frontiers | Research Topics

Nutrition and metabolic aging

Edited by
Zachary Clayton and Devin Wahl

Published in
Frontiers in Nutrition

ISSN 1664-8714
ISBN 978-2-8325-2220-2
DOI 10.3389/978-2-8325-2220-2

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Nutrition and metabolic aging

Topic editors
Zachary Clayton — University of Colorado Boulder, United States
Devin Wahl — Colorado State University, United States

Topic Coordinator
Jingqi Fang — Buck Institute for Research on Aging, United States

Citation
Clayton, Z., Wahl, D., eds. (2023). *Nutrition and metabolic aging*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-2220-2

Table of contents

05 Editorial: Nutrition and metabolic aging
Devin Wahl and Zachary S. Clayton

08 Prevalence of Undernutrition and Risk of Undernutrition in Overweight and Obese Older People
Claire Sulmont-Rossé, Virginie Van Wymelbeke-Delannoy and Isabelle Maître

16 Late-Onset Calorie Restriction Improves Lipid Metabolism and Aggravates Inflammation in the Liver of Old Wistar Rats
Ana Teofilović, Miloš Vratarić, Nataša Veličković, Danijela Vojnović Milutinović, Aleksandra Mladenovic, Milica Prvulovic and Ana Djordjevic

28 Citrate Synthase Insufficiency Leads to Specific Metabolic Adaptations in the Heart and Skeletal Muscles Upon Low-Carbohydrate Diet Feeding in Mice
Kanako Sumi, Yuiko Hatanaka, Reina Takahashi, Naoko Wada, Chihiro Ono, Yuri Sakamoto, Hirohito Sone and Kaoruko Iida

39 Effects of Variability in Glycemic Indices on Longevity in Chinese Centenarians
Sheng-han Ji, Chen Dong, Rou Chen, Chen-chen Shen, Jing Xiao, Yun-juan Gu and Jian-lin Gao

46 Saturated fatty acid biomarkers and risk of cardiometabolic diseases: A meta-analysis of prospective studies
Zhaoqing Li, Haoyuan Lei, Hong Jiang, Yahui Fan, Jia Shi, Chao Li, Fangyao Chen, Baibing Mi, Mao Ma, Jing Lin and Le Ma

57 Non-alcoholic fatty liver disease and dairy products consumption: Results from FASA Persian cohort study
Zahra Keshavarz, Mehran Rahimlou, Mojtaba Farjam, Reza Homayounfar, Mahmoud Khodadost, Ashkan Abdollahi and Reza Tabrizi

69 Effect of dietary protein content shift on aging in elderly rats by comprehensive quantitative score and metabolomics analysis
Wenxuan Zheng, Ruiding Li, Yang Zhou, Fengcui Shi, Yao Song, Yanting Liao, Fan Zhou, Xiaohua Zheng, Jingwen Lv and Quanyang Li

87 The temporal trend of disease burden attributable to metabolic risk factors in China, 1990–2019: An analysis of the Global Burden of Disease study
Yingzhao Jin, Ho So, Ester Cerin, Anthony Barnett, Sumaira Mubarik, Kamal Hezam, Xiaoqi Feng, Ziyue Wang, Junjie Huang, Chenwen Zhong, Khezar Hayat, Fang Wang, Ai-Min Wu, Suowen Xu, Zhiyong Zou, Lee-Ling Lim, Jiao Cai, Yimeng Song, Lai-shan Tam and Dongze Wu

105 Western diet augments metabolic and arterial dysfunction in a sex-specific manner in outbred, genetically diverse mice
Xiangyu Zheng, Zhuoxin Li, Jennifer Berg Sen, Luaye Samarah, Christina S. Deacon, Joseph Bernardo and Daniel R. Machin

121 Dietary magnesium and calcium intake is associated with lower risk of hearing loss in older adults: A cross-sectional study of NHANES
Xinmin Wei

frontiers | Frontiers in Nutrition

TYPE Editorial
PUBLISHED 05 April 2023
DOI 10.3389/fnut.2023.1191958

OPEN ACCESS

EDITED AND REVIEWED BY
Ellen E. Blaak,
Maastricht University, Netherlands

*CORRESPONDENCE
Zachary S. Clayton
✉ Zachary.Clayton@colorado.edu

SPECIALTY SECTION
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

RECEIVED 22 March 2023
ACCEPTED 27 March 2023
PUBLISHED 05 April 2023

CITATION
Wahl D and Clayton ZS (2023) Editorial: Nutrition and metabolic aging. *Front. Nutr.* 10:1191958. doi: 10.3389/fnut.2023.1191958

