Emerging imaging technologies or novel non-invasive methods that address the drawbacks of DXA and MRI could also be explored. Despite being less precise, BIA is more practical for regular monitoring due to its simplicity and non-invasiveness, though its accuracy can be affected by factors such as hydration status and body composition. A key limitation of BIA is its tendency to overestimate body fat in obese individuals due to the reliance on electrical conductivity, as fat tissue has higher resistance to electrical currents. This can result in inflated body fat estimates in individuals with greater fat mass, though it tends to provide more reliable estimates in lean populations, such as the children studied in our research. BIA was selected for this study based on its practicality. Regular monitoring of body composition in conjunction with functional and metabolic outcomes may offer deeper insights into the unique growth patterns in children with SB, aiding in personalized clinical care and interventions.

5. Conclusions

In conclusion, this study emphasizes several key findings related to obesity and body composition in children with SB:

- (i) Childhood obesity is a significant global concern, and children with SB are at an even higher risk due to altered body composition and physical limitations.
- (ii) Traditional BMI measurements may underestimate obesity in SB patients, as they do not account for the unique body composition seen in this population, particularly the imbalance between muscle mass and fat tissue.
- (iii) BIA offers a non-invasive and more accurate method for assessing body composition in SB patients, revealing higher obesity rates compared to BMI alone.
- (iv) Obesity in SB patients is associated with an increased risk of metabolic syndrome and bone disease, emphasizing the need for early intervention and the use of precise assessment methods to manage these risks effectively.

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

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References

1. Rocque, B.G.; Hopson, B.D.; Blount, J.P. Caring for the child with spina bifida. *Pediatr. Clin. N. Am.* **2021**, *68*, 915–927. [CrossRef] [PubMed]
2. Gour-Provençal, G.; Costa, C. Metabolic syndrome in children with myelomeningocele and the role of physical activity: A narrative review of the literature. *Top. Spinal Cord. Inj. Rehabil.* **2022**, *28*, 15–40. [CrossRef] [PubMed]
3. Oliveros, E.; Somers, V.K.; Sochor, O.; Goel, K.; Lopez-Jimenez, F. The concept of normal weight obesity. *Prog. Cardiovasc. Dis.* **2014**, *56*, 426–433. [CrossRef] [PubMed]
4. Ward, L.C. Bioelectrical impedance analysis for body composition assessment: Reflections on accuracy, clinical utility, and standardisation. *Eur. J. Clin. Nutr.* **2019**, *73*, 194–199. [CrossRef] [PubMed]

5. Lyons-Reid, J.; Derraik, J.G.B.; Ward, L.C.; Tint, M.T.; Kenealy, T.; Cutfield, W.S. Bioelectrical impedance analysis for assessment of body composition in infants and young children-A systematic literature review. *Clin. Obes.* **2021**, *11*, e12441. [CrossRef]
6. Orsso, C.E.; Gonzalez, M.C.; Maisch, M.J.; Haqq, A.M.; Prado, C.M. Using bioelectrical impedance analysis in children and adolescents: Pressing issues. *Eur. J. Clin. Nutr.* **2022**, *76*, 659–665. [CrossRef]
7. Kozioł-Kozakowska, A.; Wójcik, M.; Stochel-Gaudyn, A.; Szczudlik, E.; Suder, A.; Piórecka, B. The Severity of Obesity Promotes Greater Dehydration in Children: Preliminary Results. *Nutrients* **2022**, *14*, 5150. [CrossRef]
8. Palamarchuk, O.S.; Leshko, M.M.; Klushyn, V.O.; Feketa, V.P. A differentiated approach to the diagnosis of overweight and obesity in children based on bioimpedance analysis of body composition. *Wiad. Lek.* **2024**, *77*, 402–408. [CrossRef]
9. Schoenmakers, M.A.; Gulmans, V.A.; Gooskens, R.H.; Pruijs, J.E.; Helden, P.J. Spinal fusion in children with spina bifida: Influence on ambulation level and functional abilities. *Eur. Spine J.* **2005**, *14*, 415–422. [CrossRef]
10. Centers for Disease Control and Prevention. CDC Extended BMI-for-Age Growth Charts 2022. Available online: <https://www.cdc.gov/growthcharts/extended-bmi.htm> (accessed on 15 December 2022).
11. World Health Organization (WHO). Growth Chart for Children 5–19 Years 2024. Available online: <https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/bmi-for-age> (accessed on 3 February 2024).
12. Plachta-Danielzik, S.; Gehrke, M.I.; Kehden, B.; Kromeyer-Hauschild, K.; Grillenberger, M.; Willhöft, C.; Bosy-Westphal, A.; Müller, M.J. Body fat percentiles for German children and adolescents. *Obes. Facts* **2015**, *5*, 77–90. [CrossRef]
13. Macek, P.; Biskup, M.; Terek-Derszniak, M.; Stachura, M.; Krol, H.; Gozdz, S.; Zak, M. Optimal body fat percentage cut-off values in predicting the obesity-related cardiovascular risk factors: A cross-sectional cohort study. *Diabetes Metab. Syndr. Obes.* **2020**, *12*, 1587–1597. [CrossRef]
14. Salton, N.; Kern, S.; Interator, H.; Lopez, A.; Moran-Lev, H.; Lebenthal, Y.; Brenner, A. Muscle-to-fat ratio for predicting metabolic syndrome components in children with overweight and obesity. *Child. Obes.* **2022**, *18*, 132–142. [CrossRef]
15. O’Neil, J.; Fuqua, J.S. Short stature and the effect of human growth hormone: Guidelines for the care of people with spina bifida. *J. Pediatr. Rehabil. Med.* **2020**, *13*, 549–555. [CrossRef]
16. Simmonds, M.; Llewellyn, A.; Owen, C.G.; Woolcott, N. Predicting adult obesity from childhood obesity: A systematic review and meta-analysis. *Obes. Rev.* **2016**, *17*, 95–107. [CrossRef]
17. McPherson, A.C.; Swift, J.A.; Yung, E.; Lyons, J.; Church, P. The assessment of weight status in children and young people attending a spina bifida outpatient clinic: A retrospective medical record review. *Disabil. Rehabil.* **2013**, *35*, 2123–2131. [CrossRef]
18. Dosa, N.P.; Foley, J.T.; Eckrich, M.; Woodall-Ruff, D.; Liptak, G.S. Obesity across the lifespan among persons with spina bifida. *Disabil. Rehabil.* **2009**, *31*, 914–920. [CrossRef]
19. Rendeli, C.; Kuczyńska, E.; Giuliano, A.C.; Chiaretti, A.; Ausili, E. Dietary approach to prevent obesity risk in Spina Bifida patients. *Childs Nerv. Syst.* **2020**, *36*, 1515–1520. [CrossRef]
20. Polfuss, M.; Liu, T.; Smith, K.; Huang, C.C.; Moosreiner, A.; Papanek, P.E.; Sawin, K.J.; Zvara, K.; Bandini, L. Weight status of children participating in the National Spina Bifida Patient Registry. *Pediatrics* **2022**, *150*, e2022057007. [CrossRef]
21. Polfuss, M.; Forseth, B.; Schoeller, D.A.; Huang, C.C.; Moosreiner, A.; Papanek, P.E.; Sawin, K.J.; Zvara, K.; Bandini, L. Accuracy of body mass index in categorizing weight status in children with intellectual and developmental disabilities. *J. Pediatr. Rehabil. Med.* **2021**, *14*, 621–629. [CrossRef]
22. Bray, G.A. Beyond BMI. *Nutrients* **2023**, *15*, 2254. [CrossRef]
23. Liu, J.S.; Dong, C.; Vo, A.X.; Dickmeyer, L.J.; Leung, C.L.; Huang, R.A.; Kielb, S.J.; Mukherjee, S. Obesity and anthropometry in spina bifida: What is the best measure. *J. Spinal Cord. Med.* **2018**, *41*, 55–62. [CrossRef]
24. Nelson, M.D.; Widman, L.M.; Abresch, R.T.; Stanhope, K.; Havel, P.J.; Styne, D.M.; McDonald, C.M. Metabolic syndrome in adolescents with spinal cord dysfunction. *J. Spinal Cord. Med.* **2007**, *30* (Suppl. 1), S127–S139. [CrossRef]
25. Zhang, J.X.; Li, W.; Tao, X.J.; Chen, C.; Wang, Q.A.; Liu, W.L.; Yang, C.; Wang, K.R.; Qiu, J.W.; Zhao, Y.; et al. Fat-to-muscle ratio as a predictor for dyslipidaemia in transitional-age youth. *Lipids Health Dis.* **2022**, *21*, 88. [CrossRef]
26. Marreiros, H. Update on bone fragility in spina bifida. *J. Pediatr. Rehabil. Med.* **2018**, *11*, 265–281. [CrossRef]
27. Trinh, A.; Wong, P.; Sakthivel, A.; Fahey, M.C.; Hennel, S.; Brown, J.; Strauss, B.J.; Ebeling, P.R.; Fuller, P.J.; Milat, F. Fat-bone interactions in adults with spina bifida. *J. Endocr. Soc.* **2017**, *1*, 1301–1311. [CrossRef]

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Article

Palmitic Acid Induces Oxidative Stress and Senescence in Human Brainstem Astrocytes, Downregulating Glutamate Reuptake Transporters—Implications for Obesity-Related Sympathoexcitation

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Abstract: Obesity has been associated with a chronic increase in sympathetic nerve activity, which can lead to hypertension and other cardiovascular diseases. Preliminary studies from our lab found that oxidative stress and neuroinflammation in the brainstem contribute to sympathetic overactivity in high-fat-diet-induced obese mice. However, with glial cells emerging as significant contributors to various physiological processes, their role in causing these changes in obesity remains unknown. In this study, we wanted to determine the role of palmitic acid, a major form of saturated fatty acid in the high-fat diet, in regulating sympathetic outflow. Human brainstem astrocytes (HBAs) were used as a cell culture model since astrocytes are the most abundant glial cells and are more closely associated with the regulation of neurons and, hence, sympathetic nerve activity. In the current study, we hypothesized that palmitic acid-mediated oxidative stress induces senescence and downregulates glutamate reuptake transporters in HBAs. HBAs were treated with palmitic acid (25 μ M for 24 h) in three separate experiments. After the treatment period, the cells were collected for gene expression and protein analysis. Our results showed that palmitic acid treatment led to a significant increase in the mRNA expression of oxidative stress markers (NQO1, SOD2, and CAT), cellular senescence markers (p21 and p53), SASP factors (TNF α , IL-6, MCP-1, and CXCL10), and a downregulation in the expression of glutamate reuptake transporters (EAAT1 and EAAT2) in the HBAs. Protein levels of Gamma H2AX, p16, and p21 were also significantly upregulated in the treatment group compared to the control. Our results showed that palmitic acid increased oxidative stress, DNA damage, cellular senescence, and SASP factors, and downregulated the expression of glutamate reuptake transporters in HBAs. These findings suggest the possibility of excitotoxicity in the neurons of the brainstem, sympathoexcitation, and increased risk for cardiovascular diseases in obesity.

Keywords: glial cells; oxidative stress; senescence; EAAT2; p21/p53; obesity

1. Introduction

Metabolic syndrome is a conglomeration of conditions that can increase the risk of stroke, heart conditions, and diabetes. Obesity, a serious public health issue, plays an important role in the development of metabolic syndrome. Key conditions associated with metabolic syndrome include increased body fat around the waist, high blood pressure, high cholesterol levels, insulin resistance, dyslipidemia, and high blood sugar, all commonly observed in obesity [1]. The prevalence of obesity almost tripled in the world between 1975 and 2016. According to the World Health Organization (WHO), around 2 billion people are overweight,

of which 33% are considered obese. Middle-aged adults in low-income countries and people of all ages and both sexes in high-income countries are affected by obesity [2].

In obesity, excess free fatty acids (FFAs) are released into circulation. Studies have shown that increased FFAs can activate proinflammatory pathways, leading to the increased production of cytokines and chemokines [3,4]. Obesity maintains the body in a state of low-grade chronic inflammation through the production of these proinflammatory molecules. The relationship between obesity and inflammation is a vicious cycle, where more weight gain causes more inflammation and vice versa [5]. Chronic inflammation in obesity can lead to neuroinflammation because of the disruption of the blood–brain barrier, allowing peripheral inflammatory molecules to enter the brain and interact with glial cells, such as astrocytes and microglia. The increased activation and proliferation of these glial cells have been reported in models of obesity [6].

Proinflammatory molecules induce oxidative stress, contributing to neuroinflammation in the brain. Oxidative stress is caused by an increase in reactive oxygen species (ROS) and a decrease in antioxidants, damaging cells at the molecular level and affecting lipids, proteins, and DNA. DNA damage can trigger cellular senescence, an irreversible cell cycle arrest [7–9]. Senescent cells produce proinflammatory molecules known as senescence-associated secretory phenotype (SASP) factors, which perpetuate neuroinflammation and create a feedback loop of inflammation and senescence [9,10]. In previous studies from our lab, we found that high-fat-diet (HFD)-induced obese mice produced oxidative stress and cellular senescence in a key brainstem region called the rostral ventrolateral medulla [7,8]. The neurons in this brainstem region are important for the regulation of blood pressure by modulating sympathetic nerve activity (SNA). However, little is known about the contribution of astrocytes in modulating sympathetic overactivity as observed with HFD-induced obesity [7,8].

Astrocytes are the most abundant glial cells and play an important role in neuronal regulation and sympathetic nerve activity. Astrocytes have glutamate reuptake transporters known as Excitatory Amino Acid Transporters (EAATs), which reuptake excess glutamate in the synapse and then convert it into glutamine via glutamine synthetase. This glutamine reenters neurons, is converted back into glutamate, and facilitates excitatory neurotransmission, known as the glutamate-glutamine cycle [11,12]. The downregulation of these transporters could lead to the excess accumulation of glutamate in the synapse and cause excitotoxicity, which in turn could be a contributing factor for sympathoexcitation observed in HFD-induced obesity. Palmitic acid (PA) is a major saturated free fatty acid found in the HFD. Numerous studies have suggested its role in promoting oxidative stress, inflammation, and metabolic dysfunction. Elevated levels of PA are also observed in the plasma of obese individuals. Furthermore, it is well-documented to induce lipotoxic effects in various cell types, including glial cells [3,4,7]. However, whether astrocytes play a role in mediating PA-induced neuroinflammation and the molecular mechanisms that could be involved is undocumented. To answer these questions, we investigated the effect of PA on human brainstem astrocytes.

2. Materials and Methods

2.1. Cell Culture and Treatments

Human brainstem astrocytes (HBAs) purchased from ScienCell Research Laboratories were used in all the experiments. HBAs were isolated from the human brainstem. These cells were cryopreserved at passage one (P1) and delivered frozen. Each vial had $>5 \times 10^5$ cells in 1 mL volume. After receiving the cells, they were stored in liquid nitrogen container until they were used. The cells were grown in T-75 flasks with astrocyte media supplied by ScienCell™ and supplemented with Dulbecco's Phosphate-Buffered Saline (PBS), Fetal Bovine Serum (FBS), Astrocyte Growth Supplement, as well as penicillin and streptomycin. The cultured cells were placed in a 37 °C incubator with 5% CO₂ until they reached approximately 75–80% confluency. After that, the cells were subcultured in different plates depending on the procedure for which the cells were cultured. Typically, we used passage 3 (P3) for all of our experiments.

2.2. Preparation of BSA-Conjugated Palmitic Acid (PA)

Palmitic acid (500 mM) with $\geq 99\%$ purity was purchased from Sigma-Aldrich and melted in 1 mL of 100% ethanol at 70 °C. Then, 10% Fraction V fatty acid-free bovine serum albumin (FFA-free BSA) was dissolved in cell culture media, sterile filtered, and incubated at 37 °C. FFA-free BSA obtained from Sigma-Aldrich was used in the experiment as fatty acids are insoluble in aqueous solutions and can damage the cells. Then, 10 μ L of dissolved PA in ethanol was added to 990 μ L of 10% FFA-free BSA and vortexed. After vortexing, the conjugate was incubated at 55 °C in a water bath for 15 min. Then, the PA-BSA conjugate was vortexed and kept in the water bath again for 15 min. Then, 10 μ L of 100% ethanol with 990 μ L of 10% FFA-free BSA was used as a control. The molar ratio of PA-BSA is 3.3:1 [13,14].

2.3. PA Treatment

After reaching 75–80% confluency, the cells from the T-75 flasks were subcultured in 6-well plates at a seeding density of 15×10^4 for the experiments. Cultured human brainstem astrocytes were treated with palmitic acid at a dose rate of 25 μ M for 24 h. The dosage of PA treatment was fixed based on the preliminary experiments from our lab. After the treatment period, the cells were collected in Trizol reagent and processed for real-time PCR analysis.

2.4. Oil Red O Staining

Oil Red O staining was performed to stain lipid droplets inside the cells. The Oil Red O stock solution was prepared by dissolving 60 mg of Oil Red O (ORO) in 20 mL of 100% isopropanol and allowed to sit for 20 min. In the meantime, the cell culture media were removed from all the wells and washed twice gently with 1X PBS. After that, the cells were fixed with 10% formalin and incubated for 30 min. The formalin was removed and washed gently with distilled water twice. After that, 60% isopropanol was added to each well and incubated for 5 min. Then the ORO working solution was prepared by adding 3 parts isopropanol to 2 parts of water and added to each well and incubated for 10–20 min. ORO solution is removed after the staining period and washed with distilled water as needed. Then each well was imaged under a light microscope where, in the positive cells, lipid droplets appear red, and nuclei appear blue.

2.5. Dihydroethidium Staining

The dihydroethidium staining was performed in 24 well plates with cells growing on the coverslips. After the treatment period, the culture media were removed, and the plates were rinsed once with 1X PBS. The cells were then incubated with five μ M dihydroethidium (DHE; Thermo Fisher Scientific, Waltham, MA, USA) diluted with DMSO and PBS in a light-protected humidified chamber for 30 min at 37 °C. After the incubation period, each well was washed thrice with 1X PBS. Then, the coverslips were inverted onto a slide with prolonged diamond antifade mountant and allowed to rest for 24 h. After that, the slides were imaged under a Biotek Cytation 5 multimode imager for the fluorescent identification of DHE-stained cells.

2.6. RNA Extraction and Real-Time PCR Analysis

Human brainstem astrocytes from 6-well plates were collected and lysed in 300 μ L Trizol reagent. The Direct-zol RNA Miniprep kit (Zymo Research, Irvine, CA, USA) was used to extract the RNA from the cell culture samples according to the manufacturer's instructions. Reverse transcription of equal amounts of RNA was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Waltham, MA, USA) to obtain cDNA. We used 10 ng of cDNA per reaction, and real-time PCR analysis was carried out with iTaq Universal SYBR Green mix (BioRad, Hercules, CA, USA).

The human primers for p16, p21, p53, IL-1 α , IL-1 β , IL6, IL8, TNF- α , MMP3, MMP13, MCP-1, CXCL1, CXCL10, NQO1, SOD2, CAT, EAAT1, EAAT2, Lamin B1, Plin1, Plin2, and the housekeeping gene GAPDH were designed using Primer3-Blast software and

synthesized by Integrated DNA Technologies (primer sequences are listed in Table 1). Real-time PCR data were analyzed using the $2^{-\Delta\Delta CT}$ method.

Table 1. Primer genes and their gene accession numbers for real-time PCR analysis.

Human Gene	Forward Primer	Reverse Primer	Gene Accession No.
<i>p16</i>	GATCCAGGTGGGTAGAGGGTC	CCCCTGCAAACCTTCGTCTT	NM_058197.5
<i>p21</i>	TGTCCGTCAGAACCCATGC	AAAGTCGAAGTTCCATCGCTC	NM_001374511.1
<i>p53</i>	CAGCACATGACGGAGGTTGT	TCATCCAAATACTCCACACGC	NM_001407269.1
<i>IL1α</i>	CGCCAATGACTCAGAGGAAGA	AGGGCGTCATTTCAGGATGAA	NM_000575.5
<i>IL1β</i>	AATCTGTACCTGTCCTGCGTGTT	TGGGTAATTTTGGGATCTACACTCT	NM_000576.3
<i>IL6</i>	CCGGGAACGAAAGAGAAGCT	GCGCTTGTGGAGAAGGAGTT	NM_000600.5
<i>IL8</i>	CTTGGCAGCCTTCCTGATTT	TTCTTTAGCACTCCTTGCCAAAA	NM_001354840.3
<i>TNF-α</i>	CCCAGGGACCTCTCTCTAATCA	AGCTGCCCCCTCAGCTTGAG	NM_000594.4
<i>MMP3</i>	GACACCAGCATGAACCTTGTT	GGAACCGAGTCAGGTCTGTG	NM_002422.5
<i>MMP13</i>	GGCTCCGAGAAATGCAGTCTTTCTT	ATCAAATGGGTAGAAGTCGCCATGC	NM_002427.4
<i>MCP-1</i>	AATCAATGCCCCAGTCACCT	CTTCTTTGGGACACTTGCTGC	NM_002982.4
<i>CXCL1</i>	GAAAGCTTGCCCTCAATCCTG	CACCAGTGAGCTTCCTCCTC	NM_001511.4
<i>CXCL10</i>	AACCTCCAGTCTCAGCACCATGAA	AGGTACAGCGTAAGGTTCTAGAGAG	NM_001565.4
<i>NQO1</i>	ACTGATCGTACTGGCTCACTC	AGTTCATGGCATAGAGGTCCG	NM_001025434.2
<i>SOD2</i>	GTTGGGGTTGGCTTGGTTTC	GTTCTTGCAGTGGATCCTGA	NM_001322819.2
<i>CAT</i>	CTTTCTGTTGAAGATGCGGCG	AGTCCAGGAGGGGTACTTTCC	NM_001752.4
<i>EAAT1</i>	TGCCCTGGGTCTAGTTGTCT	CCAGTCTCATGATGGCTTCGT	NM_004172.5
<i>EAAT2</i>	CTTGGCATCTCCCATCCACC	GCTGGAGATGATTAGAGGGAGAA	NM_001252652.2
<i>Lamin B1</i>	AAGGCGAAGAAGAGAGGTTGAAG	GCGGAATGAGAGATGCTAACACT	NM_001198557.2
<i>Plin1</i>	CAGAAACAGCATCAGCGTTCC	CAGCAAATCCGCAGTGTCTC	NM_001145311.2
<i>Plin2</i>	TCACAGGGGTGATGGACAAG	TTTCTACGCCACTGCTCACG	NM_001122.4
<i>GAPDH</i>	GCCGTCTAGAAAAACCTGCC	ACCACCTGGTGCTCAGTGTA	NM_001357943.2

2.7. Immunofluorescence Staining

Immunofluorescence staining was performed for the DNA damage marker γ H2AX and the senescent markers p16 and p21 to determine the presence of senescence in PA-treated cells. For this, we used a 24-well plate with coverslips, and the cells were seeded on top of the coverslips. After the seeding and PA treatment period, the culture media were removed and gently washed with 1X PBS at room temperature (RT). Then, the cells were fixed with 4% paraformaldehyde in PBS at RT for 5 min., after which the coverslips were washed in 1X PBS for 5 min. After that, the permeabilization step was performed with 0.5% Triton X-100 in PBS at RT for 5 min and washed once with 1X PBS for 5 min. Then, the coverslips with cells were blocked with 1% BSA in PBS for 1 h, after which the primary antibody solution (Rabbit polyclonal Gamma H2AX, Catalog No. ab11174, 1:500 dilution; Rabbit polyclonal p16^{INK4a}, Catalog No. A0262, 1:100 dilution; and Rabbit polyclonal p21CIP1, Catalog No. A1483, 1:100 dilution) was added to the cells and incubated overnight at 4 °C. The next day, the primary antibody solution was removed, and the coverslips were washed three times in 1X PBS for 5 min each. Then, an appropriate fluorochrome-conjugated secondary antibody (Goat Anti-Rabbit IgG Alexa Fluor 594, Catalog No. ab150080, 1:500 dilution; and Goat Anti-Rabbit IgG Alexa Fluor 488, Catalog No. ab150077, 1:500 dilution) in 1% BSA was added to the cells for 1 h at RT in a dark environment. After that, the cells were gently washed with 1X PBS thrice for 5 min each. After the washing, the coverslips were stained with the nuclear stain DAPI

(1:500 dilution in water) for 5 min and washed with 1X PBS twice. Then, the coverslips were mounted on a slide with a drop of prolonged antifade diamond mounting media and allowed to cure overnight. The next day, the slides were imaged using a Biotek Cytation 5 multimodal imager for fluorescent imaging. Finally, the fluorescent intensity of the images was quantified using ImageJ Version 1.54, and the graphs were plotted.

2.8. Statistical Analysis

Data were analyzed by Student's *t*-test and expressed as mean $\pm$ SEM. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Validation of Palmitic Acid (PA) Treatment in the HBAs

First, we wanted to test if the palmitic acid treatment was successful in the HBAs. To achieve this, we performed gene expression analysis of the lipid-droplet-associated proteins, perilipin (PLIN) 1 and 2. Perilipins coat the lipid droplets, and increased concentrations of fatty acids will induce lipid droplet accumulation [15]. We found that the mRNA expression of perilipin 1 and 2 was significantly higher in the PA-treated group than in the control groups (Figure 1A, B). Then, we also performed Oil Red O staining to stain the lipid droplets and found that PA-treated cells had a higher number of positive cells, indicating that the palmitic acid treatment was successful and was able to mimic the obese environment in the HBAs (Figure 1C).

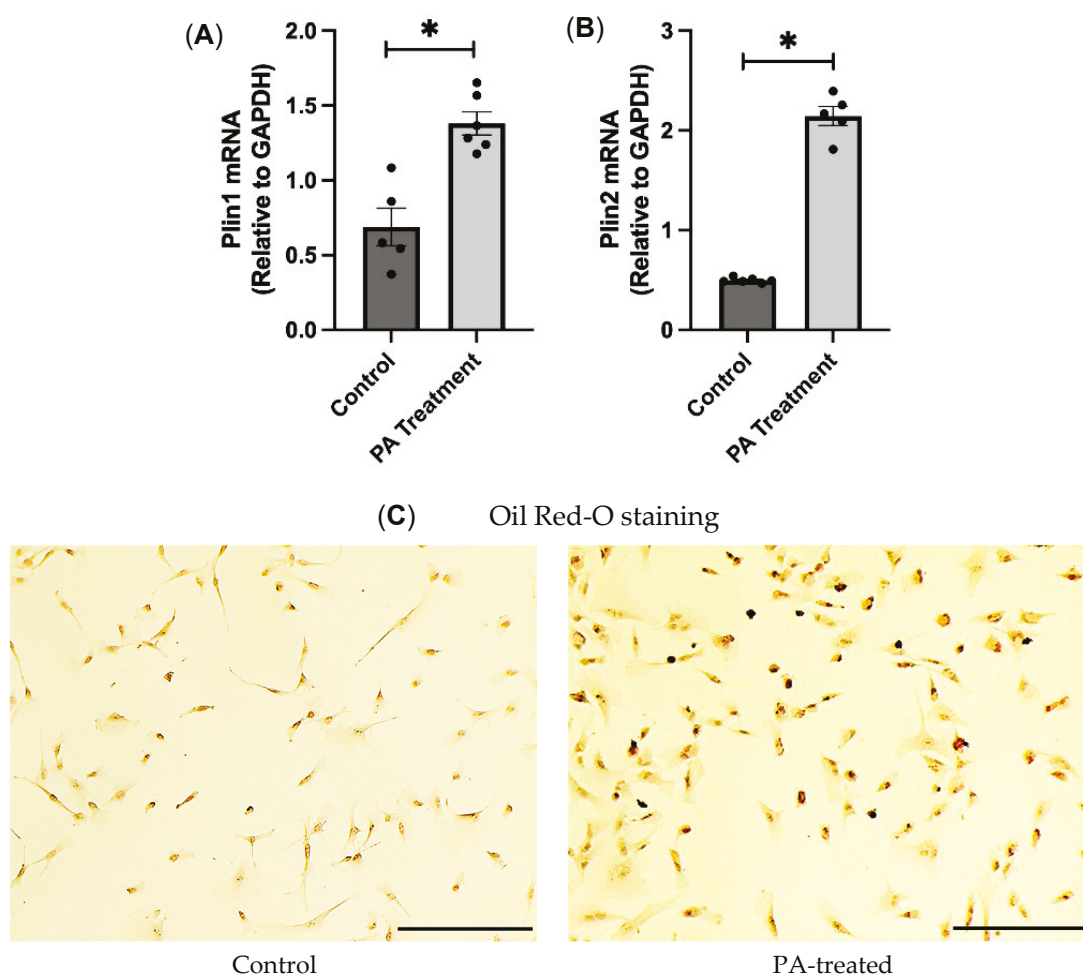


Figure 1. Validation of PA treatment and effects of PA treatment on oxidative stress markers in the HBAs. (A,B): changes in mRNA levels of lipid droplet associated genes, namely, Plin1 and Plin2 in

the human brainstem astrocytes between control and PA-treated group. (C): Oil Red-O staining of the control and PA-treated cells at 20X. Scale bar indicates 100 μ m. Data are means $\pm$ SEM ($n = 4$ –6 replicates per group). * significant difference ($p < 0.05$) between the groups.

3.2. PA Induces Oxidative Stress in the HBAs

Studies have shown that PA can enhance the production of reactive oxygen species (ROS) and can cause oxidative stress. PA causes mitochondrial dysfunction and oxidative stress through the NOX pathway. We found that the PA treatment significantly increased the gene expression levels of oxidative stress markers NQO1, SOD2, and catalase (Figure 2A,C). Additionally, we found that the DHE staining of the PA-treated cells indicated that the PA-treated group had significantly higher fluorescence, indicating that PA induces oxidative stress in human brainstem astrocytes (Figure 2D).

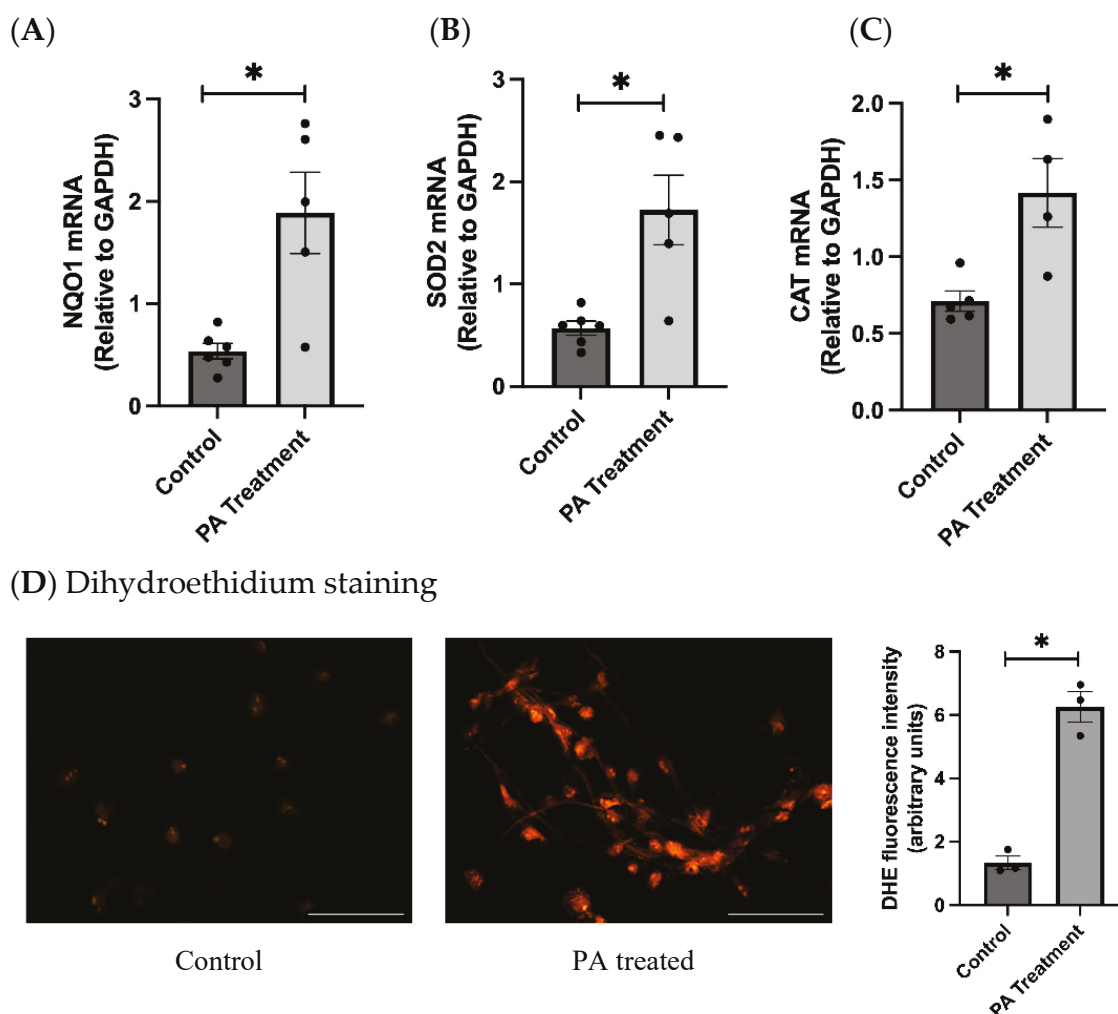


Figure 2. Effects of PA treatment on oxidative stress markers in HBAs. (A–C): changes in mRNA levels of oxidative stress markers NQO1, SOD2, and CAT in human brainstem between control and PA-treated groups. (D): dihydroethidium staining of the control and PA-treated group with bar graphs for fluorescence intensity. Data are means $\pm$ SEM ($n = 3$ –6 replicates per group). Scale bar indicates 100 μ m. * significant difference ($p < 0.05$) between groups.

3.3. PA Induces DNA Damage in the HBAs

After we found that PA treatment induced oxidative stress in the HBAs, we next wanted to see if excess oxidative stress leads to DNA damage. So, we performed immunofluorescence for the DNA damage marker, Gamma H2AX (γ -H2AX), and found that

the γ -H2AX fluorescence intensity was significantly upregulated in the PA-treated cells, indicating that the PA treatment induced DNA damage in the HBAs (Figure 3).

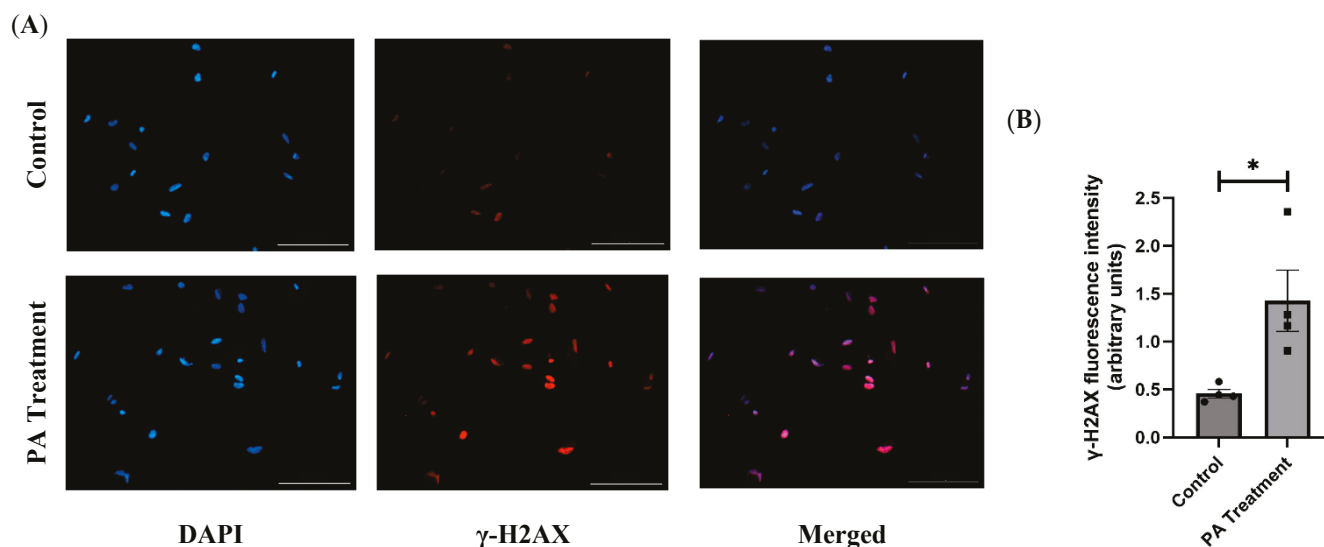


Figure 3. Immunofluorescence staining of Gamma H2AX (γ -H2AX) in the HBAs. (A): immunofluorescence staining for the DNA damage marker γ -H2AX in the human brainstem astrocytes between control and PA-treated groups. (B): bar graphs showing fluorescence intensity of γ -H2AX between the control and PA-treated groups. Data are means $\pm$ SEM ($n = 4$ replicates per group). Scale bar indicates 100 μ m. * significant difference ($p < 0.05$) between groups.

3.4. PA Causes Cellular Senescence and SASP in the HBAs

PA treatment has been shown to induce cellular senescence, a state of irreversible cell cycle arrest, through oxidative stress and the resulting DNA damage. Consistent with this earlier finding, we observed that PA treatment in the HBAs significantly increased the gene expression levels of senescence markers p21 and p53 (Figure 4A,C), along with elevated expression of oxidative stress and DNA damage markers. However, we did not observe a significant difference in p16 mRNA expression between the groups. In addition, Lamin B1, a component of the nuclear envelope, was significantly downregulated in the PA treatment group consistent with the senescence (Figure 4D).

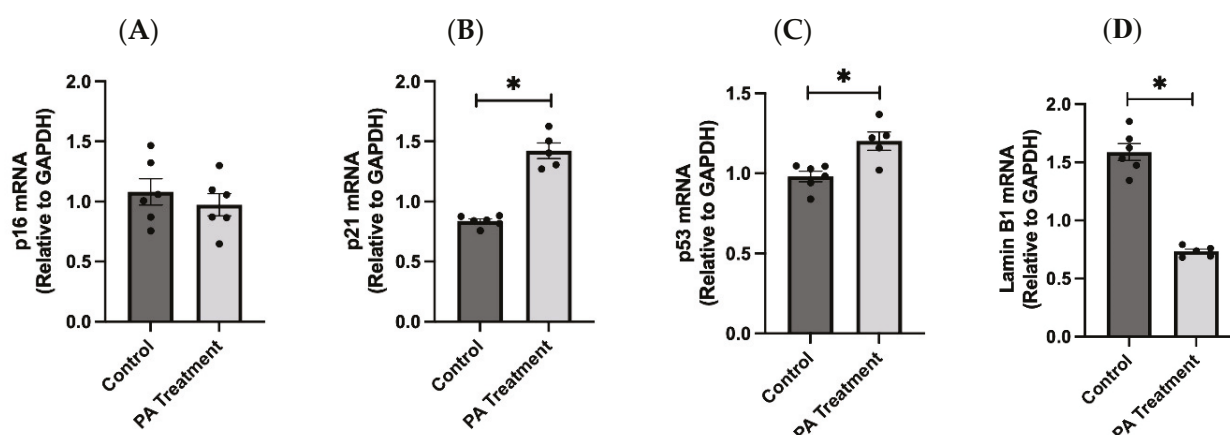


Figure 4. PA-induced oxidative stress causes cellular senescence in the HBAs. (A–D): Changes in the mRNA levels of senescence markers p16, p21, p53 and the nuclear envelope gene Lamin B1 in the human brainstem astrocytes between control and PA-treated groups. Data are means $\pm$ SEM ($n = 5$ –6 replicates per group). * Significant difference ($p < 0.05$) between the groups.

By immunofluorescence staining of the PA-treated cells with p16 and p21 antibodies, we found that the PA-treated cells have significantly higher p16 and p21 levels, further confirming that PA treatment causes cellular senescence (Figures 5 and 6).

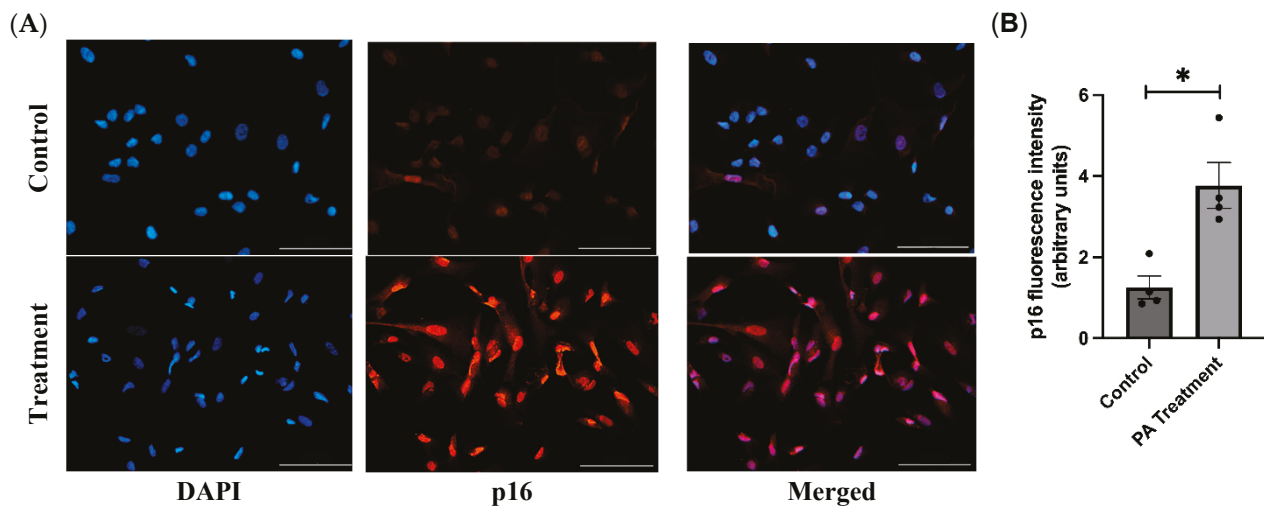


Figure 5. Immunofluorescence staining of p16^{INK4a} in the HBAs. (A): immunofluorescence staining for the senescence marker p16^{INK4a} in the human brainstem astrocytes between control and PA-treated groups. (B): bar graphs showing fluorescence intensity of p16^{INK4a} between the control and PA-treated groups. Data are means $\pm$ SEM ($n = 4$ replicates per group). Scale bar indicates 100 μ m. * significant difference ($p < 0.05$) between the groups.

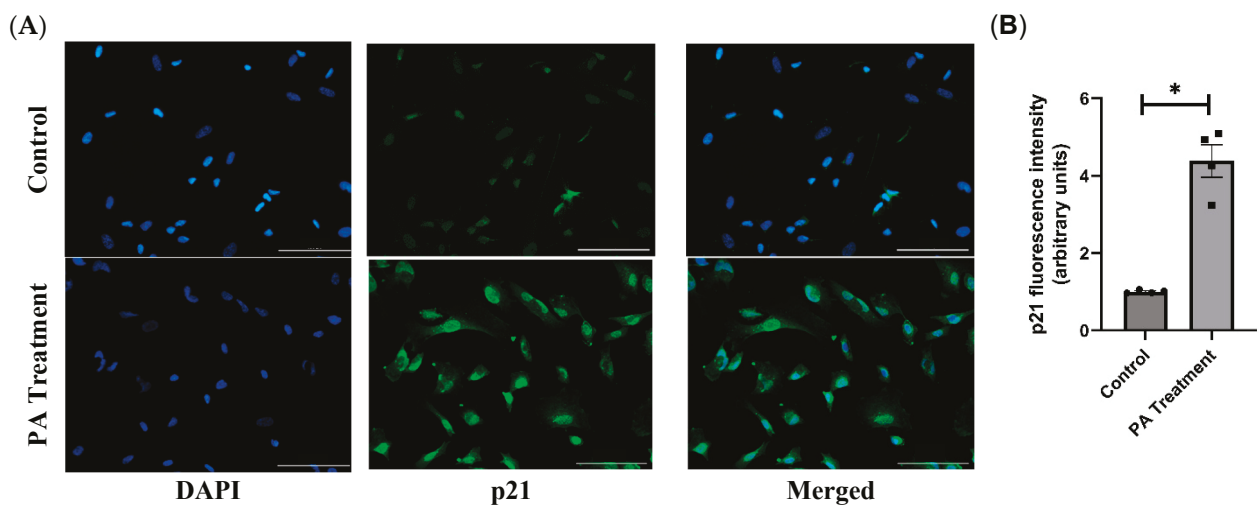


Figure 6. Immunofluorescence staining of p21^{CIP1} in the HBAs. (A): immunofluorescence staining for the senescence marker p21^{CIP1} in the human brainstem astrocytes between control and PA-treated groups. (B): bar graphs showing fluorescence intensity of p21^{CIP1} between the control and PA-treated groups. Data are means $\pm$ SEM ($n = 4$ replicates per group). Scale bar indicates 100 μ m. * significant difference ($p < 0.05$) between the groups.

Senescent cells, in addition to arresting growth, also secrete various proinflammatory factors, known as SASP factors, which can affect neighboring cells in a paracrine fashion. We found that there is a significant increase in the gene expression levels of SASP factors IL1 α , TNF α , IL6, MCP1, and CXCL10 (Figure 7), indicating that SASP factors, which are a potential source of neuroinflammation, are produced with PA treatment.

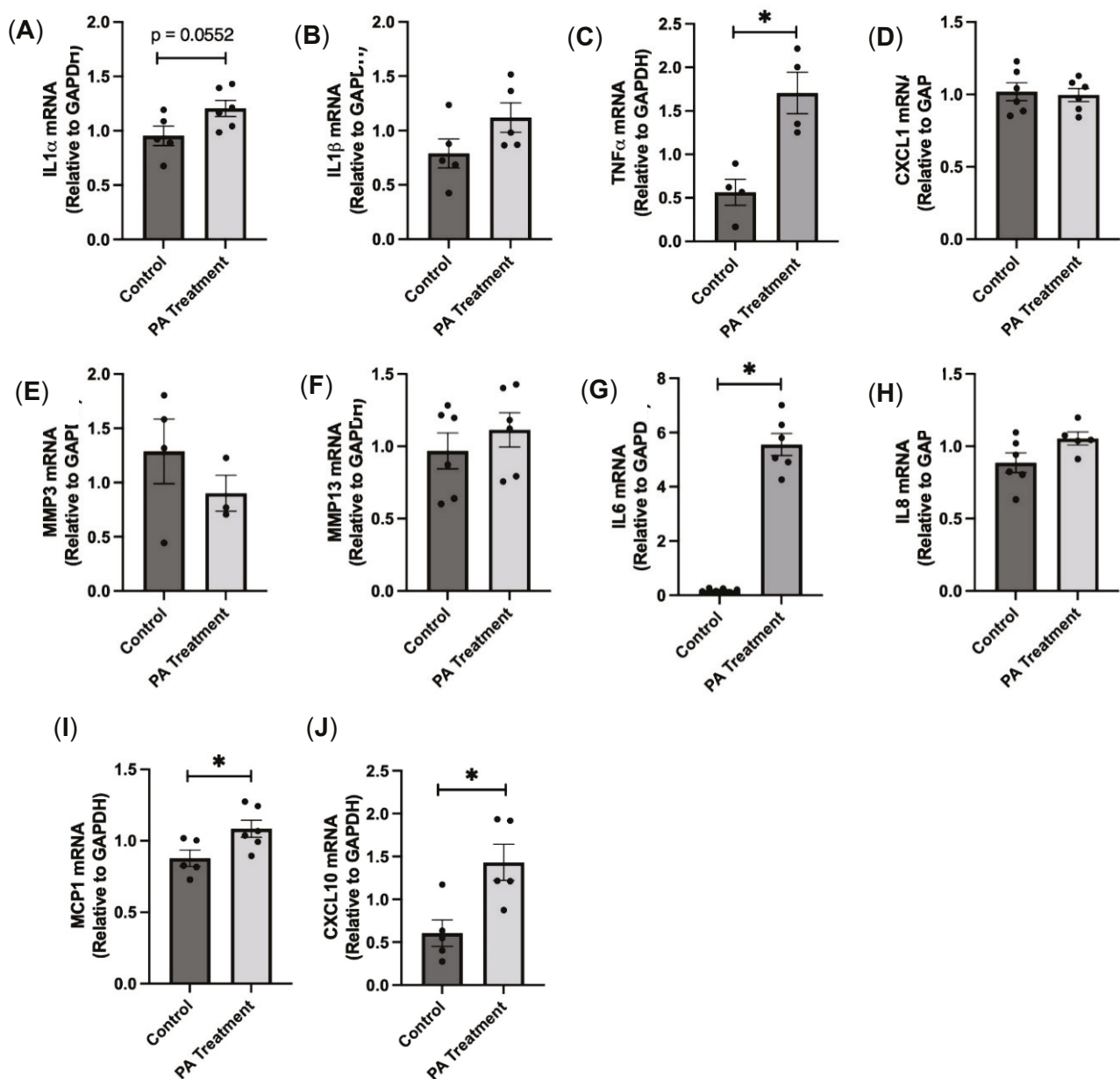


Figure 7. PA-induced senescence causes SASP factor production in the HBAs. (A–J): changes in the mRNA levels of various proinflammatory SASP factors in the human brainstem astrocytes between control and PA-treated groups. Data are means $\pm$ SEM ($n = 5$ –6 replicates per group). * significant difference ($p < 0.05$) between the groups.