Editorial: Nutrition and metabolic aging

Devin Wahl[1,2] and Zachary S. Clayton[3]*

[1]Center for Healthy Aging, Colorado State University, Fort Collins, CO, United States, [2]Department of Health and Exercise Science, Colorado State University, Fort Collins, CO, United States, [3]Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO, United States

KEYWORDS

nutrition, aging, metabolic health, calorie restriction, health span

Editorial on the Research Topic
Nutrition and metabolic aging

Aging is the primary non-modifiable risk factor for most chronic and metabolic diseases including cardiovascular disorders, cancers, and neurodegeneration (1) (Jin et al.). As such, identifying interventions that slow the aging process and reduce age-associated diseases is a biomedical research priority. In recent years, several nutritional interventions have been identified that profoundly impact the aging process. These interventions can increase healthspan (the component of life that is generally healthy and devoid of chronic disorders) and lifespan. In this Research Topic, original research studies eloquently demonstrate how nutrition affects healthspan in pre-clinical models and humans (Figure 1).

Perhaps the most robust nutritional intervention to reduce age-associated disease and increase healthspan in pre-clinical models is calorie restriction (CR) (2), a reduction in total caloric intake by 10–50% *without malnutrition* (3). CR extends healthspan and lifespan in many pre-clinical models, and in this Research Topic, one study builds upon these findings. Teofilović et al. demonstrate that 40% CR, even if commenced later in life, improves lipid metabolism by attenuating liver fatty acid synthesis, stimulating beta-oxidation, and reducing triglyceride synthesis in rats. Interestingly, the authors find that these improvements were associated with increased liver inflammation, which may have been partly due to the older age of the rats. The results of this study underscore the need to carefully consider several factors when investigating the effects of CR in health and disease, including the age of an individual, nutritional status, and treatment duration (4).

In addition to CR, the role of dietary macronutrients (protein, carbohydrates, and fats) in modulating health has been the focus of much research (5). In this Research Topic, several studies explore the impact of macronutrient consumption in health and disease. Here, Sumi et al. investigate the effects of a low carbohydrate ketogenic diet or a high-fat, high carbohydrate diet in mice with reduced citrate synthase activity [an enzyme most often responsible for catalyzing the first reaction of the citric acid (TCA) cycle]. Interestingly, the findings of this study show that in the presence of a low-carbohydrate diet, these mice exhibit tissue-specific metabolic adaptations including decreased glycogen levels, suppression of growth pathways, and muscle atrophy. These results provide an overview of how different organs use fuel under varying energy conditions and provide insight into how metabolic disorders are related to impaired TCA cycle metabolism.

Like dietary fats and carbohydrates, dietary protein also has a profound impact on aging (6). Here, Zheng W. et al. provide insight into optimal protein consumption in older age by examining the influence of *ad-libitum* lower protein or higher protein diets on metabolic health in rats, and whether increasing dietary protein intake in older age improves health.