3.5. PA Downregulates Glutamate Reuptake Transporter Expression in the HBAs

The downregulation of glutamate reuptake transporters can cause excitotoxicity due to excess glutamate in the synapse, which in turn can increase sympathetic nerve activity to the end organs and damage them. We wanted to test if PA treatment downregulates these glutamate transporters. We found that the mRNA expression levels of EAAT 1 and EAAT 2 were significantly downregulated in the PA-treated group compared with the controls, indicating that PA downregulates glutamate reuptake transporter expression (Figure 8).

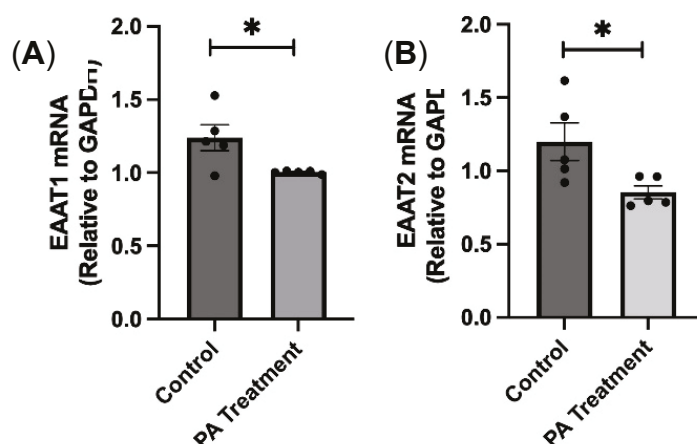


Figure 8. PA treatment downregulates glutamate reuptake transporter expression in the HBAs. (A,B): changes in the mRNA levels glutamate re-uptake transporters, EAAT1 and EAAT2 in the human brainstem astrocytes between control and PA-treated groups. Data are means $\pm$ SEM ($n = 4$ –6 replicates per group). * significant difference ($p < 0.05$) between the groups.

4. Discussion

The major findings of this study include: (i) palmitic acid treatment increased the expression of oxidative stress and DNA damage markers in human brainstem astrocytes; (ii) palmitic acid elevated the mRNA and protein expression of cellular senescence markers; (iii) palmitic acid upregulated the mRNA expression of SASP factors; and (iv) palmitic acid treatment induced the downregulation of glutamate reuptake transporters, EAAT1 and EAAT2.

It is widely known that elevated free fatty acids, such as palmitic acid (PA), in circulation can contribute to increased oxidative stress in an obese environment [3,4]. Our findings align with these studies, demonstrating that PA treatment significantly increased oxidative stress markers such as NQO1, SOD2, and CAT. Similar to the work by Ogrodnik et al. (2019), which showed that obesity leads to DNA damage and accumulation of senescent glial cells, we found that PA-induced oxidative stress in the HBAs also resulted in DNA damage, as determined by γ H2AX staining. Our study further extends these findings by showing that DNA damage occurring in the HBAs due to oxidative stress can stimulate a DNA damage response (DDR), which, when sustained, can cause them to enter a state of cellular senescence [16,17].

Cellular senescence is a complex biological process characterized by irreversible growth arrest, involving cell cycle inhibitors (p16, p21, and p53) and a proinflammatory state (SASP factors) [18]. With PA treatment, we found that senescence markers p21 and p53 were increased in the HBAs. We further explored the cell cycle inhibitors at the protein level and confirmed that both p16 and p21 were significantly upregulated with PA treatment, similar to other obese animal studies [19]. To examine whether senescent glial cells created a proinflammatory state [20,21], we measured the levels of SASP factors such as TNF α , IL6, MCP1, and CXCL10 and found them to be upregulated after PA treatment. Other studies have shown that chronic exposure to SASP factors could cause damage to healthy cells in a paracrine manner and could be responsible for tissue dysfunction in obesity and aging [22].

One significant finding from our current study is the observation that PA treatment leads to the downregulation of glutamate reuptake transporters (EAAT1 and EAAT2). The importance of glutamate reuptake transporters has been described in various studies, and the dysregulation of glutamate homeostasis is a key pathological feature, even in the development of neurodegenerative diseases [23,24]. The astrocytes closely associated with the synapse are responsible for the synthesis of glutamine, which eventually produces glutamate. Under normal circumstances, excess glutamate is taken up by astrocytes using the reuptake transporters [25]. However, the reduced reuptake transporters after

PA treatment suggests that there would be an excess accumulation of glutamate at the synapse, which could be responsible for chronic sympathoexcitation as seen in high-fat diet-induced obesity [7,26,27]. To summarize, this study provides evidence that palmitic acid treatment increased the oxidative stress, cellular senescence, and downregulation of glutamate reuptake transporters in HBAs.

One of the main limitations of this present study is that the effects observed are only in the HBAs in isolation, without exploring interactions with neurons and microglia. To overcome this limitation, our future studies will employ co-culture and/or animal experiments to investigate glia–neuron crosstalk and glia–glia interaction. The second limitation is that we only used palmitic acid in our experiments and didn't compare it with any other fatty acids, such as oleic acid, or other stressors like glucose, insulin, and H₂O₂. These will be explored in our future studies.

5. Conclusions

Our findings demonstrate that elevated PA in obesity can impair astrocyte function, leading to oxidative stress, DNA damage, cellular senescence, and downregulation of glutamate reuptake transporters. These changes can potentially disrupt glutamate homeostasis, contributing to excitotoxicity and neurodegeneration. Therefore, modulating these transporters could be a novel therapeutic approach to reduce obesity-related sympathetic overactivity and its associated cardiovascular complications. However, future studies in animals would be required to reaffirm these preliminary in vitro findings.

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References

1. Han, T.S.; Lean, M.E. A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. *JRSM Cardiovasc. Dis.* **2016**, *5*, 2048004016633371. [CrossRef]
2. Swinburn, B.A.; Sacks, G.; Hall, K.D.; McPherson, K.; Finegood, D.T.; Moodie, M.L.; Gortmaker, S.L. The global obesity pandemic: Shaped by global drivers and local environments. *Lancet* **2011**, *378*, 804–814. [CrossRef]
3. Boden, G.; She, P.; Mozzoli, M.; Cheung, P.; Gumireddy, K.; Reddy, P.; Xiang, X.; Luo, Z.; Ruderman, N. Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. *Diabetes* **2005**, *54*, 3458–3465. [CrossRef] [PubMed]
4. Xu, H.; Barnes, G.T.; Yang, Q.; Tan, G.; Yang, D.; Chou, C.J.; Sole, J.; Nichols, A.; Ross, J.S.; Tartaglia, L.A.; et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Investig.* **2003**, *112*, 1821–1830. [CrossRef]
5. Hildebrandt, X.; Ibrahim, M.; Peltzer, N. Cell death and inflammation during obesity: “Know my methods, WAT(son)”. *Cell Death Differ.* **2023**, *30*, 279–292. [CrossRef]
6. Castanon, N.; Luheshi, G.; Laye, S. Role of neuroinflammation in the emotional and cognitive alterations displayed by animal models of obesity. *Front. Neurosci.* **2015**, *9*, 229. [CrossRef] [PubMed]
7. Balasubramanian, P.; Asirvatham-Jeyaraj, N.; Monteiro, R.; Sivasubramanian, M.K.; Hall, D.; Subramanian, M. Obesity-induced sympathoexcitation is associated with Nrf2 dysfunction in the rostral ventrolateral medulla. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2020**, *318*, R435–R444. [CrossRef]

8. Balasubramanian, P.; Branen, L.; Sivasubramanian, M.K.; Monteiro, R.; Subramanian, M. Aging is associated with glial senescence in the brainstem—Implications for age-related sympathetic overactivity. *Aging* **2021**, *13*, 13460–13473. [CrossRef] [PubMed]
9. Balasubramanian, P.; Hall, D.; Subramanian, M. Sympathetic nervous system as a target for aging and obesity-related cardiovascular diseases. *Geroscience* **2019**, *41*, 13–24. [CrossRef]
10. Birch, J.; Gil, J. Senescence and the SASP: Many therapeutic avenues. *Genes Dev.* **2020**, *34*, 1565–1576. [CrossRef]
11. Pajarillo, E.; Rizer, A.; Lee, J.; Aschner, M.; Lee, E. The role of astrocytic glutamate transporters GLT-1 and GLAST in neurological disorders: Potential targets for neurotherapeutics. *Neuropharmacology* **2019**, *161*, 107559. [CrossRef]
12. Rose, C.R.; Felix, L.; Zeug, A.; Dietrich, D.; Reiner, A.; Henneberger, C. Astroglial Glutamate Signaling and Uptake in the Hippocampus. *Front. Mol. Neurosci.* **2017**, *10*, 451. [CrossRef]
13. Alsabeeh, N.; Chausse, B.; Kakimoto, P.A.; Kowaltowski, A.J.; Shirihai, O. Cell culture models of fatty acid overload: Problems and solutions. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2018**, *1863*, 143–151. [CrossRef]
14. Kim, J.Y.; Lee, H.J.; Lee, S.J.; Jung, Y.H.; Yoo, D.Y.; Hwang, I.K.; Seong, J.K.; Ryu, J.M.; Han, H.J. Palmitic Acid-BSA enhances Amyloid-beta production through GPR40-mediated dual pathways in neuronal cells: Involvement of the Akt/mTOR/HIF-1alpha and Akt/NF-kappaB pathways. *Sci. Rep.* **2017**, *7*, 4335. [CrossRef]
15. Schelbert, S.; Schindeldecker, M.; Drebber, U.; Witzel, H.R.; Weinmann, A.; Dries, V.; Schirmacher, P.; Roth, W.; Straub, B.K. Lipid Droplet-Associated Proteins Perilipin 1 and 2: Molecular Markers of Steatosis and Microvesicular Steatotic Foci in Chronic Hepatitis C. *Int. J. Mol. Sci.* **2022**, *23*, 15456. [CrossRef] [PubMed]
16. Feringa, F.M.; Raaijmakers, J.A.; Hadders, M.A.; Vaarting, C.; Macurek, L.; Heitink, L.; Krenning, L.; Medema, R.H. Persistent repair intermediates induce senescence. *Nat. Commun.* **2018**, *9*, 3923. [CrossRef]
17. Ishida, T.; Ishida, M.; Tashiro, S.; Takeishi, Y. DNA Damage and Senescence-Associated Inflammation in Cardiovascular Disease. *Biol. Pharm. Bull.* **2019**, *42*, 531–537. [CrossRef]
18. Rodier, F.; Campisi, J. Four faces of cellular senescence. *J. Cell Biol.* **2011**, *192*, 547–556. [CrossRef]
19. Ogrodnik, M.; Zhu, Y.; Langhi, L.G.P.; Tchkonja, T.; Kruger, P.; Fielder, E.; Victorelli, S.; Ruswhandi, R.A.; Giorgadze, N.; Pirtskhalava, T.; et al. Obesity-Induced Cellular Senescence Drives Anxiety and Impairs Neurogenesis. *Cell Metab.* **2019**, *29*, 1061–1077.e8. [CrossRef]
20. Wiley, C.D.; Campisi, J. The metabolic roots of senescence: Mechanisms and opportunities for intervention. *Nat. Metab.* **2021**, *3*, 1290–1301. [CrossRef]
21. Ohtani, N. The roles and mechanisms of senescence-associated secretory phenotype (SASP): Can it be controlled by senolysis? *Inflamm. Regen.* **2022**, *42*, 11. [CrossRef]
22. Nelson, G.; Wordsworth, J.; Wang, C.; Jurk, D.; Lawless, C.; Martin-Ruiz, C.; von Zglinicki, T. A senescent cell bystander effect: Senescence-induced senescence. *Aging Cell* **2012**, *11*, 345–349. [CrossRef] [PubMed]
23. O'Donovan, S.M.; Sullivan, C.R.; McCullumsmith, R.E. The role of glutamate transporters in the pathophysiology of neuropsychiatric disorders. *NPJ Schizophr.* **2017**, *3*, 32. [CrossRef] [PubMed]
24. Hotz, A.L.; Jamali, A.; Rieser, N.N.; Niklaus, S.; Aydin, E.; Myren-Svelstad, S.; Lalla, L.; Jurisch-Yaksi, N.; Yaksi, E.; Neuhauss, S.C.F. Loss of glutamate transporter eaat2a leads to aberrant neuronal excitability, recurrent epileptic seizures, and basal hypoactivity. *Glia* **2022**, *70*, 196–214. [CrossRef] [PubMed]
25. Hertz, L.; Rodrigues, T.B. Astrocytic-Neuronal-Astrocytic Pathway Selection for Formation and Degradation of Glutamate/GABA. *Front. Endocrinol.* **2014**, *5*, 42. [CrossRef]
26. Armada-Moreira, A.; Gomes, J.I.; Pina, C.C.; Savchak, O.K.; Goncalves-Ribeiro, J.; Rei, N.; Pinto, S.; Morais, T.P.; Martins, R.S.; Ribeiro, F.F.; et al. Going the Extra (Synaptic) Mile: Excitotoxicity as the Road Toward Neurodegenerative Diseases. *Front. Cell Neurosci.* **2020**, *14*, 90. [CrossRef]
27. Dong, X.X.; Wang, Y.; Qin, Z.H. Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacol. Sin.* **2009**, *30*, 379–387. [CrossRef]

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Review

Microbiota-Based Intervention Alleviates High-Fat Diet Consequences Through Host-Microbe Environment Remodeling

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Abstract: A high-fat diet leads to metabolic disturbances, which are important factors in the development of obesity. Gut microbial composition and diversity are altered by a high-fat diet. In general, a high-fat diet resulted in increased Firmicutes abundance and decreased alpha diversity. Bile acids (BAs) are involved in the digestion and absorption of fats in the small intestine and are also the metabolic substrates of microorganisms with bile salt hydrolase (BSH) activity. High-fat diets (HFDs) have been shown to alter gut microbiota composition and BA profiles in murine models. Similarly, probiotic supplementation reverses HFD-induced adverse effects. This review focuses on the energy composition characteristics of a high-fat diet and its effects on body weight, plasma lipid-related biochemical markers, changes in gut microbiome characteristics, and the important role of BAs. The regular mechanism by which a high-fat diet affects the intestinal microenvironment was attempted to be found.

Keywords: high-fat diet; obesity; intestinal; microbes

1. Introduction

Obesity is a recognized global public health problem and is influenced by many factors such as age, gender, genetics, diet, geographic location, etc. [1–3]. A strong link between diet and obesity has been observed by changing dietary patterns [4,5]. Dietary patterns can generally be divided into three types: 1. Healthy diet, 2. Western diet, 3. Mixed diet [6]. A healthy diet is high in vegetables, fruits, and whole legumes. The Western diet is characterized by refined grains, solid fats, and snacks. A mixed diet is somewhere between a healthy diet and a Western diet. It is well known that the Western diet is the main dietary pattern that contributes to obesity. Therefore, the simple summary is a high-fat diet.

The digestion and absorption of nutrients in the gut is a dynamic process in which microorganisms play an important role. The gut microbiota is an important environmental factor that affects the host's energy acquisition and storage from the diet and can induce de novo synthesis of fat in the liver by promoting the absorption of monosaccharides in

the gut [7]. There were differences in the number of microorganisms in different intestinal segments. An amount of 10^4 – 10^8 microorganisms per mL were present in the contents of the small intestine, and microbial colonization was limited due to the short residence time of the chyme. The microbial density of the large intestine can reach 10^{10} per mL, which is the intestinal segment with the highest intestinal microbial density [8]. Microbiome study is popular all over the world, and the rapid development of high-throughput sequencing technology makes it possible to observe the gut microbial environment [9]. About 90% of the microorganisms in the large intestine belong to the phyla Bacteroidetes and Firmicutes and also include Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia [10].

A translational perspective is established through systematic analysis of high-fat diet-induced physiological and microbial alterations, focusing on three critical aspects: (1) the operational definition of high-fat diets, (2) identification of diet-associated gut microbiota signatures, and (3) evaluation of therapeutic potential from microbial supplementation strategies.

2. Nutrient Characteristics of a High-Fat Diet

2.1. High-Fat Diet Induces Fat Deposition

High-fat diets are characterized by low carbohydrate levels and relatively unchanged protein levels, with a fat composition of more than 30% of total dietary energy (Table 1). Body weight and adipose tissue weight increased significantly, and plasma cholesterol concentrations increased. Dietary fats are emulsified by bile salts in the small intestine, and then pancreatic juice secreted by the pancreas begins to digest the lipids. The free fatty acids produced by lipolysis are absorbed by the small intestine, resynthesize triglycerides (TGs), and enter the blood circulation in the form of chylomicrons. The TGs released in the liver are combined with apolipoproteins and transported to extrahepatic tissues for utilization [11]. At the same time, a high-fat diet also caused significant upregulation of the lipase genes adipose triglyceride lipase (*ATGL*), hormone-sensitive triglyceride lipase (*HSL*), and fatty acid synthase gene *FASN* in the liver of mice, indicating that the lipid metabolism activity of mice fed a high-fat diet was more rigorous than that of the mice fed a low-fat diet [12].

A short-term (within 2 weeks) high-fat diet has no significant effect on the body weight of mice, and the deposition of fat in the body takes a period of time. When dietary fat was increased to 50% for one week, there were no significant differences in body weight, body fat, and fat mass compared with a diet containing 30% fat [13]. Diets containing 30% vegetable oil significantly increased body weight compared with 20% [14]. A 4-week high-fat diet experiment, designed to have significantly higher cholesterol levels than the control group, then measured weekly body weights in Sprague-Dawley rats, which were nearly the same weight as the control group in the first and second weeks and began to rise slowly after the second week [15]. The feeding efficiency of C57BL/6 mice fed a high-fat diet increased, but the body mass was almost no different from the control group at 0–15 days, and the increase was less than 1 g at 15–20 days [16].

Table 1. Body weight, body composition, or serum biochemical markers affected by high-fat diet.

Reference	Species	Sex	Age or Weight	Groups	Energy Content	Main Fat Source of High-Fat Diet	Term	Effects
Nagai et al., 2005 [17]		Men	23.6 years old	Low-fat meal	70% Carbohydrates, 10% protein, and 20% fat	Butter, high-fat cream	210 min after meal	Thermoregulatory sympathetic nervous system (SNS) activity and a greater level of fat oxidation ↑ ($p < 0.05$)
				High-fat meal	20% carbohydrates, 10% protein, and 70% fat			
Meksawan et al., 2004 [13]	human	Men and women	Male (24.8 ± 1.0 years old)	Regular diet	54% carbohydrates, 16% protein, and 30% fat	Not found	7 d	HDL ↑ ($p < 0.05$); no difference in body weight
			Female (22.3 ± 1.3 years old)	High-fat diet	31% carbohydrates, 19% protein, and 50% fat			
Rowlands and Hopkins, 2002 [18]		Men	27 ± 5 years old	High-carbohydrate diet	70% carbohydrate, 15% protein, and 15% fat	High-fat meats, eggs and dairy products, nuts and seeds, low-starch vegetable products, and oils	Three 2-week dietary treatment	During exercise: 10%–20% plasma-glucose concentration ↑ ($p < 0.01$); plasma triacylglycerol ↑ ($p < 0.05$); 2.5–2.9 fold increase in the peak fat-oxidation rate ($p < 0.0001$)
				High-fat diet	15% carbohydrate, 15% protein, and 70% fat			
Linehan et al., 2018 [15]	Sprague–Dawley rats	Male	3 weeks old	Standard diet	58.5% carbohydrates, 28.7% protein, and 12.7% fat	Not found	7~28 d	No difference in body weight
				High-fat diet	44% Carbohydrates, 16% protein, and 40% fat			
Woodie and Blythe, 2018 [19]			6 weeks old	Control diet	44.3% carbohydrate and 5.8% fat	Not found	63 d	Final body weight, body weight change, fat pad weight, food intake, and kcal consumed ↑ ($p < 0.05$); no difference in fasting blood glucose
				High-fat diet	20% carbohydrate and 60% fat			

Table 1. Cont.

Reference	Species	Sex	Age or Weight	Groups	Energy Content	Main Fat Source of High-Fat Diet	Term	Effects
Cheng et al., 2017 [20]	Sprague–Dawley rats	Male	3 weeks old	Control diet	70% carbohydrates, 20% protein, and 10% lipid	Corn oil, milk fat	56 d	Central obesity, systolic and diastolic hypertension, impaired fasting glucose, hypertriglyceridaemia, and elevated non-HDL cholesterol level
				High-fat diet	20% carbohydrates, 20% protein, and 60% lipid			
Huang et al., 2004 [21]	Sprague–Dawley rats	Male	7 weeks old	Standard diet	57.99% carbohydrates, 28.50% protein, and 13.49% fat	Lard	56 d	Body weight, liver weight, adipose tissue, and relative liver weight ↑ ($p < 0.05$); the plasma cholesterol concentration, a-Amylase, b-Hydroxybutyrate, and Leptin ↑
				High-fat diet	AIN-76 diet, containing 20% fat			
Kanthé et al., 2021 [22]	albino Wistar rats	Not found	180–220 g	Control diet	60% carbohydrates, 18% protein, and 20% fat	Not found	21 d	Body weight ↑ ($p < 0.05$)
				High-fat diet	50% carbohydrates, 18% protein, and 30% fat			
Patil et al., 2019 [14]				Control group	60% carbohydrate, 18% protein, and 20% fat	Vegetable oil	22 d	Final body weight ↑ ($p < 0.05$); lipid peroxidation and oxidative stress ↑
				High-fat diet	50% carbohydrate, 18% protein, and 30% fat			

Table 1. Cont.

Reference	Species	Sex	Age or Weight	Groups	Energy Content	Main Fat Source of High-Fat Diet	Term	Effects
Maejima et al., 2020 [23]	Wistar rats	Male	8-week-old	Normal chow diet	20.5% protein and 10.1% fat	Not found	72 d	Body weight, energy intake, visceral fat, and subcutaneous fat ↑ ($p < 0.05$); muscle ↓
				High-fat diet	20.5% protein and 56.7% fat			
He et al., 2020 [24]	Wistar rats	Male	Not found	Standard diet	55.5% carbohydrates, 33.3% protein, and 11.2% fat	Not found	70 d	No difference in TG, TC, LDL-C, and HDL-C (serum)
				High-fat diet	28.6% carbohydrates, 26.2% protein, and 45.2% fat			
Schanuel et al., 2019 [16]	C57BL/6 mice	Male	12 weeks old	Standard chow	76% carbohydrates, 14% protein, and 10% lipids	Soybean oil, lard	20 d	Body weight and average fasting blood glucose were no significant differences between groups; inflammatory and fibroblast-like cells ↑ (10 days after, $p < 0.05$)
				High-fat chow	26% carbohydrates, 14% protein, and 60% lipids			

Table 1. Cont.

Reference	Species	Sex	Age or Weight	Groups	Energy Content	Main Fat Source of High-Fat Diet	Term	Effects
Emelyanova et al., 2019 [25]			6 weeks old	Standard chow	64.5% carbohydrate, 23.6% protein, and 11.9% fat	Lard	70 d	Body weight, gain of body weight ↑ ($p < 0.05$)
				High-fat diet	20% carbohydrate, 20% protein, and 60% fat			
Pang et al., 2016 [26]	C57BL/6 mice	Male	8 weeks old	Normal diet	65.42% carbohydrate, 22.47% protein, and 12.11% fat	Not found	90 d	Energy efficiency ↑ ($p < 0.01$); epididymal and perirenal fat weight ↑ ($p < 0.01$); insulin and glucose concentrations ↑ ($p < 0.05$)
				High-fat diet	20% carbohydrate, 20% protein, and 60% fat			
Topal et al., 2019 [27]	Swiss albino mice	Female	8–10 weeks old	Standard chow	66% carbohydrate, 24% protein, and 10% fat	Not found	63 d	Body weight, intraperitoneal adipose tissue; adrenal gland weight ↑ ($p < 0.01$)
				High-fat diet	23% carbohydrate, 17% protein, and 60% fat			

Note: ↑ and ↓ represent higher (↑) or lower (↓) values in the HFD group compared with the standard diet group or the low-fat diet group.

Feeding a high-fat diet for more than 8 weeks resulted in obesity in mice, regardless of whether the dietary fat source was lard, vegetable oil, peanut oil, corn oil, or soybean oil. High fat led to lower food intake but higher body weight and total fat pad weight [19]. Normal obesity on a high-fat diet in childhood may develop into overweight obesity in adulthood [23].

2.2. Changes in Serum Lipid Indexes

TG molecules represent the major storage and transport form of fatty acids within cells and in plasma. The liver is the central organ of fatty acid metabolism. Dietary fat is mainly hydrolyzed by pancreatic lipase and then emulsified by bile acid (BA), and the resulting lipid molecules are absorbed to synthesize TG [28]. Up to 70% of dietary fat is ingested by the body, and plasma TG levels are elevated during exercise [18]. The diet containing 60% fat was fed to mice for 56 days, and the mice developed hypertriglyceridemia [20]. However, one study showed that 45.2% dietary fat did not affect the plasma levels of total cholesterol (TC), TG, low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) in male Wistar rats [24].

The type of lipids in the diet affects the levels of plasma lipid profiles. A total of 21% lard in the diet induced a significant increase in body weight gain in Wistar rats and a significant increase in TG content in the plasma lipid profile, while other parameters were not affected [29]. C57BL/6 mice were fed diets containing lard, sunflower oil, soybean oil, lard mixed with sunflower oil, and lard mixed with soybean oil for 12 weeks and found that the lard diet resulted in increased TG levels, although the vegetable oil diet did not cause obesity but will cause cholesterol metabolism disorder [30]. Oils rich in unsaturated fatty acids, such as safflower oil, sunflower oil, and rapeseed, are more effective at lowering LDL-C than foods rich in saturated fatty acids, such as butter or lard [31].

Nonalcoholic fatty liver disease (NAFLD) is caused by excessive fat deposition in liver cells caused by a high-fat diet. A mixed diet of lard and soybean oil attenuated low-fat-high-carbohydrate diet-induced NAFLD by modulating genes and BA profiles in C57BL/6 mice. A lard-soybean oil mixture alleviates NAFLD by down-regulating fatty acid binding protein 2 (*FABP2*), fatty acid synthase (*FAS*), tumor necrosis factor receptor-associated factor 2 (*TRAF2*), activator protein-1 (*AP-1*), mitochondrially encoded cytochrome b (*MT-Cytb*), interleukin 6 (*IL-6*), and interleukin 1 (*IL-1*) genes; upregulating protein kinase AMP-activated catalytic subunit alpha 2 (*AMPK α 2*) and *HSL* genes and promoting the binding of BAs and BAs signaling receptor takeda G protein-coupled receptor 5 (TGR5) protein [31].

3. High-Fat Diet Alters Host Gut Microbiota Abundance and Diversity

3.1. High-Fat Diet Affects Gut Microbiome Composition

A high-fat diet will affect the composition and abundance of intestinal microbiota. The digested chyme stays in the large intestine for a long time. Therefore, more articles are studying the microbes of the large intestine and feces. Microbial composition in mouse and rat large intestines and feces was analyzed using 16S rRNA sequencing technology. When the taxonomic level is phylum, Firmicutes, Bacteroidetes, and Proteobacteria account for more than 90%. At the genus level, *Parabacteroides*, *Lachnospirillum*, *Oscillibacter*, *Lactobacillus*, *Akkermansia*, *Bacteroides*, and *Alistipes* appeared more frequently (Table 2).

Table 2. Gut microbial composition under high-fat diet treatment.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Song et al., 2021 [32]	C57BL/6 mice	Male	4 weeks old	70% carbohydrate, 10% fat, 20% protein	35% carbohydrate, 45% fat, 20% protein	14 weeks	Feces	Phylum: about 66% Firmicutes, 27% Bacteroidetes, 5% Proteobacteria, and 1% Tenericutes Genus: <i>Parabacteroides</i> , <i>Clostridiales</i> , <i>Lactobacillus</i> , <i>Akkermansia</i> , <i>Bacteroides</i> , and <i>Alistipes</i>	Increase: Firmicutes, Proteobacteria, Tenericutes, and <i>Lactobacillus</i> Reduce: Bacteroidetes, Parabacteroides, <i>Akkermansia</i> , <i>Bacteroides</i> , and <i>Alistipes</i>	Alpha: Not found Beta: the respective aggregation areas do not overlap
Han et al., 2021 [33]	C57BL/6 mice	Male	19–22 g	55.9% carbohydrate, 5.2% fat, 18% protein	41% carbohydrate, 30% fat, 13% protein	14 weeks	Feces	Phylum: about 40% Bacteroidetes, 29% Firmicutes, and 24% Proteobacteria Genus: Not found	Increase: Firmicutes and Proteobacteria Reduce: Bacteroidetes	Alpha: Not found Beta: the respective aggregation areas do not overlap
Lu et al., 2021 [34]	C57BL/6 mice	Male	7 weeks old	70% carbohydrate, 10% fat, 20% protein	20% carbohydrate, 60% fat, 20% protein	12 weeks	Feces	Phylum: about 55% Firmicutes, 40% Bacteroidetes, and 2% Proteobacteria Genus: <i>Alistipes</i> , <i>Blautia</i> , <i>Oscillibacter</i> , <i>Rikenella</i> , <i>Ruminiclostridium</i> , <i>Ruminococcaceae_UCG-014</i> , <i>Lachnospirillum</i> , <i>Lactococcus</i> , <i>Streptococcus</i>	Increase: Firmicutes, <i>Oscillibacter</i> Reduce: Bacteroidetes, Proteobacteria	Alpha: Not found Beta: the respective aggregation areas do not overlap

Table 2. Cont.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Wu et al., 2021 [35]	C57BL/6 mice	Male	20 ± 2 g	73.5% corn, 20% wheat bran, 5% fish meal, 1% farina, and 0.5% salt	20.8% carbohydrate, 60.9% fat, 18.3% protein, and 228 mg/kg cholesterol	16 weeks	Feces	<p>Phylum: Firmicutes, Bacteroidetes, TM7, Tenericutes, and Actinobacteria</p> <p>Genus: <i>Anaerostipes</i>, <i>Coprococcus</i>, <i>Blautia</i>, <i>Oscillospira</i>, <i>Ruminococcus</i>, <i>Allobaculum</i>, <i>Clostridium</i>, <i>Parabacteroides</i>, <i>Paraprevotella</i>, <i>Prevotella</i>, <i>Odoribacter</i>, <i>Butyricimonas</i>, AF12, <i>Bacteroides</i>, <i>Desulfovibrio</i>, and <i>Bilophila</i></p>	<p>Increase: <i>Anaerostipes</i>, <i>Coprococcus</i>, <i>Blautia</i>, <i>Oscillospira</i>, <i>Ruminococcus</i>, <i>Allobaculum</i>, <i>Clostridium</i>, <i>Odoribacter</i>, <i>Butyricimonas</i>, AF12, <i>Bacteroides</i>, <i>Desulfovibrio</i>, and <i>Bilophila</i></p> <p>Reduce: <i>Lactobacillus</i>, <i>Parabacteroides</i>, <i>Paraprevotella</i>, <i>Prevotella</i>, and <i>Bifidobacterium</i></p>	Alpha: Chao1, Observed species, Shannon ↓ Beta: CG and HF groups formed two distinct clusters
Islam et al., 2021 [36]	C57BL/6 mice	Male	5 weeks old	Not found	35% carbohydrate, 45% fat, 20% protein	14 weeks	Cecal contents	<p>Phylum: about 57% Firmicutes, 30% Bacteroidetes, and 12% Verrucomicrobia</p> <p>Family: Lachnospiraceae, Muribaculaceae, Ruminococcaceae, Akkermansiaceae, Erysipelotrichaceae, Bacteroidaceae, Clostridiaceae 1, Peptostreptococcaceae, and Burkholderiaceae</p>	Not found	Not found

Table 2. Cont.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Peng et al., 2020 [37]	C57BL/6 mice	Male	6 weeks old	70% carbohydrate, 10% fat, 20% protein	35% carbohydrate, 45% fat, 20% protein	12 weeks	Feces	Phylum: about 54% Bacteroidetes, and 42% Firmicutes Genus: <i>Ruminococcaceae_UCG_014</i> , <i>Eisenbergiella</i> , <i>Faecalibaculum</i> , <i>Prevotellaceae_UCG_001</i> , <i>Alloprevotella</i> , <i>Akkermansia</i> , and <i>Ruminococcus_2</i>	Increase: Bacteroidetes, <i>Ruminococcaceae_UCG_014</i> , <i>Eisenbergiella</i> , <i>Faecalibaculum</i> , <i>Prevotellaceae_UCG_001</i> , <i>Alloprevotella</i> , <i>Akkermansia</i> , and <i>Ruminococcus_2</i> Reduce: Firmicutes	Alpha: Sobs $\uparrow$ ($p < 0.01$), Shannon $\uparrow$ ($p > 0.05$) Beta: the respective aggregation areas do not overlap
Peng et al., 2020 [37]	C57BL/6 mice	Female	6 weeks old	70% carbohydrate, 10% fat, 20% protein	35% carbohydrate, 45% fat, 20% protein	12 weeks	Feces	Phylum: about 62% Firmicutes, 27% Bacteroidetes, and 8% Proteobacteria Genus: <i>Escherichia_Shigella</i> , <i>Blautia</i> , <i>Parabacteroides</i> , <i>Erysipetotoclostridium</i> , <i>Anaerotruncus</i> , <i>Ruminiclostridium_9</i> , <i>Lachnoclostridium</i> , <i>Ruminococcaceae_UCG_004</i> , <i>Streptococcus</i> , and <i>Acinetobacter</i>	Increase: Proteobacteria, <i>Escherichia_Shigella</i> , <i>Blautia</i> , <i>Parabacteroides</i> , <i>Erysipetotoclostridium</i> , <i>Anaerotruncus</i> , <i>Ruminiclostridium_9</i> , <i>Lachnoclostridium</i> , <i>Ruminococcaceae_UCG_004</i> , <i>Streptococcus</i> , <i>Lactococcus</i> , and <i>Acinetobacter</i> Reduce: Firmicutes and Bacteroidetes	Alpha: Sobs $\downarrow$ ($p < 0.01$), Shannon $\downarrow$ ($p < 0.05$) Beta: the respective aggregation areas do not overlap

Table 2. Cont.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Wang et al., 2020 [38]	C57BL/6 mice	Male	6 weeks old	Containing 10% fat by energy	Containing 60% fat by energy	16 weeks	Feces	Phylum: about 70% Firmicutes, 17% Bacteroidetes, 14% Proteobacteria Genus: <i>Allobaculum</i> , <i>Bacteroides</i> , <i>Lachnospiraceae</i> , <i>nspiraceae</i> , <i>NK4A136_group</i> , <i>Ruminiclostridium_9</i> , <i>Desulfovibrio</i> , <i>Ruminiclostridium_9</i> , <i>Alistipes</i> , <i>Coriobacteriaceae</i> , <i>UCC-002</i> , <i>Lactobacillus</i> , <i>Helicobacter</i> , and <i>Alloprevotella</i>	Increase: <i>Proteobacteria</i> , <i>Bacteroides</i> , <i>Lachnospiraceae</i> , <i>NK4A136_group</i> , <i>Desulfovibrio</i> , <i>Ruminiclostridium_9</i> , <i>Alistipes</i> , <i>Coriobacteriaceae</i> , <i>UCC-002</i> , <i>Lactobacillus</i> , and <i>Helicobacter</i> Reduce: Firmicutes, Bacteroidetes, and <i>Allobaculum</i>	Alpha: Not found Beta: the respective aggregation areas do not overlap
Xu et al., 2020 [39]	C57BL/6 mice	Male	4 weeks old	Normal chow diet	20% carbohydrate, 60% fat, 20% protein	9 weeks	Feces	Phylum: about 57% Firmicutes, 36% Bacteroidetes, 5% Proteobacteria, and 1% Verrucomicrobia Genus: <i>Allobaculum</i> , <i>Bacteroides</i> , <i>Lactococcus</i> , <i>Parabacteroides</i> , and <i>Odoribacter</i>	Increase: Firmicutes, Proteobacteria, Verrucomicrobia, and <i>Odoribacter</i> Reduce: Bacteroidetes, <i>Bacteroides</i> , and <i>Parabacteroides</i>	Alpha: Observed species, Chao, ACE, and Simpson ↓ Beta: the respective aggregation areas do not overlap

Table 2. Cont.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Won et al., 2020 [40]	C57BL/6 mice	Male	5 weeks old	Containing 10% fat by energy	Containing 60% fat by energy	10 weeks	Cecal contents	Phylum: about 44% Firmicutes, 28% Verrucomicrobia, 22% Proteobacteria, and 3% Bacteroidetes Family: Lachnospiraceae, Ruminococcaceae, Desulfovibrionaceae, Bacteridaceae, Helicobacteraceae, and Muribaculaceae	Increase: Firmicutes, Verrucomicrobia, Lachnospiraceae, and Desulfovibrionaceae Reduce: Bacteroidetes, Proteobacteria, Ruminococcaceae, Helicobacteraceae, and Muribaculaceae	Alpha: No significant change in the alpha diversity index Beta: the respective aggregation areas do not overlap
Jing et al., 2022 [11]	C57BL/6 mice	Male	7 weeks old	70% carbohydrate, 10% fat, 20% protein	35% carbohydrate, 45% fat, 20% protein	8 weeks	Cecal contents	Phylum: Firmicutes, Proteobacteria, Actinobacteria, Bacteroidetes Genus: <i>Aerococcus</i> , <i>Staphylococcus</i> , <i>Bacteroides</i> , <i>Adlercreutzia</i> , <i>Alistipes</i> , <i>Akkermansia</i> , <i>Parabacteroides</i> , <i>Turicibacter</i> , <i>Lachnospiraceae</i> NK4A136_group, and <i>norank_f_Lachnospiraceae</i>	Increase: Proteobacteria, Actinobacteria, <i>Turicibacter</i> , and <i>Lachnospiraceae</i> NK4A136_group Reduce: Firmicutes, Bacteroidetes, <i>Aerococcus</i> , <i>Adlercreutzia</i> , <i>Alistipes</i> , <i>Akkermansia</i> , <i>Parabacteroides</i> , and <i>norank_f_Lachnospiraceae</i>	Alpha: Chao, Shannon ↓ Beta: the respective aggregation areas do not overlap

Table 2. Cont.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Van et al., 2020 [41]	C57BL/6 mice	Male	9 weeks old	70% carbohydrate, 10% fat, 20% protein	Containing 60% fat by energy	8 weeks	Cecal contents	Phylum: Firmicutes, Bacteroidetes, Proteobacteria, and Verrucomicrobia Genus: <i>Akkermansia</i> , <i>Allobaculum</i> , <i>Ruminococcus</i> , <i>Oscillospira</i> , <i>Odoribacter</i> , <i>Parabacteroides</i> , and <i>Bacteroides</i>	Increase: <i>Ruminococcus</i> , <i>Oscillospira</i> , and <i>Odoribacter</i> Reduce: <i>Allobaculum</i> , <i>Parabacteroides</i> , and <i>Bacteroides</i>	Alpha: Shannon, Simpson, Chao 1 ↓ Beta: the respective aggregation areas do not overlap
Gu et al., 2019 [42]	C57BL/6 mice	Male	6 weeks old	48.2% cornstarch, 16.1% maltodextrin, 6.4% sucrose, 2.4% soybean oil, 1.6% lard	22.5% maltodextrin, 8.9% sucrose, 3.3% soybean oil, 30.1% lard	8 weeks	Cecal contents	Phylum: about 52% Firmicutes, 42% Bacteroidetes, and 5% Proteobacteria Genus: <i>Butyricimonas</i> , <i>Butyricicoccus</i> , <i>Rikenella</i> , <i>Anaerotruncus</i> , <i>Xylanibacter</i> , and <i>Parasutterella</i>	Increase: Bacteroidetes, Proteobacteria, <i>Butyricimonas</i> , <i>Rikenella</i> , <i>Xylanibacter</i> , <i>Butyricicoccus</i> , <i>Anaerotruncus</i> , and <i>Parasutterella</i> Reduce: Firmicutes, <i>Oscillibacter</i> , <i>Lachnospiraceae_incertae_sedis</i> , <i>Flavonifractor</i> , <i>Helicobacter</i> , <i>Bacteroides</i> , and <i>Pseudoflavonifractor</i>	Alpha: no significant difference in Shannon and Chao1 Beta: the respective aggregation areas do not overlap

Table 2. Cont.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Liu et al., 2019 [43]	C57BL/6 mice	Male	7 weeks old	Containing 9.4% fat by energy	Containing 40% fat by energy	28 weeks	Feces	Phylum: 53% Bacteroidetes, 39% Firmicutes, 7% Proteobacteria, and 1% Verrucomicrobia	Increase: Bacteroidetes, Proteobacteria, Bacteroidales_S24-7_group, Ruminococcaceae, Rikenellaceae, Helicobacteraceae, Desulfovibrionaceae, and Peptostreptococcaceae Reduce: Firmicutes, Verrucomicrobia, Lachnospiraceae, Erysipelotrichaceae, Lactobacillaceae, and Verrucomicrobiaceae	Alpha: Sobs, Shannon ↓ Beta: the respective aggregation areas do not overlap
								Family: Bacteroidales_S24-7_group, Lachnospiraceae, Ruminococcaceae, Rikenellaceae, Bacteroidaceae, Erysipelotrichaceae, Porphyromonadaceae, Lactobacillaceae, Helicobacteraceae, Desulfovibrionaceae, Verrucomicrobiaceae, and Peptostreptococcaceae		
Cao et al., 2020 [44]	C57BL/6 mice	Male	4 weeks old	All mice were fed a high-fat diet, and fat mice were compared with lean mice	7.2% cornstarch, 9.9% maltodextrin, 17.0% sucrose, 5.5% soybean oil, 39.4% lard	16 weeks	Colon contents	Phylum: about 56% Bacteroidetes, 35% Firmicutes and 2% Actinobacteria Genus: about 12% Alistipes, 10% Bacteroides, 6% Oscillibacter, 5% Ruminiclostridium, 3% Lachnospiraceae, 2% Lactobacillus, and 2% Faecalibaculum	Increase: Bacteroidetes, Alistipes, Oscillibacter, Ruminiclostridium, Odoribacter, and Alloprevotella Reduce: Firmicutes, Faecalibaculum, Lactobacillus, Bacteroides, Lachnospiraceae, and Akkermansia	Alpha: Simpson ↑ ($p < 0.05$); no significant difference in Sobs, Shannon, Chao1, ACE, and PD whole tree Beta: the microbial communities of the two groups were clustered separately and in close proximity

Table 2. Cont.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Watanabe et al., 2018 [45]	C57BL/6 mice	Male	7 weeks old	Not found	26% carbohydrate, 61% fat, 23% protein	4 weeks	Feces	<p>Phylum: about 73% Firmicutes, 15% Bacteroidetes, 4% Proteobacteria, and 1% Actinobacteria</p> <p>Family: Lachnospiraceae, Peptostrept-ococcaceae, Clostridiaceae, Defemb-acteraceae, Helicobacteraceae, Porph-yrromonadaceae, Rikenellaceae, Corio-bacteriaceae, Desulfovbrionaceae, Bcte-roidaceae, Peptococcaceae, Ruminoc-occaceae, Lactobacillaceae, and Streptococcaceae</p>	Not found	<p>Alpha: /</p> <p>Beta: the samples were closely clustered, and the microbiota composition between samples was similar</p>
Zheng et al., 2018 [46]	C57BL/6 mice	Male	6 weeks old	70% carbohydrate, 10% fat, 20% protein	35% carbohydrate, 45% fat, 20% protein	5 months	Feces	<p>Phylum: about 47% Firmicutes, 27% Bacteroidetes, 17% Proteobacteria, and 6% Actinobacteria</p> <p>Family: Coriobacteriaceae, Erysipel-otrichaceae, S24-7, Desulfovibrionaceae, Ruminococcaceae, Lachnospiraceae, Peptostreptococcaceae, Porphyromonadaceae, and Rikenellaceae</p>	<p>Increase: Bacteroidetes, Proteobacteria, Peptostreptococaceae, Porphyromonadaceae, and Coriobacteriaceae</p> <p>Reduce: Firmicutes, Actinobacteria, Bifidobacteriaceae, and Lactobacillaceae</p>	<p>Alpha: Not found</p> <p>Beta: there is a certain distance between the two sets of sample points</p>

Table 2. Cont.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Huang et al., 2021 [47]	Sprague-Dawley rats	Male	Not found	Standard chow diet (The Medical Laboratory-Animal Center in Guangdong, China)	15% lard, 20% sucrose, 10% casein, 1.2% cholesterol, 0.2% sodium cholate, and 53.6% standard chow diet	9 weeks	Colon contents	Phylum: about 91% Firmicutes, 5% Proteobacteria, and 3.4% Bacteroidetes Genus: <i>Bacteroides</i> , <i>Allobaculum</i> , <i>Blautia</i> , <i>Lachnospirillum</i> , <i>Parabacteroides</i> , <i>Staphylococcus</i> , <i>Fusicatenibacter</i> , <i>Shuttleworthia</i> , and <i>Ralstonia</i>	Increase: Firmicutes, Bacteroidetes, and <i>Ralstonia</i> Reduce: Proteobacteria, <i>Turicibacter</i> , <i>Acinetobacter</i> , <i>Brevundimonas</i> , and <i>Bacillus</i>	Alpha: Sobs, Shannon, Chao1, PD whole tree $\downarrow$ ($p < 0.01$); Goods coverage $\uparrow$ ($p < 0.01$) Beta: the respective aggregation areas do not overlap
Li et al., 2018 [48]	Sprague-Dawley rats	Male	200–220 g	Low-fat diet	80% low-fat diet feed + 10% egg yolk powder + 10% lard	8 weeks	Feces	Phylum: about 73% Firmicutes and 24% Bacteroidetes Genus: <i>Lactobacillus</i> , <i>Barnesiella</i> , <i>Prevotella</i> , <i>Pseudoflavonifractor</i> , <i>Lachnospirillum</i> , <i>Flavonifractor</i> , <i>Desulfovibrio</i> , <i>Oscillibacter</i> , and <i>Ruminiclostridium</i>	Increase: <i>Lactobacillus</i> , <i>Barnesiella</i> , <i>Desulfovibrio</i> , and <i>Oscillibacter</i> Reduce: <i>Lachnospirillum</i> , <i>Prevotella</i> , and <i>Pseudoflavonifractor</i>	Not found
Choi et al., 2017 [49]	ICR mice	Female	8 weeks old	Normal chow diet	Containing 60% fat by energy	12 weeks	Mixed colon and cecal contents	Phylum: about 86% Firmicutes, 8% Actinobacteria, 7% Bacteroidetes Genus: <i>Lactobacillus</i> , <i>Akkermansia</i> , <i>Bacteroides</i> , <i>Prevotella</i> , <i>Ruminococcus</i> , and <i>Dorea</i>	Increase: Firmicutes, Actinobacteria, <i>Lactobacillus</i> , and <i>Ruminococcus</i> Reduce: Bacteroidetes, <i>Prevotella</i> , and <i>Rikenellaceae</i>	Alpha: Not found Beta: the respective aggregation areas do not overlap

Table 2. Cont.

Reference	Species	Sex	Age or Weight	Control Group (CG) Diet	High-Fat Diet	Terms	Sample	Dominant Flora of HF	Compared with the CG	Diversity (HF vs. CG)
Zhao et al., 2017 [50]	Wistar rats	Male	160–180 g	70% carbohydrate, 10% fat, 20% protein	35% carbohydrate, 45% fat, 20% protein	10 weeks	Feces	Phylum: about 84% Firmicutes, 8% Bacteroidetes, and 2% Proteobacteria Genus: <i>Lachnospirillum</i> , <i>Ruminococcaceae_UCG-014</i> , <i>Bacteroidales_S24-7_group_norank</i> , <i>Ruminococcaceae_UCG-005</i> , <i>Bilophila</i> , <i>Eubacterium_coprostanoligenes_group</i> , and <i>Akkermansia</i>	Increase: Firmicutes, <i>Lachnospirillum</i> , and <i>Bilophila</i> Reduce: Bacteroidetes, Proteobacteria, <i>Ruminococcaceae_UCG-014</i> , <i>Bacteroidales_S24-7_group_norank</i> , <i>Ruminococcaceae_UCG-005</i> , <i>Eubacterium_coprostanoligenes_group</i> and <i>Akkermansia</i>	Alpha: Shannon ↓ Beta: the respective aggregation areas do not overlap

Note: ↑ and ↓ represent higher (↑) or lower (↓) values in the HFD group compared with the CG group.

The ratio of F/B (Firmicutes/Bacteroidetes) is generally considered to be associated with obesity [51]. Some studies suggest that F/B is elevated in obese individuals, while others show no significant change in F/B [52]. This suggests that elevated F/B in obese individuals is not inevitable. If we focus solely on the changes in both phyla, the level of dietary fat content will have an impact on composition. Notably, gut microbiota composition in high-fat diet-induced obese mice exhibited distinct shifts in phylum-level dominance, with Firmicutes and Bacteroidetes showing differential responsiveness to dietary fat content. While Firmicutes prevalence was generally amplified in mice consuming elevated dietary fat levels, Bacteroidetes abundance displayed an inverse correlation pattern under equivalent conditions. However, the results in some studies demonstrated outcomes contrary to the predominant trend described above.

A high-fat diet caused increased body weight, body composition, serum TGs, and cholesterol levels in mice. The gut microenvironment is influenced by diet, and microbial composition and diversity are altered to varying degrees. Therefore, there may be a link between changes in the microbiome and body weight and serum markers. A high-fat diet can affect the composition and abundance of the gut microbiota, triggering disturbances in the gut microbiota, which may cause a range of health problems. Christensenellaceae, Porphyromonadaceae, Rikenellaceae, *Ruminococcaceae* UCG 014, and *Ruminococcaceae* UCG 005 were negatively associated with obesity [50].

3.2. Alterations in Alpha Diversity May Be Related to Lipid Types

A considerable number of studies have shown that a high-fat diet can lead to a decrease in the Simpson, Shannon, and Chao index of gut microbes [35,37,39,41,43,47,53]. The decrease in gut microbial alpha diversity may not only be due to the effect of a high-fat diet but may also be related to interactions between lipid metabolism, inter-microbial, and microbial metabolites. Contrary to the popular belief that a high-fat diet reduces gut microbial diversity, it was elevated in a high-fat diet with lard as the main fat source compared with a low-fat diet, and linear discriminant analysis effect size (LEfSe) results indicated that the taxa features that best characterize the differences between the high-fat diet and low-fat diet groups were mainly those of the Rikenellaceae, Deferribacteraceae, Streptococcaceae, Christensenellaceae, and Peptococcaceae families [12].

High-fat diets typically use corn oil, peanut oil, soybean oil, and lard as the main fat sources. However, the fat source of the diet inducing obesity in mice was mainly lard. Different fat treatments affected the community composition of gut microbiota; for example, the abundance of Proteobacteria showed a decreasing trend in the lard, walnut, and peanut oil intervention groups, while flaxseed oil, olive oil, and canola oil showed a downward trend or increasing trend [54]. Lard has a synergistic effect with *Coriobacteriaceae*_UCG-002 in the cecum of Kunming mice (half male and female), and vegetable oil has a synergistic effect with *Akkermansia*, *Roseburia*, and *Enteractinococcus*. Among them, *Coriobacteriaceae*_UCG-002 showed a significant negative correlation with Glycolysis/Gluconeogenesis. *Roseburia* was most strongly associated with starch and sucrose metabolism [55].

4. Bile Acid—Fat Metabolism and Microbial Action

By binding to glycine (human) or taurine (rodent), BAs help limit passive reabsorption, promote micelle formation, and facilitate digestion and absorption of fats in the small intestine [56]. Bile acidolysis conjugation is carried out by bacteria with bile salt hydrolase (BSH) activity, such as *Lactobacillus*, *Bifidobacterium*, *Clostridium*, and *Bacteroidetes* with this functional BSH, resulting in a small amount of BA not being reabsorbed by the intestine back to the liver [57]. Another microbial metabolic pathway for BAs is catalyzed by bacteria

with hydroxysteroid dehydrogenases found in Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes [58].

In general, impaired gut microbial diversity affected by a high-fat diet was characterized by the highest abundance of Firmicutes, but elevated microbial diversity was characterized by the highest abundance of Bacteroidetes (Table 2). *Lactobacillus* is a member of the Firmicutes phylum. *Lactobacillus* abundance was positively correlated with the concentration of free BAs, which inhibit gut bacteria and modulate gut microflora [59]. *Parabacteroides distasonis* alleviates obesity-related metabolic dysregulation by producing succinate, which directly activates intestinal gluconeogenesis via fructose-1,6-bisphosphatase binding, paired with BA-mediated farnesoid X receptor (FXR) signaling to synergistically improve glucose and lipid homeostasis [60].

There is an interaction between microorganisms and BAs, and BAs inhibit the growth of BA-sensitive bacteria by promoting the growth of BA-metabolizing bacteria. BAs exert direct antibacterial effects through bacterial membrane damage, a mechanism demonstrable both in vitro and in vivo within the gut, and indirectly by activating ileal epithelial FXR signaling, which induces antimicrobial peptide expression in vivo [61,62]. This is one of the reasons for the altered diversity of the microbiota. In addition, a study suggests that BA metabolites produced by colonic microbes build a proinflammatory gut microenvironment that may further develop into various types of intestinal inflammation [63]. The enrichment of intestinal *Clostridia* causes an increase in the free BA content in the intestine, which stimulates the gastrointestinal tract and causes diarrhea [64].

5. Beneficial Effects of Probiotics on Mice Fed with High-Fat Diet

HFD has been shown to alter gut microbiota composition and BA profiles in murine models. Similarly, probiotic supplementation reverses HFD-induced adverse effects. The advent of high-throughput sequencing technologies has facilitated a surge in gut microbiome studies. Recognized as the “second genome” of animals, gut microbiota regulates a spectrum of physiological and biochemical processes. However, given the intricate regulatory networks among microbial communities, research focusing on strain-specific interventions to counteract HFD-associated dysbiosis has gained significant attention.

5.1. *Lactobacillus*

Certain strains within the *Lactobacillus* (specific strains to be subsequently described) have been identified as probiotics and have attracted considerable attention due to their ability to alleviate obesity and adipose tissue accumulation. Different strains have different effects on high-fat diet mice (Supplementary Table S1). *Lactiplantibacillus plantarum* FZU3013 reduced body weight and serum TG, TC, and LDL-c and increased the mRNA levels of liver cholesterol 7 α -hydroxylase (CYP7A1) and bile salt export pump (BSEP), indicating that BA synthesis was enhanced and the excretion of BA through feces was promoted [65]. *Lactiplantibacillus plantarum* NKK20 reduced TC and TG concentrations, increased the abundance of colonic *Akkermansia* and the concentration of short-chain fatty acids (SCFAs), and regulated BA anabolism [66]. *Lactiplantibacillus plantarum* strain CNCM I-4459 reduced LDL-c concentrations and downregulated liver *FAS*, perilipin (*PLIN*), and carnitine palmitoyltransferase-I-alpha (*CPT1 α*) genes [67]. *Lactiplantibacillus plantarum* FRT10 reduced body weight, fat weight, and liver TG concentration; upregulated the mRNA expression levels of liver peroxisome proliferator-activated receptor alpha (*PPAR α*) and carnitine palmitoyltransferase-1 alpha (*CPT1 α*); and downregulated the mRNA expression levels of liver sterol regulatory element-binding protein 1 (*SREBP-1*) and diacylglycerol acyltransferase 1 (*DGAT1*) [68]. *Lactiplantibacillus plantarum* NCHBL-004 induced glucagon-like peptide 1 (*GLP-1*) production and increased fecal SCFA levels [69]. *Lactiplantibacillus*

plantarum CQPC01 inhibited the increase in adipocyte volume, increased IL-4 and IL-10 content, downregulated the expression of CCAAT/enhancer binding protein alpha (*C/EBP-α*) and peroxisome proliferator-activated receptor gamma (*PPARγ*) mRNA, and upregulated the expression of *CYP7A1*, *CPT1*, lipoprotein lipase (*LPL*), catalase (*CAT*), superoxide dismutase 1 (*SOD1*), and *SOD2* [70]. *Lactiplantibacillus plantarum* SKO-001 promoted the increase in serum adiponectin, decreased the levels of leptin, insulin, TC, LDL-c, free fatty acid (FFA), and TG, and decreased the mRNA levels of *SREBP-1c* and *PPARγ* [71]. The extract of *Lactiplantibacillus plantarum* LMT1-48 reduced liver weight and TG levels and downregulated the lipogenic genes *PPARγ*, *HSL*, stearoyl-CoA desaturase-1 (*SCD-1*), and fatty acid translocase (*FAT* or *CD36*) in the liver, leading to a reduction in body weight and fat volume [72]. *Lactiplantibacillus plantarum* CQPC03 alleviated inflammation by increasing the levels of IL-4 and IL-10 and reducing the levels of proinflammatory factors, including IL-6, IL-1β, tumor necrosis factor-alpha (*TNF-α*), and interferon-gamma (*IFN-γ*) [73]. *Lactiplantibacillus plantarum* Shinshu N-07 reduces epididymal adipose tissue weight and adipocyte area and inhibits hepatic steatosis [74]. *Lactiplantibacillus plantarum* Y44 inhibited the expression of FAS and acetyl CoA carboxylases (*ACC*) in the liver of obese mice, up-regulated the expression of colonic tight junction proteins such as claudin-1 and occludin, reduced serum IL-8 and *TNF-α* levels, and increased the content of SCFA in feces [75]. *Lactiplantibacillus plantarum* HF02 inhibited pancreatic lipase activity in small intestinal contents, increased fecal TG levels, and reduced serum lipopolysaccharide (*LPS*), IL-1β, and *TNF-α* levels [76]. *Lactiplantibacillus plantarum* E2_MCCKT leads to upregulation of *PPAR-α* mRNA, downregulation of adipogenesis and fatty acid synthesis genes (*SREBP-1c*, *ACC*, and *FAS*), and downregulation of proinflammatory cytokine (IL-1Ra and *TNF-α*) expression [77]. *Lactiplantibacillus plantarum* KAD 8 restores metabolic health by normalizing glycemia, lipidomics, liver parameters, oxidative stress, and inflammatory parameters [78]. *Lactiplantibacillus plantarum* BXM2 reversed intestinal dysbiosis by increasing the ratio of villus height to crypt depth and the number of intestinal goblet cells and normalizing the mRNA expression of *TNF-α* and IL-6 [79]. *Lactiplantibacillus plantarum* DSR330 reduced the expression of *SREBP-1c*, *ACC1*, *FAS*, 1-aminocyclopropane-1-carboxylic acid oxidase (*ACO*), *PPARα*, and *CPT-1* in hepatocytes [80]. *Lactiplantibacillus plantarum* A29 downregulated the expression of lipogenic genes (*PPAR-γ*, *C/EBP-α*, and *C/EBP-β*) in adipocytes and alleviated the development of obesity by increasing phosphorylation and activation of p38 mitogen-activated protein kinase (*MAPK*), p44/42, and *AMPK-α* [81]. *Lactiplantibacillus plantarum* dfa1 reduced inflammatory cytokines in blood and colon tissue and decreased the relative abundance of Proteobacteria [82]. *Lactiplantibacillus plantarum* strain Ln4 reduced body weight and epididymal fat mass and decreased the protein levels of C-reactive protein (*CRP*), insulin-like growth factor binding protein-3 (*IGFBP-3*), and monocyte chemoattractant protein-1 (*MCP-1*) in white adipose tissue [83]. *Lactiplantibacillus plantarum* DSM20174 improved glucose and lipid homeostasis and reduced white adipose inflammation [84]. *Lactiplantibacillus plantarum* KC28 significantly upregulated *PPAR-gamma* co-activator-1 alpha (*PGC1-α*) and *CPT1-α* in the liver and downregulated *ACOX-1*, *PPAR-γ*, and *FAS* expression in mesenteric adipose tissue [85].

Lactocaseibacillus paracasei S0940 and *Streptococcus thermophilus* ldbm1 reduced serum and liver TC and TG levels in high-fat-fed mice [86]. *Bifidobacterium longum* BORI reduced the body weight of mice, *Lactobacillus acidophilus* AD031 and *Bifidobacterium bifidum* BGN4 reduced the TG level in the liver of mice, while *Bifidobacterium longum* BORI reduced the TC level in the liver [87]. *Lactocaseibacillus paracasei* 24 reduced body weight and fat deposition, decreased the ratio of Firmicutes/Bacteroidetes, and increased the abundance of *Akkermansia* [88]. *Lactocaseibacillus paracasei* K56 reduced the expression of *FAS* and *PPAR-γ* in the liver [89]. *Lactocaseibacillus paracasei* N1115 reduced visceral fat, liver weight, serum insulin,

and leptin levels; altered intestinal microbiota; and increased SCFA content [90]. *Lactica-seibacillus paracasei* X-1, *Lactica-seibacillus paracasei* X-17, and *Limosilactobacillus fermentum* BM-325 inhibited the growth of adipocyte volume and stabilized fasting blood glucose [91]. *Lactica-seibacillus paracasei* BEPC22 and *Lactiplantibacillus plantarum* BLP53 reduced white adipose tissue volume and adipocyte size, reduced the expression of PPAR γ in the liver, and increased the expression of PPAR α in white adipose tissue [92]. *Lactica-seibacillus paracasei* FZU103 reduced epididymal adipocyte hypertrophy, promoted fecal excretion of BAs, and increased the relative abundance of *Ruminococcus*, *Alistipes*, *Pseudoflavonifractor* and *Helicobacter* [93]. *Lactica-seibacillus paracasei* AO356 alters the relative abundance of microorganisms involved in lipid metabolism pathways and obesity-related markers, such as *Lactobacillus*, *Bacteroides*, and *Oscillospira* [94]. *Lactobacillus casei* CRL 431 exerts beneficial effects by reducing the proinflammatory cytokines IL-6, IL-17, and TNF- α [95].

Lactobacillus sakei MJM60958 reduced the expression of FAS, ACC, and SREBP-1 in the liver; upregulated the expression of PPAR α and CPT1A; and affected the regulation of intestinal flora by increasing the production of acetate [96]. *Latilactobacillus sakei* QC9 increased the abundance of butyrate-producing bacteria and the content of SCFA to mediate the microbiota-gut-liver axis, affecting the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway in the liver and alleviating the development of T2DM [97]. *Latilactobacillus sakei* WIKIM31 reduced body weight gain, epididymal fat mass, TG, and TC levels; significantly decreased the expression of lipogenesis-related genes in epididymal adipose tissue and liver; and promoted the production of intestinal short-chain fatty acids (such as butyrate and propionate) [98]. *Lactobacillus sakei* OK67 reduced LPS levels in blood and colon contents, colonic TNF- α and IL-1 β expression, and nuclear factor-kappaB (NF- κ B) activation; increased IL-10 and tight junction protein expression; and downregulated PPAR γ , FAS, and TNF- α expression in adipose tissue [99,100]. *Lactobacillus sakei* ADM14 reduced body weight gain and blood glucose levels, decreased the expression of lipid-related genes in the epididymal fat pad, decreased the ratio of Firmicutes to Bacteroidetes, and increased the relative abundance of *Bacteroides faecichinchillae* and *Alistipes* [40]. *Lactobacillus sakei* CJLS03 reduced the average size of adipocytes, decreased the gene expression of SREBP-1c, FAS, and SCD1 in epididymal adipose tissue, and increased the levels of SCFA in serum and feces [101].

Lactobacillus acidophilus reduces body weight, fat mass, inflammation, and insulin resistance and inhibits the toll-like receptor 4 (TLR4)/NF- κ B signaling pathway [102]. *Lactobacillus acidophilus* NX2-6 activated the insulin signaling pathway; promoted glucose uptake, glycolysis, and intestinal gluconeogenesis; inhibited hepatic gluconeogenesis; effectively lowered blood glucose levels and improved glucose tolerance; and improved liver energy metabolism through the fibroblast growth factor 21 (FGF21)/AMPK α /PGC-1 α /nuclear respiratory factor 1 (NRF1) pathway [103]. *Lactobacillus acidophilus* NS1 increased the expression of SREBP2 and low-density lipoprotein receptor (LDLR) in the liver [104]. *Lactobacillus acidophilus* LA5 reduced obesity, intestinal permeability defect, endotoxemia, and serum cytokines in mice and increased the relative abundance of *Akkermansia muciniphila* [105]. *Lactobacillus acidophilus* GOLDGUT-LA100 has high BSH activity, good gastric acid and bile salt tolerance, and alleviates the pathophysiological symptoms of high-fat diet-induced obese mice [106].

Lactobacillus fermentum CECT5716 increased the relative abundance of *Akkermansia* sp. and the proportion of Bacteroidetes [107]. *Limosilactobacillus fermentum* HNU312 reduced body weight, serum TG, TC, and LDL-c levels, significantly reduced fat accumulation in the liver and adipose tissue, and increased SCFA production [108]. *Limosilactobacillus fermentum* MG4231 reduced the expression of PPAR γ , C/EBP α , FAS, PLIN2, and LPL in epididymal tissue and reduced SREBP1-c and FAS in liver tissue [109]. *Limosilactobacillus fermentum* MG4294 and *Lactiplantibacillus plantarum* MG5289 reduced the levels of proinflammatory cytokines TNF- α , IL-1 β , and IL-6 in intestinal tissues [110]. *Lactobacillus fermentum* LM1016 improved glucose clearance and fatty liver and reduced inflammation in gonadal white adipose tissue in mice fed a high-fat diet [111]. *Lactobacillus fermentum* CKCC1858, *Lactobacillus fermentum* CKCC1369, *Lactiplantibacillus plantarum* CKCC1312, and *Lactobacillus gasseri* CKCC1913 alleviated liver and pancreatic damage, reduced blood lipids and the secretion of proinflammatory cytokines, increased liver antioxidant enzymes, and improved hyperlipidemia, inflammation, and oxidative stress [112].

Lactobacillus rhamnosus reduced serum IL-6 levels [113]. *Lactobacillus rhamnosus* TR08 promoted the increase in the relative abundance of Bifidobacteria and Bacteroidetes, thereby reshaping the intestinal flora, reducing the abundance of pathogenic bacteria *Enterococci*, and increasing the content of SCFAs [114]. *Lactobacillus rhamnosus* strain LRH05 modulated white adipose tissue monoacylglycerol O-acyltransferase 1 (*MOGAT1*), insulin-like growth factor-1 (*IGF-1*), *MCP-1*, and *F4/80* mRNA expression and increased butyrate- and propionate-producing bacteria (*Lachnospirillum*, *Romboutsia*, and *Fusobacterium*) [115]. *Lactobacillus rhamnosus* LA68 significantly reduced TC and HDL levels, while *Lactiplantibacillus plantarum* WCFS1 was more effective in reducing TG and LDL levels [116]. *Lactobacillus rhamnosus* GG restored exogenous leptin responsiveness, increased the ratio of villus height to crypt depth, and reduced the proportion of Proteobacteria in the fecal microbiota [117]. *Lactobacillus rhamnosus* LRA05 reduced body weight, blood lipid levels, and lipid accumulation in hepatocytes and epididymal adipose tissue; reduced the abundance of the pathogen-promoting bacterium *Streptococcus*; and inhibited blood and liver glucose content [118]. *Lactobacillus rhamnosus* GR-1 reduced the development of oxidative stress and chronic inflammation in a dose-dependent manner [119].

Lactobacillus gasseri SBT2055 reduced the number of macrophages in adipose tissue, the ratio of M1 macrophages to total macrophages was significantly reduced, and the expression of C-C motif ligand 2 (*CCL2*), C-C chemokine receptor 2 (*CCR2*), and leptin (*LEP*) was downregulated [120]. *Lactobacillus paragasseri* SBT2055 increased small intestinal lipid excretion into feces by reducing the mRNA levels of *FABP1*, *FABP2*, fatty-acid transport protein 4 (*FATP4*), *CD36*, and apolipoprotein B48 (*APOB48*) [121].

Lactobacillus johnsonii 3121 and *Lactobacillus rhamnosus* 86 downregulated the expression of genes related to adipogenesis and normalized the obesity-associated intestinal microbiota [122]. *Lactobacillus johnsonii* JNU3402 reduced the expression of hepatic SREBP-1c, FAS, and ACC; inhibited SREBP-1c transcriptional activity by enhancing protein kinase A (PKA)-mediated phosphorylation; and reduced the expression of its lipogenic target genes in alpha mouse liver 12 (AML12) and HepG2 cells, thereby attenuating hepatic lipid accumulation [123].

Lactobacillus curvatus HY7601 and *Lactiplantibacillus plantarum* KY1032 upregulate the expression of cholesterol transport genes in the liver and jejunum, including liver X receptors alpha (*LXR α*), ATP-binding cassette transporters G (*ABCG*) 5 and *ABCG8*, and *CYP7A1* [124]. *Lactobacillus curvatus* HY7601 and *Lactiplantibacillus plantarum* KY1032 upregulate the expression of fatty acid oxidation-related genes (*PGC1 α* , *CPT1*, *CPT2*, and *ACOX1*) in the liver and reduce the expression of proinflammatory genes (TNF α , IL6, IL1 β , and *MCP1*) in adipose tissue [125].

Lactobacillus reuteri FN04 reduces hepatic FAS overexpression, increases SREBP1c expression, improves intestinal epithelial barrier function, and induces the intestinal microbiota to produce SCFAs [126]. *Limosilactobacillus reuteri* BIO7251 reduced subcutaneous adipose tissue mass, glucose absorption, and food intake [127]. *Lactobacillus rhamnosus* FJSYC4-1 and *Lactobacillus reuteri* FGSZY33L6 alleviated weight gain, blood sugar, and lipid disorders; regulated intestinal flora; and produced SCFA [128].

Lactobacillus amylovorus KU4 increased the expression of uncoupling protein 1 (UCP1), PPAR γ , and PGC-1 α in subcutaneous inguinal white adipose tissue, reduced receptor-interacting protein 140 (RIP140) expression, and released RIP140 to stimulate UCP1 expression, thereby increasing the interaction between PPAR γ and PGC-1 α , thereby promoting the browning of white adipocytes [129]. *Ligilactobacillus Salivarius* LCK11 inhibited food intake by significantly increasing the transcription and translation levels of peptide tyrosine tyrosine (PYY) and, ultimately, the serum PYY level, which is attributed to the activation of the TLR2/NF- κ B signaling pathway in enteroendocrine L cells by the peptidoglycan of LCK11 [130]. *Lactobacillus kefir* DH5 upregulated the expression of PPAR- α , FABP4 and CPT1 in epididymal adipose tissue, stimulated fatty acid oxidation, and reduced obesity [131]. *Lactobacillus pentosus* S-PT84 improved intestinal integrity by maintaining tight junction protein expression to inhibit LPS from entering the blood and reduced the secretion of TNF- α and MCP-1 to inhibit systemic inflammatory response [132]. *Lactobacillus delbrueckii* subsp. *lactis* CKDB001 reduced liver TG and TC levels without significantly affecting the expression of genes related to lipid metabolism [133]. *Lactobacillus coryniformis* supsp. *torquens* T3 inhibits liver inflammation and oxidative stress damage by regulating the LPS inflammatory pathway in the liver, enhances the mechanical function of the intestinal barrier, and increases the content of short-chain fatty acids [134].

5.2. Bifidobacterium (Supplementary Table S2)

Bifidobacterium longum subsp. *infantis* YB0411 reduced body weight and fat weight [135]. *Bifidobacterium longum* subsp. *infantis* FB3-14 reduced the Firmicutes/Bacteroidetes ratio and increased the abundance of *Akkermansia muciniphila*, unclassified_Muribaculaceae, *Lachnospiraceae_NK4A136_group*, and *Bifidobacterim* [136]. *Lactiplantibacillus plantarum* LC27 and *Bifidobacterium longum* LC67 increased the expression of claudin-1 and occludin in the colon and reduced the level of Firmicutes and Proteobacteria and fecal LPS [137]. *Bifidobacterium longum* subsp. *longum* BL21 reduced serum TC, TG, and LDL-c levels, improved fat vacuolization in hepatocytes and epididymal fat accumulation, and reduced the Firmicutes/Bacteroidetes ratio [138].

Bifidobacterium adolescentis IM38 increased colonic IL-10 and tight junction protein expression, downregulated NF- κ B activation and TNF expression, and reduced blood and colonic content LPS levels, as well as the ratio of Proteobacteria to Bacteroidetes [139]. *Bifidobacterium adolescentis* (BA3, BA5, Z25) and *Lactobacillus rhamnosus* (LGG, L7-1, L10-1) increased the concentration of SCFA in the intestine of mice, among which *Lactobacillus rhamnosus* LGG regulated energy metabolism and lipid metabolism, and *Lactobacillus rhamnosus* L10-1 reduced liver inflammation [140].

Bifidobacterium animalis subsp. *lactis* lkm512 improved hepatic lipid accumulation and intestinal barrier function [141]. *Bifidobacterium animalis* subsp. *lactis* MN-Gup significantly reduced fasting blood glucose levels, increased SCFA levels, increased the relative abundance of *Bifidobacterium*, and reduced the relative abundance of *Escherichia-Shigella* and *Staphylococcus* [142].

Bifidobacterium lactis IDCC 4301 reduced body weight and adipose tissue weight, increased blood lipid levels, and downregulated mRNA expression of adipogenesis-related genes [143]. *Bifidobacterium breve* strain B-3 dose-dependently inhibited body weight and

epididymal fat accumulation, increased serum TC, fasting blood glucose, and insulin levels, and significantly increased intestinal *bifidobacterium* counts [144]. *Bifidobacterium* CECT 7765 reduced serum levels of leptin, IL-6, and monocyte chemotactic protein-1 while increasing IL-4 levels [145]. *Bifidobacterium bifidum* DS0908 reduced body weight and epididymal fat accumulation and serum TG, LDL-c, and TC levels [146].

5.3. Other Probiotics (Supplementary Table S3)

Bacillus coagulans BC69 reduced body weight and increased acetate and butyrate concentrations in feces [147]. A probiotic mixture consisting of five different *Bacillus* species (*sonorensis* JJY12-3, *paralicheniformis* JJY12-8, *sonorensis* JJY13-1, *sonorensis* JJY 13-3, and *sonorensis* JJY 13-8) increased the hepatic expression of lipid oxidation genes, downregulated the expression of genes for lipid uptake and lipogenesis, and reduced lipid accumulation in subcutaneous and mesenteric adipose tissue [148]. *Bacillus amyloliquefaciens* SC06 improved the antioxidant capacity of mice through the Nrf2/Keap1 signaling pathway and reduced the ratio of Firmicutes/Bacteroidetes [149]. *Bacillus coagulans* T4 inhibits the accumulation of macrophages in white adipose tissue, converts M1 macrophages into M2 macrophages, reduces TLR4 gene mRNA expression, increases the number of *Lactobacillus* and *Faecalibacterium*, and increases propionate and acetate levels [150]. *Bacillus licheniformis* reduces body weight, serum and liver TG, and epididymal fat weight; reduces liver fat deposition; and significantly changes the colonic bacterial community of obese mice [151].

Bacteroides vulgatus leads to reduced 5-hydroxytryptamine (5-HT) synthesis in jejunal enterochromaffin cells and reduced chylomicron uptake in the jejunal mesentery after HFD in Tph1ΔIEC, thereby alleviating HFD-induced obesity and metabolic dysfunction [152]. *Bacteroides thetaiotaomicron* increases the proportion of polyunsaturated fatty acids in the liver and prevents hepatic steatohepatitis and liver damage [153]. *Bacteroides ovatus* reduces serum LPS, CD163, IL-1β, and TNF-α levels and downregulates genes for de novo lipogenesis in the liver (SREBF1, ACC, SCD1, and FAS), accompanied by upregulation of genes related to fatty acid oxidation (PPARα) [154].

Pediococcus pentosaceus PP04 reduces serum TC, TG, LDL-C, FFA, leptin, LPS, and TNF-α levels; downregulates liver SREBP-1c, FAS, and SCD1 to inhibit lipogenesis; and significantly increases the expression of tight junction proteins such as occludin, claudin-1 and zonula occludens-1 (ZO-1) to improve the abnormal increase in intestinal permeability, thereby reducing liver LPS concentration and alleviating intestinal inflammation caused by a high-fat diet through the NF-κB/Nrf2 signaling pathway [155,156]. *Clostridium cochlearium* reduces body weight, fat mass, fasting blood glucose, and SCFA levels [157]. *Clostridium tyrobutyricum* reduces liver PPARγ expression, upregulates AMPK, PPARα, ATGL, and HSL expression, reduces the expression of TNF-α, IL-6, and IL-1β in the colon, and upregulates the expression of tight junction proteins [158].

Enterococcus faecium SF68 improves intestinal barrier integrity and function in obese mice by increasing the expression of tight junction proteins and intestinal butyrate transporter [159]. *Blautia producta* can inhibit cellular lipid accumulation and improve hyperlipidemia [160]. *Leuconostoc mesenteroides* subsp. *mesenteroides* SD23 reduces the height of intestinal villi, reduces the expression of TNF-α in the liver, and increases the expression of IL-10 [161]. *Roseburia hominis* inhibits the expansion of white adipose tissue in mice fed a high-fat diet, which is partly attributed to the production of nicotinamide riboside and the upregulation of the Sirtuin1/mTOR signaling pathway [162]. *Coproccoccus* can effectively reverse HFD-induced hepatic lipid accumulation, inflammation, and fibrosis in mice [163]. *Akkermansia muciniphila* alleviated high-fat diet-induced weight gain, hepatic steatosis, and liver damage; decreased *Alistipes*, *Lactobacilli*, *Tyzzereella*, *Butyricimonas*, and *Blautia*; and increased *Clostridium*, *Oscilibacter*, *Allobaculum*, *Anaeroplasma*, and *Rikenella*. *Akkerman-*

sia muciniphila regulated the intestinal FXR-FGF15 axis and remodeled BA construction, reducing secondary BAs in the cecum and liver, including deoxycholic acid (DCA) and lithocholic acid (LCA) [164].

6. Summary

A high-fat diet is the main cause of obesity. Containing more than 30% dietary fat can be called a high-fat diet. Short-term high-fat diets of less than 2 weeks resulted in almost no weight gain, while more than 8 weeks resulted in a significant increase in fat deposition. Dietary fat is digested and absorbed into the blood in the small intestine, and when it reaches a certain amount, it will cause an increase in TC, TG, LDL-C, and HDL-C in plasma, which is related to many metabolic diseases.

High-fat diets lead to altered gut microbiome profiles. Firmicutes and Bacteroidetes usually account for more than 90%. The F/B ratio in obese mice is generally thought to be elevated, but there are exceptions. Gut microbiota composition in high-fat diet-induced obese mice exhibited distinct shifts in phylum-level dominance, with Firmicutes and Bacteroidetes showing differential responsiveness to dietary fat content. A high-fat diet leads to a decrease in the alpha diversity of gut microbes, and different lipid types may influence changes in microbial abundance. Family-level microorganisms such as Rikenellaceae, Deferribacteraceae, Streptococcaceae, Christensenellaceae, and Peptococcaceae may be important biomarkers that differentiate high-fat diets from normal diets.

BAs are key to the emulsification of fats in the small intestine. Small amounts of BAs are conjugated by BSH-active microorganisms in the gut, including *Lactobacillus*, *Bifidobacterium*, *Clostridium*, and *Bacteroides*, thereby preventing their entry into the enterohepatic circulation. Decreased gut microbial alpha diversity related to the levels of BAs. Adequate BA levels are beneficial for maintaining gut health and weight management. Supplementing probiotics can reverse the negative effects of obesity caused by a high-fat diet in mice, including weight gain, increased serum TG, TC, and LDL contents, increased adipocyte area, increased gene expression of lipogenic genes in the liver and adipose tissue, and a damaged intestinal barrier.

The murine model of HFD is widely used in obesity-related research. HFD-induced metabolic disorders and inflammatory processes exhibit bidirectional interplay with the gut microbiota (Figure 1). The advancement of high-throughput technologies has led to a growing number of studies investigating microbial strains that mitigate the adverse effects of HFD, although most of these strains belong to the *Lactobacillus*. However, research on strains from genera identified by 16S rRNA sequencing and metagenomic analyses as taxa potentially linked to probiotic properties, such as *Bifidobacterium*, *Akkermansia*, *Prevotella*, and *Oscilibacter*, remains limited, as these techniques primarily reveal taxonomic associations rather than direct evidence of probiotic functions. Future studies should prioritize mechanistic investigations of strains within these genera to evaluate their probiotic potential.

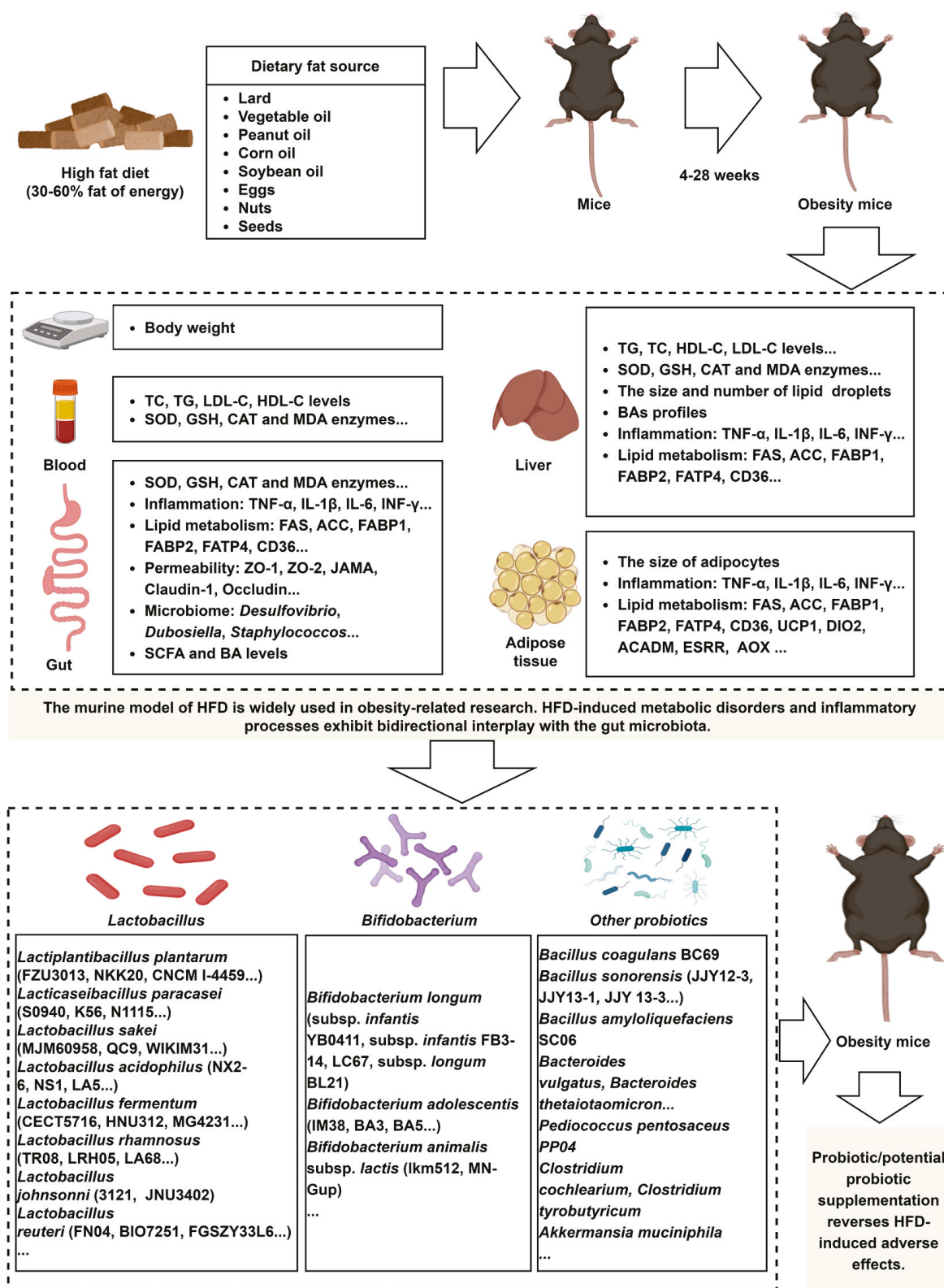


Figure 1. A HFD induces dysregulation of lipid metabolism and immune responses in mice, and probiotic supplementation effectively reverses these detrimental effects. The image material in Figure 1 comes from www.biorender.com.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/nu17091402/s1>, Table S1: Effects of oral administration of a strain (belonging to the *Lactobacillus*) on mice fed a high-fat diet; Table S2: Effects of oral administration

of a strain (belonging to the *Bifidobacterium*) on mice fed a high-fat diet; Table S3: Effects of oral administration of a strain (other probiotics/potential probiotic) on mice fed a high-fat diet.

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Abbreviations

The following abbreviations are used in this manuscript.

5-HT	5-hydroxytryptamine
ABCG	ATP-binding cassette transporters G
ACC	acetyl CoA carboxylases
ACO	1-aminocyclopropane-1-carboxylic acid oxidase
AML12	alpha mouse liver 12
AMPK α 2	kinase AMP-activated catalytic subunit alpha 2
AP-1	activator protein-1
APOB48	apolipoprotein B48
ATGL	adipose triglyceride lipase
BA	bile acid
BSEP	bile salt export pump
BSH	bile salt hydrolase
C/EBP- α	CCAAT/enhancer binding protein alpha
CAT	catalase
CCL2	C-C motif ligand 2
CCR2	C-C chemokine receptor 2
CPT1 α	carnitine palmitoyltransferase-1 alpha
CPTI α	carnitine palmitoyltransferase-I-alpha
CRP	C-reactive protein
CYP7A1	cholesterol 7 α -hydroxylase
DCA	deoxycholic acid
DGAT1	diacylglycerol acyltransferase 1
F/B	Firmicutes/Bacteroidetes
FABP2	fatty acid binding protein 2
FAS	fatty acid synthase
FAT/CD36	fatty acid translocase
FATP4	fatty-acid transport protein 4
FFA	free fatty acid
FGF21	fibroblast growth factor 21
FXR	farnesoid X receptor
GLP-1	glucagon-like peptide 1
HDL-C	high-density lipoprotein cholesterol
HFD	high fat diet
HSL	hormone-sensitive triglyceride lipase
IFN- γ	interferon-gamma

IGF-1	insulin-like growth factor-1
IGFBP-3	insulin-like growth factor binding protein-3
IL-1	interleukin 1
IL-6	interleukin 6
LCA	lithocholic acid
LDL-C	low-density lipoprotein cholesterol
LDLR	low-density lipoprotein receptor
LEfSe	linear discriminant analysis effect size
LEP	leptin
LPL	lipoprotein lipase
LPS	lipopolysaccharide
LXR α	liver X receptors alpha
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemoattractant protein-1
MOGAT1	monoacylglycerol O-acyltransferase 1
MT-Cytb	mitochondrially encoded cytochrome b
NAFLD	nonalcoholic fatty liver disease
NF- κ B	nuclear factor-kappaB
NRF1	nuclear respiratory factor 1
PGC1- α	PPAR-gamma co-activator-1 alpha
PI3K	phosphatidylinositol 3-kinase
PKA	protein kinase A
PLIN	perilipin
PPAR α	peroxisome proliferator-activated receptor alpha
PPAR γ	peroxisome proliferator-activated receptor gamma
PYY	peptide tyrosine tyrosine
RIP140	receptor-interacting protein 140
SCD-1	stearoyl-CoA desaturase-1
SCFAs	short-chain fatty acids
SNS	sympathetic nervous system
SOD1	superoxide dismutase 1
SREBP-1	sterol regulatory element-binding protein 1
TC	total cholesterol
TG	triglyceride
TGR5	takeda G protein-coupled receptor 5
TLR4	toll-like receptor 4
TNF- α	tumor necrosis factor-alpha
TRAF2	tumor necrosis factor receptor-associated factor 2
UCP1	uncoupling protein 1
ZO-1	zonula occludens-1

References

1. Lotrean, L.M.; Stan, O.; Lencu, C.; Laza, V. Dietary patterns, physical activity, body mass index, weight-related behaviours and their interrelationship among Romanian university students-trends from 2003 to 2016. *Nutr. Hosp.* **2018**, *35*, 375–383. [PubMed]
2. Rocha, N.P.; Milagres, L.C.; Filgueiras, M.S.; Suhett, L.G.; Silva, M.A.; Albuquerque, F.M.; Ribeiro, A.Q.; Vieira, S.A.; Novaes, J.F. Association of dietary patterns with excess weight and body adiposity in Brazilian children: The Pase-Brasil study. *Arq. Bras. Cardiol.* **2019**, *113*, 52–59. [CrossRef] [PubMed]
3. Ang, Q.Y.; Alba, D.L.; Upadhyay, V.; Bisanz, J.E.; Cai, J.; Lee, H.L.; Barajas, E.; Wei, G.; Noecker, C.; Patterson, A.D.; et al. The East Asian gut microbiome is distinct from colocalized White subjects and connected to metabolic health. *Elife* **2021**, *10*, e70349. [CrossRef]
4. Lampuré, A.; Castetbon, K.; Hanafi, M.; Deglaire, A.; Schlich, P.; Péneau, S.; Hercberg, S.; Méjean, C. Relative influence of socioeconomic, psychological and sensory characteristics, physical activity and diet on 5-year weight gain in French adults. *Nutrients* **2017**, *9*, 1179. [CrossRef]

5. Viola, P.C.A.F.; Carvalho, C.A.; Bragança, M.L.B.M.; França, A.K.T.D.C.; Alves, M.T.S.S.B.E.; da Silva, A.A.M. High consumption of ultra-processed foods is associated with lower muscle mass in Brazilian adolescents in the RPS birth cohort. *Nutrition* **2020**, *79–80*, 110983. [CrossRef] [PubMed]
6. Aghayan, M.; Asghari, G.; Yuzbashian, E.; Mahdavi, M.; Mirmiran, P.; Azizi, F. Secular trend in dietary patterns of Iranian adults from 2006 to 2017: Tehran lipid and glucose study. *Nutr. J.* **2020**, *19*, 110. [CrossRef]
7. Bäckhed, F.; Ding, H.; Wang, T.; Hooper, L.V.; Koh, G.Y.; Nagy, A.; Semenkovich, C.F.; Gordon, J.I. The gut microbiota as an environmental factor that regulates fat storage. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15718–15723. [CrossRef]
8. Requena, T.; Velasco, M. The human microbiome in sickness and in health. *Rev. Clínica Española* **2021**, *221*, 233–240. [CrossRef]
9. Wilson, A.S.; Koller, K.R.; Ramaboli, M.C.; Nesengani, L.T.; Ocvirk, S.; Chen, C.; Flanagan, C.A.; Sapp, F.R.; Merritt, Z.T.; Bhatti, F.; et al. Diet and the human gut microbiome: An international review. *Dig. Dis. Sci.* **2020**, *65*, 723–740. [CrossRef]
10. Mancabelli, L.; Milani, C.; Lugli, G.A.; Turrone, F.; Ferrario, C.; van Sinderen, D.; Ventura, M. Meta-analysis of the human gut microbiome from urbanized and pre-agricultural populations. *Environ. Microbiol.* **2017**, *19*, 1379–1390. [CrossRef]
11. Jing, Y.S.; Ma, Y.F.; Pan, F.B.; Li, M.S.; Zheng, Y.G.; Wu, L.F.; Zhang, D.S. An insight into antihyperlipidemic effects of polysaccharides from natural resources. *Molecules* **2022**, *27*, 1903. [CrossRef] [PubMed]
12. Wang, B.; Kong, Q.; Li, X.; Zhao, J.; Zhang, H.; Chen, W.; Wang, G. A High-fat diet increases gut microbiota biodiversity and energy expenditure due to nutrient difference. *Nutrients* **2020**, *12*, 3197. [CrossRef] [PubMed]
13. Meksawan, K.; Pendergast, D.R.; Leddy, J.J.; Mason, M.; Horvath, P.J.; Awad, A.B. Effect of low and high fat diets on nutrient intakes and selected cardiovascular risk factors in sedentary men and women. *J. Am. Coll. Nutr.* **2004**, *23*, 131–140. [CrossRef]
14. Patil, B.S.; Kanthe, P.S.; Reddy, C.R.; Das, K.K. *Emblica officinalis* (Amla) ameliorates high-fat diet induced alteration of cardiovascular pathophysiology. *Cardiovasc. Hematol. Agents Med. Chem.* **2019**, *17*, 52–63. [CrossRef]
15. Linehan, V.; Fang, L.Z.; Hirasawa, M. Short-term high-fat diet primes excitatory synapses for long-term depression in orexin neurons. *J. Physiol.* **2018**, *596*, 305–316. [CrossRef]
16. Schanuel, F.S.; Romana-Souza, B.; Monte-Alto-Costa, A. Short-term administration of a high-fat diet impairs wound repair in mice. *Lipids* **2020**, *55*, 23–33. [CrossRef] [PubMed]
17. Nagai, N.; Sakane, N.; Moritani, T. Metabolic responses to high-fat or low-fat meals and association with sympathetic nervous system activity in healthy young men. *J. Nutr. Sci. Vitaminol.* **2005**, *51*, 355–360. [CrossRef]
18. Rowlands, D.S.; Hopkins, W.G. Effects of high-fat and high-carbohydrate diets on metabolism and performance in cycling. *Metabolism* **2002**, *51*, 678–690. [CrossRef]
19. Woodie, L.; Blythe, S. The differential effects of high-fat and high-fructose diets on physiology and behavior in male rats. *Nutr. Neurosci.* **2018**, *21*, 328–336. [CrossRef]
20. Cheng, H.S.; Ton, S.H.; Phang, S.C.W.; Tan, J.B.L.; Abdul Kadir, K. Increased susceptibility of post-weaning rats on high-fat diet to metabolic syndrome. *J. Adv. Res.* **2017**, *8*, 743–752. [CrossRef]
21. Huang, B.W.; Chiang, M.T.; Yao, H.T.; Chiang, W. The effect of high-fat and high-fructose diets on glucose tolerance and plasma lipid and leptin levels in rats. *Diabetes Obes. Metab.* **2004**, *6*, 120–126. [CrossRef] [PubMed]
22. Kanthe, P.S.; Patil, B.S.; Das, K.K. Terminalia arjuna supplementation ameliorates high fat diet-induced oxidative stress in nephrotoxic rats. *J. Basic Clin. Physiol. Pharmacol.* **2021**, *33*, 409–417. [CrossRef]
23. Maejima, Y.; Yokota, S.; Horita, S.; Shimomura, K. Early life high-fat diet exposure evokes normal weight obesity. *Nutr. Metab.* **2020**, *17*, 48. [CrossRef] [PubMed]
24. He, Y.; Shi, M.; Wu, J.; Sun, Z.; Guo, J.; Liu, Y.; Han, D. Effects of a high-fat diet on intracellular calcium (Ca²⁺) handling and cardiac remodeling in Wistar rats without hyperlipidemia. *Ultrastruct. Pathol.* **2020**, *44*, 42–51. [CrossRef]
25. Emelyanova, L.; Boukatina, A.; Myers, C.; Oyarzo, J.; Lustgarten, J.; Shi, Y.; Jahangir, A. High calories but not fat content of lard-based diet contribute to impaired mitochondrial oxidative phosphorylation in C57BL/6J mice heart. *PLoS ONE* **2019**, *14*, e0217045. [CrossRef]
26. Pang, J.; Xi, C.; Huang, X.; Cui, J.; Gong, H.; Zhang, T. Effects of excess energy intake on glucose and lipid metabolism in C57BL/6 mice. *PLoS ONE* **2016**, *11*, e0146675. [CrossRef]
27. Topal, F.; Goren, H.; Yucel, F.; Sahinturk, V.; Aydar, Y. Effect of consuming high-fat diet on the morphological parameters of adrenal gland. *Bratisl. Med. J.* **2019**, *120*, 593–600. [CrossRef] [PubMed]
28. Alves-Bezerra, M.; Cohen, D.E. Triglyceride metabolism in the liver. *Compr. Physiol.* **2017**, *8*, 1–8. [CrossRef]
29. Muniz, L.B.; Alves-Santos, A.M.; Camargo, F.; Martins, D.B.; Celes, M.R.N.; Naves, M.M.V. High-lard and high-cholesterol diet, but not high-lard diet, leads to metabolic disorders in a modified dyslipidemia model. *Arq. Bras. De Cardiol.* **2019**, *113*, 896–902. [CrossRef]
30. Yan, S.; Liu, S.; Qu, J.; Li, X.; Hu, J.; Zhang, L.; Liu, X.; Li, X.; Wang, X.; Wen, L.; et al. A lard and soybean oil mixture alleviates low-fat-high-carbohydrate diet-induced nonalcoholic fatty liver disease in mice. *Nutrients* **2022**, *14*, 560. [CrossRef]
31. Schwingshackl, L.; Bogensberger, B.; Benčić, A.; Knüppel, S.; Boeing, H.; Hoffmann, G. Effects of oils and solid fats on blood lipids: A systematic review and network meta-analysis. *J. Lipid Res.* **2018**, *59*, 1771–1782. [CrossRef] [PubMed]

32. Song, H.; Shen, X.; Wang, F.; Li, Y.; Zheng, X. Black current anthocyanins improve lipid metabolism and modulate gut microbiota in high-fat diet-induced obese mice. *Mol. Nutr. Food Res.* **2021**, *65*, e2001090. [CrossRef] [PubMed]
33. Han, H.S.; Lee, H.H.; Gil, H.S.; Chung, K.S.; Kim, J.K.; Kim, D.H.; Yoon, J.; Chung, E.K.; Lee, J.K.; Yang, W.M.; et al. Standardized hot water extract from the leaves of *Hydrangea serrata* (Thunb.) Ser. alleviates obesity via the AMPK pathway and modulation of the gut microbiota composition in high fat diet-induced obese mice. *Food Funct.* **2021**, *12*, 2672–2685. [CrossRef] [PubMed]
34. Lu, L.; Tang, M.; Li, J.; Xie, Y.; Li, Y.; Xie, J.; Zhou, L.; Liu, Y.; Yu, X. Gut microbiota and serum metabolic signatures of high-fat-induced bone loss in mice. *Front. Cell. Infect. Microbiol.* **2021**, *11*, 788576. [CrossRef]
35. Wu, L.; Yan, Q.; Chen, F.; Cao, C.; Wang, S. Bupleuri radix extract ameliorates impaired lipid metabolism in high-fat diet-induced obese mice via gut microbiota-mediated regulation of FGF21 signaling pathway. *Biomed. Pharmacother.* **2021**, *135*, 111187. [CrossRef]
36. Islam, T.; Koboziev, I.; Albracht-Schulte, K.; Mistretta, B.; Scoggin, S.; Yosofvand, M.; Moussa, H.; Zabet-Moghaddam, M.; Ramalingam, L.; Gunaratne, P.H.; et al. Curcumin reduces adipose tissue inflammation and alters gut microbiota in diet-induced obese male mice. *Mol. Nutr. Food Res.* **2021**, *65*, e2100274. [CrossRef]
37. Peng, C.; Xu, X.; Li, Y.; Li, X.; Yang, X.; Chen, H.; Zhu, Y.; Lu, N.; He, C. Sex-specific association between the gut microbiome and high-fat diet-induced metabolic disorders in mice. *Biol. Sex Differ.* **2020**, *11*, 5. [CrossRef]
38. Wang, P.; Li, D.; Ke, W.; Liang, D.; Hu, X.; Chen, F. Resveratrol-induced gut microbiota reduces obesity in high-fat diet-fed mice. *Int. J. Obes.* **2020**, *44*, 213–225. [CrossRef]
39. Xu, Y.; Wang, N.; Tan, H.Y.; Li, S.; Zhang, C.; Zhang, Z.; Feng, Y. *Panax notoginseng* saponins modulate the gut microbiota to promote thermogenesis and beige adipocyte reconstruction via leptin-mediated AMPK α /STAT3 signaling in diet-induced obesity. *Theranostics* **2020**, *10*, 11302–11323. [CrossRef] [PubMed]
40. Won, S.M.; Chen, S.; Lee, S.Y.; Lee, K.E.; Park, K.W.; Yoon, J.H. *Lactobacillus sakei* ADM14 induces anti-obesity effects and changes in gut microbiome in high-fat diet-induced obese mice. *Nutrients* **2020**, *12*, 3703. [CrossRef]
41. Van Hul, M.; Karnik, K.; Canene-Adams, K.; De Souza, M.; Van den Abbeele, P.; Marzorati, M.; Delzenne, N.M.; Everard, A.; Cani, P.D. Comparison of the effects of soluble corn fiber and fructooligosaccharides on metabolism, inflammation, and gut microbiome of high-fat diet-fed mice. *Am. J. Physiol. Endocrinol. Metab.* **2020**, *319*, E779–E791. [CrossRef] [PubMed]
42. Gu, Y.; Liu, C.; Zheng, N.; Jia, W.; Zhang, W.; Li, H. Metabolic and gut microbial characterization of obesity-prone mice under a high-fat diet. *J. Proteome Res.* **2019**, *18*, 1703–1714. [CrossRef] [PubMed]
43. Liu, J.; Hao, W.; He, Z.; Kwek, E.; Zhao, Y.; Zhu, H.; Liang, N.; Ma, K.Y.; Lei, L.; He, W.S.; et al. Beneficial effects of tea water extracts on the body weight and gut microbiota in C57BL/6J mice fed with a high-fat diet. *Food Funct.* **2019**, *10*, 2847–2860. [CrossRef]
44. Cao, W.; Chin, Y.; Chen, X.; Mi, Y.; Xue, C.; Wang, Y.; Tang, Q. The role of gut microbiota in the resistance to obesity in mice fed a high fat diet. *Int. J. Food Sci. Nutr.* **2020**, *71*, 453–463. [CrossRef] [PubMed]
45. Watanabe, K.; Igarashi, M.; Li, X.; Nakatani, A.; Miyamoto, J.; Inaba, Y.; Sutou, A.; Saito, T.; Sato, T.; Tachibana, N.; et al. Dietary soybean protein ameliorates high-fat diet-induced obesity by modifying the gut microbiota-dependent biotransformation of bile acids. *PLoS ONE* **2018**, *13*, e0202083. [CrossRef]
46. Zheng, J.; Cheng, G.; Li, Q.; Jiao, S.; Feng, C.; Zhao, X.; Yin, H.; Du, Y.; Liu, H. Chitin oligosaccharide modulates gut microbiota and attenuates high-fat-diet-induced metabolic syndrome in mice. *Mar. Drugs* **2018**, *16*, 66. [CrossRef]
47. Huang, J.; Xiao, N.; Sun, Y.; Wu, S.; Tian, W.; Lai, Y.; Li, P.; Du, B. Supplementation of *Bacillus* sp. DU-106 reduces hypercholesterolemia and ameliorates gut dysbiosis in high-fat diet rats. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 287–299.
48. Li, D.; Wu, H.; Dou, H.; Guo, L.; Huang, W. Microcapsule of sweet orange essential oil changes gut microbiota in diet-induced obese rats. *Biochem. Biophys. Res. Commun.* **2018**, *505*, 991–995. [CrossRef]
49. Choi, S.; Hwang, Y.J.; Shin, M.J.; Yi, H. Difference in the gut microbiome between ovariectomy-induced obesity and diet-induced obesity. *J. Microbiol. Biotechnol.* **2017**, *27*, 2228–2236. [CrossRef]
50. Zhao, L.; Zhang, Q.; Ma, W.; Tian, F.; Shen, H.; Zhou, M. A combination of quercetin and resveratrol reduces obesity in high-fat diet-fed rats by modulation of gut microbiota. *Food Funct.* **2017**, *8*, 4644–4656. [CrossRef]
51. Rosenbaum, M.; Knight, R.; Leibel, R.L. The gut microbiota in human energy homeostasis and obesity. *Trends Endocrinol. Metab.* **2015**, *26*, 493–501. [CrossRef] [PubMed]
52. Schwieritz, A.; Taras, D.; Schäfer, K.; Beijer, S.; Bos, N.A.; Donus, C.; Hardt, P.D. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity* **2010**, *18*, 190–195. [CrossRef] [PubMed]
53. Jing, N.; Liu, X.; Jin, M.; Yang, X.; Hu, X.; Li, C.; Zhao, K. Fubrick tea attenuates high-fat diet induced fat deposition and metabolic disorder by regulating gut microbiota and caffeine metabolism. *Food Funct.* **2020**, *11*, 6971–6986. [CrossRef] [PubMed]
54. An, J.; Wang, Q.; Yi, S.; Liu, X.; Jin, H.; Xu, J.; Wen, G.; Zhu, J.; Tuo, B. The source of the fat significantly affects the results of high-fat diet intervention. *Sci. Rep.* **2022**, *12*, 4315. [CrossRef]
55. Qiao, B.; Li, X.; Wu, Y.; Guo, T.; Tan, Z. Comparative analysis of the gut microbiota in mice under lard or vegetable blend oil diet. *J. Oleo Sci.* **2022**, *71*, 1613–1624. [CrossRef] [PubMed]

56. Monte, M.J.; Marin, J.J.; Antelo, A.; Vazquez-Tato, J. Bile acids: Chemistry, physiology, and pathophysiology. *World J. Gastroenterol.* **2009**, *15*, 804–816. [CrossRef]
57. Jones, B.V.; Begley, M.; Hill, C.; Gahan, C.G.; Marchesi, J.R. Functional and comparative metagenomic analysis of bile salt hydrolase activity in the human gut microbiome. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 13580–13585. [CrossRef]
58. Kisiela, M.; Skarka, A.; Ebert, B.; Maser, E. Hydroxysteroid dehydrogenases (HSDs) in bacteria: A bioinformatic perspective. *J. Steroid Biochem. Mol. Biol.* **2012**, *129*, 31–46. [CrossRef] [PubMed]
59. Wang, Q.; Hao, C.; Yao, W.; Zhu, D.; Lu, H.; Li, L.; Ma, B.; Sun, B.; Xue, D.; Zhang, W. Intestinal flora imbalance affects bile acid metabolism and is associated with gallstone formation. *BMC Gastroenterol.* **2020**, *20*, 59. [CrossRef]
60. Wang, K.; Liao, M.; Zhou, N.; Bao, L.; Ma, K.; Zheng, Z.; Wang, Y.; Liu, C.; Wang, W.; Wang, J.; et al. Parabacteroides distasonis alleviates obesity and metabolic dysfunctions via production of succinate and secondary bile acids. *Cell Rep.* **2019**, *26*, 222–235.e5. [CrossRef]
61. Inagaki, T.; Moschetta, A.; Lee, Y.K.; Peng, L.; Zhao, G.; Downes, M.; Yu, R.T.; Shelton, J.M.; Richardson, J.A.; Repa, J.J.; et al. Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 3920–3925. [CrossRef]
62. Kurdi, P.; Kawanishi, K.; Mizutani, K.; Yokota, A. Mechanism of growth inhibition by free bile acids in lactobacilli and bifidobacteria. *J. Bacteriol.* **2006**, *188*, 1979–1986. [CrossRef] [PubMed]
63. Wang, L.; Gong, Z.; Zhang, X.; Zhu, F.; Liu, Y.; Jin, C.; Du, X.; Xu, C.; Chen, Y.; Cai, W.; et al. Gut microbial bile acid metabolite skews macrophage polarization and contributes to high-fat diet-induced colonic inflammation. *Gut Microbes* **2020**, *12*, 1–20. [CrossRef] [PubMed]
64. Zhao, L.; Yang, W.; Chen, Y.; Huang, F.; Lu, L.; Lin, C.; Huang, T.; Ning, Z.; Zhai, L.; Zhong, L.L.; et al. Clostridia-rich microbiota enhances bile acid excretion in diarrhea-predominant irritable bowel syndrome. *J. Clin. Investig.* **2020**, *130*, 438–450. [CrossRef] [PubMed]
65. Chen, M.; Guo, W.L.; Li, Q.Y.; Xu, J.X.; Cao, Y.J.; Liu, B.; Yu, X.D.; Rao, P.F.; Ni, L.; Lv, X.C. The protective mechanism of *Lactobacillus plantarum* FZU3013 against non-alcoholic fatty liver associated with hyperlipidemia in mice fed a high-fat diet. *Food Funct.* **2020**, *11*, 3316–3331. [CrossRef]
66. Sun, C.; Qiu, C.; Zhang, Y.; Yan, M.; Tan, J.; He, J.; Yang, D.; Wang, D.; Wu, L. *Lactiplantibacillus plantarum* NKK20 Alleviates High-Fat-Diet-Induced Nonalcoholic Fatty Liver Disease in Mice through Regulating Bile Acid Anabolism. *Molecules* **2023**, *28*, 4042. [CrossRef]
67. Jacouton, E.; Mondot, S.; Langella, P.; Bermúdez-Humarán, L.G. Impact of Oral Administration of *Lactiplantibacillus plantarum* Strain CNCM I-4459 on Obesity Induced by High-Fat Diet in Mice. *Bioengineering* **2023**, *10*, 1151. [CrossRef]
68. Cai, H.; Wen, Z.; Li, X.; Meng, K.; Yang, P. *Lactobacillus plantarum* FRT10 alleviated high-fat diet-induced obesity in mice through regulating the PPAR α signal pathway and gut microbiota. *Appl. Microbiol. Biotechnol.* **2020**, *104*, 5959–5972. [CrossRef]
69. Jang, A.R.; Jung, D.H.; Lee, T.S.; Kim, J.K.; Lee, Y.B.; Lee, J.Y.; Kim, S.Y.; Yoo, Y.C.; Ahn, J.H.; Hong, E.H.; et al. *Lactobacillus plantarum* NCHBL-004 modulates high-fat diet-induced weight gain and enhances GLP-1 production for blood glucose regulation. *Nutrition* **2024**, *128*, 112565. [CrossRef]
70. Gan, Y.; Tang, M.W.; Tan, F.; Zhou, X.R.; Fan, L.; Xie, Y.X.; Zhao, X. Anti-obesity effect of *Lactobacillus plantarum* CQPC01 by modulating lipid metabolism in high-fat diet-induced C57BL/6 mice. *J. Food Biochem.* **2020**, *44*, e13491. [CrossRef]
71. Choi, M.J.; Yu, H.; Kim, J.I.; Seo, H.; Kim, J.G.; Kim, S.K.; Lee, H.S.; Cheon, H.G. Anti-obesity effects of *Lactiplantibacillus plantarum* SKO-001 in high-fat diet-induced obese mice. *Eur. J. Nutr.* **2023**, *62*, 1611–1622. [CrossRef] [PubMed]
72. Choi, W.J.; Dong, H.J.; Jeong, H.U.; Ryu, D.W.; Song, S.M.; Kim, Y.R.; Jung, H.H.; Kim, T.H.; Kim, Y.H. *Lactobacillus plantarum* LMT1-48 exerts anti-obesity effect in high-fat diet-induced obese mice by regulating expression of lipogenic genes. *Sci. Rep.* **2020**, *10*, 869. [CrossRef] [PubMed]
73. Gan, Y.; Chen, H.; Zhou, X.R.; Chu, L.L.; Ran, W.T.; Tan, F.; Zhao, X. Regulating effect of *Lactobacillus plantarum* CQPC03 on lipid metabolism in high-fat diet-induced obesity in mice. *J. Food Biochem.* **2020**, *44*, e13495.
74. Yin, T.; Bayanjargal, S.; Fang, B.; Inaba, C.; Mutoh, M.; Kawahara, T.; Tanaka, S.; Watanabe, J. *Lactobacillus plantarum* Shinshu N-07 isolated from fermented *Brassica rapa* L. attenuates visceral fat accumulation induced by high-fat diet in mice. *Benef. Microbes* **2020**, *11*, 655–667. [CrossRef]
75. Liu, Y.; Gao, Y.; Ma, F.; Sun, M.; Mu, G.; Tuo, Y. The ameliorative effect of *Lactobacillus plantarum* Y44 oral administration on inflammation and lipid metabolism in obese mice fed with a high fat diet. *Food Funct.* **2020**, *11*, 5024–5039. [CrossRef]
76. Chen, H.; Zhao, H.; Qi, X.; Sun, Y.; Ma, Y.; Li, Q. *Lactobacillus plantarum* HF02 alleviates lipid accumulation and intestinal microbiota dysbiosis in high-fat diet-induced obese mice. *J. Sci. Food Agric.* **2023**, *103*, 4625–4637. [CrossRef]
77. Das, T.K.; Kar, P.; Panchali, T.; Khatun, A.; Dutta, A.; Ghosh, S.; Chakrabarti, S.; Pradhan, S.; Mondal, K.C.; Ghosh, K. Anti-obesity potentiality of *Lactiplantibacillus plantarum* E2_MCCCKT isolated from a fermented beverage, haria: A high fat diet-induced obese mice model study. *World J. Microbiol. Biotechnol.* **2024**, *40*, 168. [CrossRef]

78. Ghosh, S.; Ghosh, A.J.; Islam, R.; Sarkar, S.; Saha, T. *Lactobacillus plantarum* KAD protects against high-fat diet-induced hepatic complications in Swiss albino mice: Role of inflammation and gut integrity. *PLoS ONE* **2024**, *19*, e0313548. [CrossRef]
79. Cai, X.; Huang, J.; Yu, T.; Guan, X.; Sun, M.; Zhao, D.; Zheng, Y.; Wang, Q. *Lactiplantibacillus plantarum* BXM2 Treatment Alleviates Disorders Induced by a High-Fat Diet in Mice by Improving Intestinal Health and Modulating the Gut Microbiota. *Nutrients* **2025**, *17*, 407. [CrossRef]
80. Lee, N.K.; Lee, Y.; Shin, D.S.; Ra, J.; Choi, Y.M.; Ryu, B.H.; Lee, J.; Park, E.; Paik, H.D. Hepatoprotective Effect of *Lactiplantibacillus plantarum* DSR330 in Mice with High Fat Diet-Induced Nonalcoholic Fatty Liver Disease. *J. Microbiol. Biotechnol.* **2024**, *34*, 399–406. [CrossRef]
81. Soundharajan, I.; Kuppusamy, P.; Srisesharam, S.; Lee, J.C.; Sivanesan, R.; Kim, D.; Choi, K.C. Positive metabolic effects of selected probiotic bacteria on diet-induced obesity in mice are associated with improvement of dysbiotic gut microbiota. *FASEB J.* **2020**, *34*, 12289–12307. [CrossRef]
82. Ondee, T.; Pongpirul, K.; Janchot, K.; Kanacharoen, S.; Lertmongkolaksorn, T.; Wongsaroj, L.; Somboonna, N.; Ngamwongsatit, N.; Leelahavanichkul, A. *Lactiplantibacillus plantarum* dfa1 Outperforms *Enterococcus faecium* dfa1 on Anti-Obesity in High Fat-Induced Obesity Mice Possibly through the Differences in Gut Dysbiosis Attenuation, despite the Similar Anti-Inflammatory Properties. *Nutrients* **2021**, *14*, 80. [CrossRef] [PubMed]
83. Lee, E.; Jung, S.R.; Lee, S.Y.; Lee, N.K.; Paik, H.D.; Lim, S.I. *Lactobacillus plantarum* Strain Ln4 Attenuates Diet-Induced Obesity, Insulin Resistance, and Changes in Hepatic mRNA Levels Associated with Glucose and Lipid Metabolism. *Nutrients* **2018**, *10*, 643. [CrossRef] [PubMed]
84. Riezu-Boj, J.I.; Barajas, M.; Pérez-Sánchez, T.; Pajares, M.J.; Araña, M.; Milagro, F.I.; Urtasun, R. *Lactiplantibacillus plantarum* DSM20174 Attenuates the Progression of Non-Alcoholic Fatty Liver Disease by Modulating Gut Microbiota, Improving Metabolic Risk Factors, and Attenuating Adipose Inflammation. *Nutrients* **2022**, *14*, 5212. [CrossRef] [PubMed]
85. Huang, E.; Kim, S.; Park, H.; Park, S.; Ji, Y.; Todorov, S.D.; Lim, S.D.; Holzapfel, W.H. Modulation of the Gut Microbiome and Obesity Biomarkers by *Lactobacillus Plantarum* KC28 in a Diet-Induced Obesity Murine Model. *Probiotics Antimicrob. Proteins* **2021**, *13*, 677–697. [CrossRef]
86. Kuerman, M.; Bao, Y.; Guo, M.; Jiang, S. Safety assessment of two strains and anti-obese effects on mice fed a high-cholesterol diet. *Biochem. Biophys. Res. Commun.* **2021**, *572*, 131–137. [CrossRef]
87. Li, Z.; Jin, H.; Oh, S.Y.; Ji, G.E. Anti-obese effects of two *Lactobacilli* and two *Bifidobacteria* on ICR mice fed on a high fat diet. *Biochem. Biophys. Res. Commun.* **2016**, *480*, 222–227. [CrossRef]
88. Liu, Z.; Zhou, X.; Wang, W.; Gu, L.; Hu, C.; Sun, H.; Xu, C.; Hou, J.; Jiang, Z. *Lactobacillus paracasei* 24 Attenuates Lipid Accumulation in High-Fat Diet-Induced Obese Mice by Regulating the Gut Microbiota. *J. Agric. Food Chem.* **2022**, *70*, 4631–4643. [CrossRef]
89. Miao, Z.; Zheng, H.; Liu, W.H.; Cheng, R.; Lan, H.; Sun, T.; Zhao, W.; Li, J.; Shen, X.; Li, H.; et al. *Lactocaseibacillus paracasei* K56 Attenuates High-Fat Diet-Induced Obesity by Modulating the Gut Microbiota in Mice. *Probiotics Antimicrob. Proteins* **2023**, *15*, 844–855. [CrossRef]
90. Miao, Z.H.; Wang, J.N.; Shen, X.; Zhou, Q.Q.; Luo, Y.T.; Liang, H.J.; Wang, S.J.; Qi, S.H.; Cheng, R.Y.; He, F. Long-term use of *Lactocaseibacillus paracasei* N1115 from early life alleviates high-fat-diet-induced obesity and dysmetabolism in mice. *Benef Microbes* **2022**, *13*, 407–416. [CrossRef]
91. Galindez, U.; Erdenebold, U.; Batnasan, G.; Ganzorig, O.; Batdorj, B. Anti-obesity effects of potential probiotic *Lactobacillus* strains isolated from Mongolian fermented dairy products in high-fat diet-induced obese rodent model. *Braz. J. Microbiol.* **2024**, *55*, 2501–2509. [CrossRef] [PubMed]
92. Lee, N.R.; Kwon, T.J.; Chung, E.C.; Bae, J.; Soung, S.H.; Tak, H.J.; Choi, J.Y.; Lee, Y.E.; Won Hwang, N.; Lee, J.S.; et al. Combination of *Lactocaseibacillus paracasei* BEPC22 and *Lactiplantibacillus plantarum* BLP53 attenuates fat accumulation and alters the metabolome and gut microbiota in mice with high-fat diet-induced obesity. *Food Funct.* **2024**, *15*, 647–662. [CrossRef] [PubMed]
93. Lv, X.C.; Chen, M.; Huang, Z.R.; Guo, W.L.; Ai, L.Z.; Bai, W.D.; Yu, X.D.; Liu, Y.L.; Rao, P.F.; Ni, L. Potential mechanisms underlying the ameliorative effect of *Lactobacillus paracasei* FZU103 on the lipid metabolism in hyperlipidemic mice fed a high-fat diet. *Food Res. Int.* **2021**, *139*, 109956. [CrossRef] [PubMed]
94. Song, E.J.; Lee, E.S.; Kim, Y.I.; Shin, D.U.; Eom, J.E.; Shin, H.S.; Lee, S.Y.; Nam, Y.D. Gut microbial change after administration of *Lactocaseibacillus paracasei* AO356 is associated with anti-obesity in a mouse model. *Front. Endocrinol.* **2023**, *14*, 1224636. [CrossRef]
95. Novotny Núñez, I.; Maldonado Galdeano, C.; de Moreno de LeBlanc, A.; Perdigon, G. *Lactobacillus casei* CRL 431 administration decreases inflammatory cytokines in a diet-induced obese mouse model. *Nutrition* **2015**, *31*, 1000–1007. [CrossRef]
96. Nguyen, H.T.; Gu, M.; Werlinger, P.; Cho, J.H.; Cheng, J.; Suh, J.W. *Lactobacillus sakei* MJM60958 as a Potential Probiotic Alleviated Non-Alcoholic Fatty Liver Disease in Mice Fed a High-Fat Diet by Modulating Lipid Metabolism, Inflammation, and Gut Microbiota. *Int. J. Mol. Sci.* **2022**, *23*, 13436. [CrossRef]

97. Chen, M.; Pan, P.; Zhang, H.; Li, R.; Ren, D.; Jiang, B. *Latilactobacillus sakei* QC9 alleviates hyperglycaemia in high-fat diet and streptozotocin-induced type 2 diabetes mellitus mice via the microbiota-gut-liver axis. *Food Funct.* **2024**, *15*, 8008–8029. [CrossRef]
98. Park, S.S.; Lim, S.K.; Lee, J.; Park, H.K.; Kwon, M.S.; Yun, M.; Kim, N.; Oh, Y.J.; Choi, H.J. *Latilactobacillus sakei* WIKIM31 Decelerates Weight Gain in High-Fat Diet-Induced Obese Mice by Modulating Lipid Metabolism and Suppressing Inflammation. *J. Microbiol. Biotechnol.* **2021**, *31*, 1568–1575. [CrossRef]
99. Lim, S.M.; Jeong, J.J.; Woo, K.H.; Han, M.J.; Kim, D.H. *Lactobacillus sakei* OK67 ameliorates high-fat diet-induced blood glucose intolerance and obesity in mice by inhibiting gut microbiota lipopolysaccharide production and inducing colon tight junction protein expression. *Nutr. Res.* **2016**, *36*, 337–348. [CrossRef]
100. Jang, H.M.; Han, S.K.; Kim, J.K.; Oh, S.J.; Jang, H.B.; Kim, D.H. *Lactobacillus sakei* Alleviates High-Fat-Diet-Induced Obesity and Anxiety in Mice by Inducing AMPK Activation and SIRT1 Expression and Inhibiting Gut Microbiota-Mediated NF- κ B Activation. *Mol. Nutr. Food Res.* **2019**, *63*, e1800978. [CrossRef]
101. Ji, Y.; Park, S.; Chung, Y.; Kim, B.; Park, H.; Huang, E.; Jeong, D.; Jung, H.Y.; Kim, B.; Hyun, C.K.; et al. Amelioration of obesity-related biomarkers by *Lactobacillus sakei* CJLS03 in a high-fat diet-induced obese murine model. *Sci. Rep.* **2019**, *9*, 6821. [CrossRef]
102. Kang, Y.; Kang, X.; Yang, H.; Liu, H.; Yang, X.; Liu, Q.; Tian, H.; Xue, Y.; Ren, P.; Kuang, X.; et al. *Lactobacillus acidophilus* ameliorates obesity in mice through modulation of gut microbiota dysbiosis and intestinal permeability. *Pharmacol. Res.* **2022**, *175*, 106020. [CrossRef] [PubMed]
103. Tang, C.; Cao, G.; Zhao, W.; Bie, X.; Lu, F.; Lu, Z.; Lu, Y. *Lactobacillus acidophilus* NX2-6 Improved High-Fat Diet-Induced Glucose Metabolism Disorder Independent of Promotion of Insulin Secretion in Mice. *J. Agric. Food Chem.* **2021**, *69*, 15598–15610. [CrossRef]
104. Song, M.; Park, S.; Lee, H.; Min, B.; Jung, S.; Park, S.; Kim, E.; Oh, S. Effect of *Lactobacillus acidophilus* NS1 on plasma cholesterol levels in diet-induced obese mice. *J. Dairy Sci.* **2015**, *98*, 1492–1501. [CrossRef]
105. Ondee, T.; Pongpirul, K.; Visitchanakun, P.; Saisorn, W.; Kanacharoen, S.; Wongsaroj, L.; Kullapanich, C.; Ngamwongsatit, N.; Settachaimongkon, S.; Somboonna, N.; et al. *Lactobacillus acidophilus* LA5 improves saturated fat-induced obesity mouse model through the enhanced intestinal *Akkermansia muciniphila*. *Sci. Rep.* **2021**, *11*, 6367. [CrossRef] [PubMed]
106. Zheng, Y.; Zhang, S.; Zhang, Z.; Zhang, T.; Teng, X.; Xiao, G.; Huang, S. Isolation of *Lactobacillus acidophilus* strain and its anti-obesity effect in a diet induced obese murine model. *Lett. Appl. Microbiol.* **2024**, *77*, ovae021. [CrossRef] [PubMed]
107. Molina-Tijeras, J.A.; Diez-Echave, P.; Vezza, T.; Hidalgo-García, L.; Ruiz-Malagón, A.J.; Rodríguez-Sojo, M.J.; Romero, M.; Robles-Vera, I.; García, F.; Plaza-Díaz, J.; et al. *Lactobacillus fermentum* CECT5716 ameliorates high fat diet-induced obesity in mice through modulation of gut microbiota dysbiosis. *Pharmacol. Res.* **2021**, *167*, 105471. [CrossRef]
108. Li, J.; Zhang, Z.; Xu, Y.; Li, W.; Jiang, S.; Zhang, J.; Xue, H. *Limosilactobacillus fermentum* HNU312 alleviates lipid accumulation and inflammation induced by a high-fat diet: Improves lipid metabolism pathways and increases short-chain fatty acids in the gut microbiome. *Food Funct.* **2024**, *15*, 8878–8892. [CrossRef]
109. Kim, S.J.; Choi, S.I.; Jang, M.; Jeong, Y.A.; Kang, C.H.; Kim, G.H. Combination of *Limosilactobacillus fermentum* MG4231 and MG4244 attenuates lipid accumulation in high-fat diet-fed obese mice. *Benef. Microbes* **2021**, *12*, 479–491. [CrossRef]
110. Lee, J.Y.; An, M.; Heo, H.; Park, J.Y.; Lee, J.; Kang, C.H. *Limosilactobacillus fermentum* MG4294 and *Lactiplantibacillus plantarum* MG5289 Ameliorates Nonalcoholic Fatty Liver Disease in High-Fat Diet-Induced Mice. *Nutrients* **2023**, *15*, 2005. [CrossRef]
111. Yoon, Y.; Kim, G.; Noh, M.G.; Park, J.H.; Jang, M.; Fang, S.; Park, H. *Lactobacillus fermentum* promotes adipose tissue oxidative phosphorylation to protect against diet-induced obesity. *Exp. Mol. Med.* **2020**, *52*, 1574–1586. [CrossRef] [PubMed]
112. Song, H.; Xue, H.; Zhang, Z.; Wang, J.; Li, A.; Zhang, J.; Luo, P.; Zhan, M.; Zhou, X.; Chen, L.; et al. Amelioration of Type 2 Diabetes Using Four Strains of *Lactobacillus* Probiotics: Effects on Gut Microbiota Reconstitution-Mediated Regulation of Glucose Homeostasis, Inflammation, and Oxidative Stress in Mice. *J. Agric. Food Chem.* **2023**, *71*, 20801–20814. [CrossRef] [PubMed]
113. Schmidt, N.P.; Molz, P.; Fraga, B.S.; Bondarczuk, N.H.; Silveira, P.D.; Ferri, M.H.; Crestani, T.B.; Breyer, G.M.; Guimarães, G.R.; Motta, A.S.D.; et al. Effect of Probiotic *Lactocaseibacillus rhamnosus* LB1.5 on Anxiety-like Behavior, Neuroprotection and Neuroinflammation Markers of Male Mice Fed a High-Fat Diet. *Nutrients* **2024**, *16*, 879. [CrossRef] [PubMed]
114. Feng, X.; Ding, L.; Ma, G.; Zhang, Y.; Sun, Y.; Li, Z.; Tao, X.; Ali, A.; Wang, D.; Wu, L. *Lactobacillus rhamnosus* TR08 Improves Dyslipidemia in Mice Fed with a High Fat Diet by Regulating the Intestinal Microbiota, Reducing Systemic Inflammatory Response, and Promoting Sphingomholipid Metabolism. *Molecules* **2022**, *27*, 7357. [CrossRef]
115. Chen, Y.T.; Chiou, S.Y.; Hsu, A.H.; Lin, Y.C.; Lin, J.S. *Lactobacillus rhamnosus* Strain LRH05 Intervention Ameliorated Body Weight Gain and Adipose Inflammation via Modulating the Gut Microbiota in High-Fat Diet-Induced Obese Mice. *Mol. Nutr. Food Res.* **2022**, *66*, e2100348. [CrossRef]
116. Ivanovic, N.; Minic, R.; Dimitrijevic, L.; Radojevic Skodric, S.; Zivkovic, I.; Djordjevic, B. *Lactobacillus rhamnosus* LA68 and *Lactobacillus plantarum* WCFS1 differently influence metabolic and immunological parameters in high fat diet-induced hypercholesterolemia and hepatic steatosis. *Food Funct.* **2015**, *6*, 558–565. [CrossRef]

117. Cheng, Y.C.; Liu, J.R. Effect of *Lactobacillus rhamnosus* GG on Energy Metabolism, Leptin Resistance, and Gut Microbiota in Mice with Diet-Induced Obesity. *Nutrients* **2020**, *12*, 2557. [CrossRef]
118. Sun, M.; Wu, T.; Zhang, G.; Liu, R.; Sui, W.; Zhang, M.; Geng, J.; Yin, J.; Zhang, M. *Lactobacillus rhamnosus* LRa05 improves lipid accumulation in mice fed with a high fat diet via regulating the intestinal microbiota, reducing glucose content and promoting liver carbohydrate metabolism. *Food Funct.* **2020**, *11*, 9514–9525. [CrossRef]
119. Fang, Y.; Chen, H.Q.; Zhang, X.; Zhang, H.; Xia, J.; Ding, K.; Fang, Z.Y. Probiotic administration of *Lactobacillus rhamnosus* GR-1 attenuates atherosclerotic plaque formation in ApoE^{−/−} mice fed with a high-fat diet. *Eur. Rev. Med. Pharmacol. Sci.* **2019**, *23*, 3533–3541.
120. Ukibe, K.; Miyoshi, M.; Kadooka, Y. Administration of *Lactobacillus gasseri* SBT2055 suppresses macrophage infiltration into adipose tissue in diet-induced obese mice. *Br. J. Nutr.* **2015**, *114*, 1180–1187. [CrossRef]
121. Kim, M.J.; Shin, S.K.; Han, J.W.; Kim, J.E.; Lee, M.J.; Bae, H.R.; Kwon, E.Y. *Lactobacillus paragasseri* SBT2055 attenuates obesity via the adipose tissue-muscle-gut axis in obese mice. *Microbiol. Res.* **2025**, *290*, 127972. [CrossRef]
122. Lee, C.S.; Park, M.H.; Kim, B.K.; Kim, S.H. Antiobesity Effect of Novel Probiotic Strains in a Mouse Model of High-Fat Diet-Induced Obesity. *Probiotics Antimicrob. Proteins* **2021**, *13*, 1054–1067. [CrossRef] [PubMed]
123. Hong, E.; Kang, H.; Yang, G.; Oh, S.; Kim, E. The PKA-SREBP1c Pathway Plays a Key Role in the Protective Effects of *Lactobacillus johnsonii* JNU3402 Against Diet-Induced Fatty Liver in Mice. *Mol. Nutr. Food Res.* **2023**, *67*, e2200496. [CrossRef] [PubMed]
124. Lee, K.; Kim, H.J.; Kim, J.Y.; Shim, J.J.; Lee, J.H. A Mixture of *Lactobacillus* HY7601 and KY1032 Regulates Energy Metabolism in Adipose Tissue and Improves Cholesterol Disposal in High-Fat-Diet-Fed Mice. *Nutrients* **2024**, *16*, 2570. [CrossRef]
125. Park, D.Y.; Ahn, Y.T.; Park, S.H.; Huh, C.S.; Yoo, S.R.; Yu, R.; Sung, M.K.; McGregor, R.A.; Choi, M.S. Supplementation of *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 in diet-induced obese mice is associated with gut microbial changes and reduction in obesity. *PLoS ONE* **2013**, *8*, e59470. [CrossRef]
126. Li, S.; Qi, C.; Zhu, H.; Yu, R.; Xie, C.; Peng, Y.; Yin, S.W.; Fan, J.; Zhao, S.; Sun, J. *Lactobacillus reuteri* improves gut barrier function and affects diurnal variation of the gut microbiota in mice fed a high-fat diet. *Food Funct.* **2019**, *10*, 4705–4715. [CrossRef] [PubMed]
127. Abot, A.; Pomié, N.; Astre, G.; Jaomanjaka, F.; Marchand, P.; Cani, P.D.; Roudier, N.; Knauf, C. *Limosilactobacillus reuteri* BIO7251 administration improves metabolic phenotype in obese mice fed a high fat diet: An inter-organ crosstalk between gut, adipose tissue and nervous system. *Int. J. Food. Sci. Nutr.* **2024**, *75*, 58–69. [CrossRef]
128. Zheng, F.; Wang, Z.; Stanton, C.; Ross, R.P.; Zhao, J.; Zhang, H.; Yang, B.; Chen, W. *Lactobacillus rhamnosus* FJSYC4-1 and *Lactobacillus reuteri* FGSZY33L6 alleviate metabolic syndrome via gut microbiota regulation. *Food Funct.* **2021**, *12*, 3919–3930. [CrossRef]
129. Park, S.S.; Lee, Y.J.; Kang, H.; Yang, G.; Hong, E.J.; Lim, J.Y.; Oh, S.; Kim, E. *Lactobacillus amylovorus* KU4 ameliorates diet-induced obesity in mice by promoting adipose browning through PPAR γ signaling. *Sci. Rep.* **2019**, *9*, 20152. [CrossRef]
130. Liang, C.; Zhou, X.H.; Jiao, Y.H.; Guo, M.J.; Meng, L.; Gong, P.M.; Lyu, L.Z.; Niu, H.Y.; Wu, Y.F.; Chen, S.W.; et al. *Ligilactobacillus Salivarius* LCK11 Prevents Obesity by Promoting PYY Secretion to Inhibit Appetite and Regulating Gut Microbiota in C57BL/6j Mice. *Mol. Nutr. Food Res.* **2021**, *65*, e2100136. [CrossRef]
131. Kim, D.H.; Jeong, D.; Kang, I.B.; Kim, H.; Song, K.Y.; Seo, K.H. Dual function of *Lactobacillus kefir* DH5 in preventing high-fat-diet-induced obesity: Direct reduction of cholesterol and upregulation of PPAR- α in adipose tissue. *Mol. Nutr. Food Res.* **2017**, *61*, 1700252. [CrossRef]
132. Zeng, Y.; Zhang, H.; Tsao, R.; Mine, Y. *Lactobacillus pentosus* S-PT84 Prevents Low-Grade Chronic Inflammation-Associated Metabolic Disorders in a Lipopolysaccharide and High-Fat Diet C57/BL6j Mouse Model. *J. Agric. Food Chem.* **2020**, *68*, 4374–4386. [CrossRef] [PubMed]
133. Jang, H.; Joung, H.; Chu, J.; Cho, M.; Kim, Y.W.; Kim, K.H.; Shin, C.H.; Lee, J.; Ha, J.H. *Lactobacillus delbrueckii* subsp. *Lactis* CKDB001 Ameliorates Metabolic Complications in High-Fat Diet-Induced Obese Mice. *Nutrients* **2024**, *16*, 4260. [CrossRef] [PubMed]
134. Song, W.; Wang, T.; Cui, X.; Li, L.; Chen, B.; Li, Y.; Yue, T. *Lactobacillus coryniformis* subsp. *torquens* T3 alleviates non-alcoholic fatty liver disease via reconstruction of the gut microbiota and redox system. *J. Sci. Food Agric.* **2023**, *103*, 6814–6825. [CrossRef]
135. Rahman, M.S.; Kang, I.; Lee, Y.; Habib, M.A.; Choi, B.J.; Kang, J.S.; Park, D.S.; Kim, Y.S. *Bifidobacterium longum* subsp. *Infantis* YB0411 Inhibits Adipogenesis in 3T3-L1 Pre-adipocytes and Reduces High-Fat-Diet-Induced Obesity in Mice. *J. Agric. Food Chem.* **2021**, *69*, 6032–6042. [CrossRef] [PubMed]
136. Kou, R.; Wang, J.; Li, A.; Wang, Y.; Zhang, B.; Liu, J.; Sun, Y.; Wang, S. Ameliorating Effects of *Bifidobacterium longum* subsp. *Infantis* FB3-14 against High-Fat-Diet-Induced Obesity and Gut Microbiota Disorder. *Nutrients* **2023**, *15*, 4104. [CrossRef]
137. In Kim, H.; Kim, J.K.; Kim, J.Y.; Jang, S.E.; Han, M.J.; Kim, D.H. *Lactobacillus plantarum* LC27 and *Bifidobacterium longum* LC67 simultaneously alleviate high-fat diet-induced colitis, endotoxemia, liver steatosis, and obesity in mice. *Nutr. Res.* **2019**, *67*, 78–89. [CrossRef]

138. Wu, T.; Sun, M.; Liu, R.; Sui, W.; Zhang, J.; Yin, J.; Fang, S.; Zhu, J.; Zhang, M. *Bifidobacterium longum* subsp. *Longum* Remodeled Roseburia and Phosphatidylserine Levels and Ameliorated Intestinal Disorders and liver Metabolic Abnormalities Induced by High-Fat Diet. *J. Agric. Food Chem.* **2020**, *68*, 4632–4640.
139. Lim, S.M.; Kim, D.H. *Bifidobacterium adolescentis* IM38 ameliorates high-fat diet-induced colitis in mice by inhibiting NF- κ B activation and lipopolysaccharide production by gut microbiota. *Nutr. Res.* **2017**, *41*, 86–96. [CrossRef]
140. Wang, G.; Jiao, T.; Xu, Y.; Li, D.; Si, Q.; Hao, J.; Zhao, J.; Zhang, H.; Chen, W. *Bifidobacterium adolescentis* and *Lactobacillus rhamnosus* alleviate non-alcoholic fatty liver disease induced by a high-fat, high-cholesterol diet through modulation of different gut microbiota-dependent pathways. *Food Funct.* **2020**, *11*, 6115–6127. [CrossRef]
141. Ma, L.; Zheng, A.; Ni, L.; Wu, L.; Hu, L.; Zhao, Y.; Fu, Z.; Ni, Y. *Bifidobacterium animalis* subsp. *lactis* lkm512 Attenuates Obesity-Associated Inflammation and Insulin Resistance Through the Modification of Gut Microbiota in High-Fat Diet-Induced Obese Mice. *Mol. Nutr. Food Res.* **2022**, *66*, e2100639. [CrossRef] [PubMed]
142. Zhang, C.; Fang, B.; Zhang, N.; Zhang, Q.; Niu, T.; Zhao, L.; Sun, E.; Wang, J.; Xiao, R.; He, J.; et al. The Effect of *Bifidobacterium animalis* subsp. *Lactis* MN-Gup on Glucose Metabolism, Gut Microbiota, and Their Metabolites in Type 2 Diabetic Mice. *Nutrients* **2024**, *16*, 1691. [CrossRef] [PubMed]
143. Ban, O.H.; Lee, M.; Bang, W.Y.; Nam, E.H.; Jeon, H.J.; Shin, M.; Yang, J.; Jung, Y.H. *Bifidobacterium lactis* IDCC 4301 Exerts Anti-Obesity Effects in High-Fat Diet-Fed Mice Model by Regulating Lipid Metabolism. *Mol. Nutr. Food Res.* **2023**, *67*, e2200385. [CrossRef] [PubMed]
144. Kondo, S.; Xiao, J.Z.; Satoh, T.; Odamaki, T.; Takahashi, S.; Sugahara, H.; Yaeshima, T.; Iwatsuki, K.; Kamei, A.; Abe, K. Antiobesity effects of *Bifidobacterium breve* strain B-3 supplementation in a mouse model with high-fat diet-induced obesity. *Biosci Biotechnol. Biochem.* **2010**, *74*, 1656–1661. [CrossRef]
145. Cano, P.G.; Santacruz, A.; Trejo, F.M.; Sanz, Y. *Bifidobacterium* CECT 7765 improves metabolic and immunological alterations associated with obesity in high-fat diet-fed mice. *Obesity* **2013**, *21*, 2310–2321. [CrossRef]
146. Rahman, M.S.; Lee, Y.; Park, D.S.; Kim, Y.S. *Bifidobacterium bifidum* DS0908 and *Bifidobacterium longum* DS0950 Culture-Supernatants Ameliorate Obesity-Related Characteristics in Mice with High-Fat Diet-Induced Obesity. *J. Microbiol. Biotechnol.* **2023**, *33*, 96–105. [CrossRef]
147. Huang, J.; Jiang, R.; Wang, Y. Effects of the probiotic *Bacillus coagulans* BC69 on the metabolic and histological alterations induced by a high-sugar and high-fat diet in C57BL/6J mice. *Food Funct.* **2023**, *14*, 6596–6609. [CrossRef]
148. Kim, B.; Kwon, J.; Kim, M.S.; Park, H.; Ji, Y.; Holzapfel, W.; Hyun, C.K. Protective effects of *Bacillus* probiotics against high-fat diet-induced metabolic disorders in mice. *PLoS ONE* **2018**, *13*, e0210120. [CrossRef]
149. Wang, Y.; Wu, Y.; Wang, B.; Xu, H.; Mei, X.; Xu, X.; Zhang, X.; Ni, J.; Li, W. *Bacillus amyloliquefaciens* SC06 Protects Mice Against High-Fat Diet-Induced Obesity and Liver Injury via Regulating Host Metabolism and Gut Microbiota. *Front. Microbiol.* **2019**, *10*, 161. [CrossRef]
150. Hashemnia, S.M.R.; Meshkani, R.; Zamani-Garmsiri, F.; Shabani, M.; Tajabadi-Ebrahimi, M.; Ragerdi Kashani, I.; Siadat, S.D.; Mohassel Azadi, S.; Emamgholipour, S. Amelioration of obesity-induced white adipose tissue inflammation by *Bacillus coagulans* T4 in a high-fat diet-induced obese murine model. *Life Sci.* **2023**, *314*, 121286. [CrossRef]
151. Cao, G.T.; Dai, B.; Wang, K.L.; Yan, Y.; Xu, Y.L.; Wang, Y.X.; Yang, C.M. *Bacillus licheniformis*, a potential probiotic, inhibits obesity by modulating colonic microflora in C57BL/6J mice model. *J. Appl. Microbiol.* **2019**, *127*, 880–888. [CrossRef]
152. Wen, X.; Feng, X.; Xin, F.; An, R.; Huang, H.; Mao, L.; Liu, P.; Zhang, J.; Huang, H.; Liu, X.; et al. *B. vulgatus* ameliorates high-fat diet-induced obesity through modulating intestinal serotonin synthesis and lipid absorption in mice. *Gut Microbes* **2024**, *16*, 2423040. [CrossRef] [PubMed]
153. Li, H.; Wang, X.K.; Tang, M.; Lei, L.; Li, J.R.; Sun, H.; Jiang, J.; Dong, B.; Li, H.Y.; Jiang, J.D.; et al. *Bacteroides thetaiotaomicron* ameliorates mouse hepatic steatosis through regulating gut microbial composition, gut-liver folate and unsaturated fatty acids metabolism. *Gut Microbes* **2024**, *16*, 2304159. [CrossRef] [PubMed]
154. Sun, C.; Xiong, X.; Liu, M.; Liang, Q.; Zhao, Q.; Wei, G.; Shi, J.; Li, X. *Bacteroides ovatus* alleviates high-fat and high-cholesterol-induced nonalcoholic fatty liver disease via gut-liver axis. *Biomed. Pharmacother.* **2024**, *178*, 117156. [CrossRef]
155. Wang, Y.; You, Y.; Tian, Y.; Sun, H.; Li, X.; Wang, X.; Wang, Y.; Liu, J. *Pediococcus pentosaceus* PP04 Ameliorates High-Fat Diet-Induced Hyperlipidemia by Regulating Lipid Metabolism in C57BL/6N Mice. *J. Agric. Food Chem.* **2020**, *68*, 15154–15163. [CrossRef] [PubMed]
156. Wang, Y.; Tian, Y.; Zhang, N.; Li, X.; Wang, X.; Wang, W.; Zhang, J.; Piao, C.; Wang, Y.; Liu, J. *Pediococcus pentosaceus* PP04 improves high-fat diet-induced liver injury by the modulation of gut inflammation and intestinal microbiota in C57BL/6N mice. *Food Funct.* **2021**, *12*, 6851–6862. [CrossRef]
157. Yang, F.; Zhu, W.; Edirisuriya, P.; Ai, Q.; Nie, K.; Ji, X.; Zhou, K. Characterization of metabolites and biomarkers for the probiotic effects of *Clostridium cochlearium* on high-fat diet-induced obese C57BL/6 mice. *Eur. J. Nutr.* **2022**, *61*, 2217–2229. [CrossRef]
158. Luo, Y.; Jin, Y.; Wang, H.; Wang, G.; Lin, Y.; Chen, H.; Li, X.; Wang, M. Effects of *Clostridium tyrobutyricum* on Lipid Metabolism, Intestinal Barrier Function, and Gut Microbiota in Obese Mice Induced by High-Fat Diet. *Nutrients* **2024**, *16*, 493. [CrossRef]

159. Benvenuti, L.; D'Antongiovanni, V.; Pellegrini, C.; Fornai, M.; Bernardini, N.; Ippolito, C.; Segnani, C.; Di Salvo, C.; Colucci, R.; Martelli, A.; et al. Dietary Supplementation with the Probiotic SF68 Reinforces Intestinal Epithelial Barrier in Obese Mice by Improving Butyrate Bioavailability. *Mol. Nutr. Food Res.* **2023**, *67*, e2200442. [CrossRef]
160. Xu, W.; Yu, J.; Yang, Y.; Li, Z.; Zhang, Y.; Zhang, F.; Wang, Q.; Xie, Y.; Zhao, B.; Wu, C. Strain-level screening of human gut microbes identifies *Blautia producta* as a new anti-hyperlipidemic probiotic. *Gut Microbes* **2023**, *15*, 2228045. [CrossRef]
161. Castro-Rodríguez, D.C.; Reyes-Castro, L.A.; Vega, C.C.; Rodríguez-González, G.L.; Yáñez-Fernández, J.; Zambrano, E. *Leuconostoc mesenteroides* subsp. *mesenteroides* SD23 Prevents Metabolic Dysfunction Associated with High-Fat Diet-Induced Obesity in Male Mice. *Probiotics Antimicrob. Proteins* **2020**, *12*, 505–516. [CrossRef] [PubMed]
162. Huang, W.; Zhu, W.; Lin, Y.; Chan, F.K.L.; Xu, Z.; Ng, S.C. *Roseburia hominis* improves host metabolism in diet-induced obesity. *Gut Microbes* **2025**, *17*, 2467193. [CrossRef] [PubMed]
163. Lu, K.; Zhou, Y.; He, L.; Li, Y.; Shahzad, M.; Li, D. *Coprococcus* protects against high-fat diet-induced nonalcoholic fatty liver disease in mice. *J. Appl. Microbiol.* **2024**, *135*, lxae125. [CrossRef] [PubMed]
164. Wu, W.; Kaicen, W.; Bian, X.; Yang, L.; Ding, S.; Li, Y.; Li, S.; Zhuge, A.; Li, L. *Akkermansia muciniphila* alleviates high-fat-diet-related metabolic-associated fatty liver disease by modulating gut microbiota and bile acids. *Microb. Biotechnol.* **2023**, *16*, 1924–1939. [CrossRef]

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