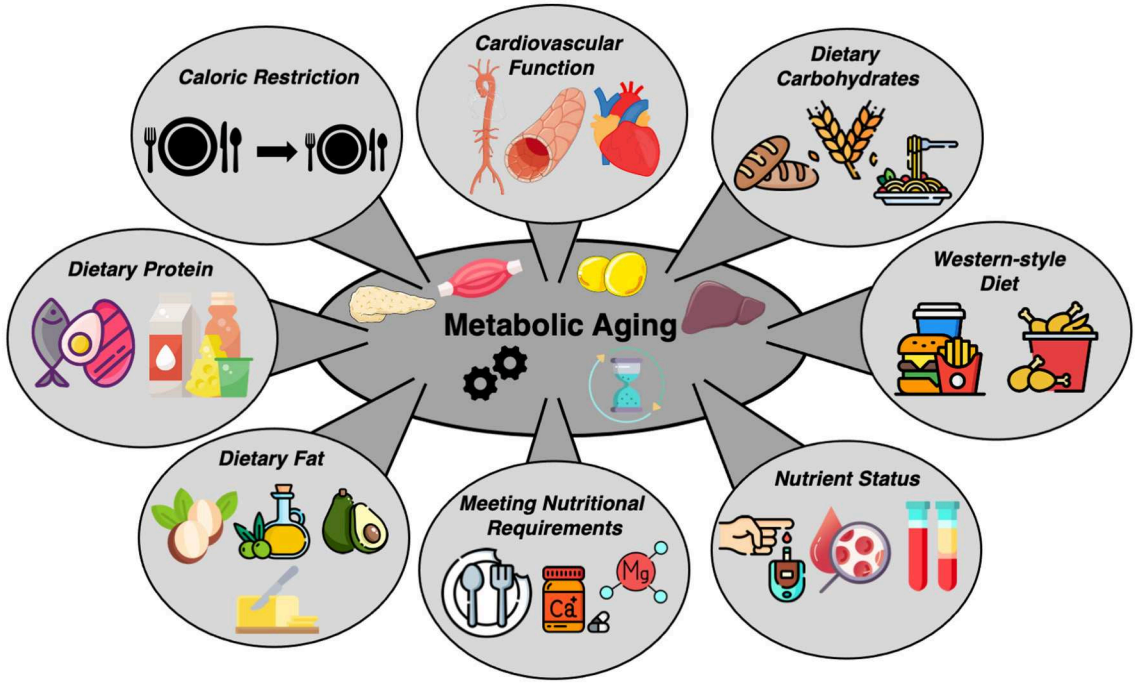


FIGURE 1
Various nutritional interventions and nutrient statuses that influence metabolic health with aging. This figure summarizes a variety of studies included in the call for papers on "Nutrition and Metabolic Aging." This collection of papers represents studies focused on caloric restriction, consumption of a Western-style diet, macro and micronutrient intake, nutrient status and cardiovascular health, all within the context of metabolic aging.

The authors performed comprehensive assessments of behavior, the circulating metabolome, inflammation, and health, and found that shifting from lower protein consumption (in younger age) to higher protein consumption (in older age) improves select health outcomes. These results provide insight into how protein requirements change with advancing age, and the importance of protein quantity in health and disease.

Dietary fat also has an important impact on aging and age-related disease. Traditionally, chronic consumption of a high-fat Western-style diet augments age-associated disease and decreases healthspan, but many of these studies have been performed in genetically homogenous murine models. In this Research Topic, Zheng X. et al. study the effects of a chronic high-fat Western-style diet on metabolic and arterial function in outbred, genetically diverse mice—a model that has greater potential for translation to humans relative to inbred strains. The authors found that consumption of a Western-style diet increased aortic stiffness and systolic blood pressure and decreased vascular endothelial function, which are all independent predictors of cardiovascular-related morbidity and mortality (7). Importantly, these effects varied between male and female mice, emphasizing the importance of considering sex as a biological variable when assessing differences in responses to certain dietary interventions.

Li et al. build upon these findings in mice by performing a meta-analysis to study the links between saturated fatty acid metabolites and cardiovascular disease in humans. The results, which include 49 prospective studies, show that a higher abundance of circulating saturated fatty acids is associated with an increased risk of cardiometabolic disease and stroke. Similarly, Keshavarz et al. examine the impact of dairy products on non-alcoholic fatty liver disease (NAFLD). The authors use measures of liver enzymes, lipid profiles, and glycemic indexes to broadly characterize liver health and find that higher milk consumption is associated with reduced incidence of NAFLD.

This Research Topic also contains studies that provide insight into how certain dietary patterns, macronutrients, or micronutrients affect health in humans. One strong measure of healthspan in humans is glucose tolerance, and several metabolic conditions coincide with higher than average blood glucose levels (8). In this Research Topic, Ji et al. expand upon this knowledge by showing that fasting blood glucose is lower and fluctuates less in centenarians (individuals $\geq$100 years of age) when compared to younger counterparts. These results highlight the role of blood glucose in aging and emphasize the need to maintain blood glucose within a certain range to maintain healthy aging.

Finally, two interesting studies in this Research Topic demonstrate the harmful effects of undernutrition in obesity, and the importance of magnesium and calcium intake to prevent hearing loss in humans. In the first study, Sulmont-Rossé et al. performed a secondary analysis of two French surveys and found that ~23% people with a BMI over 25 are at risk of undernutrition. These results highlight the fact that more work needs to be done to comprehensively understand the role of undernourishment in affecting overweight and obesity. In the second study, Wei examined the impact of dietary magnesium and calcium intake on hearing loss in older adults and found that increased consumption of these minerals is associated with improved hearing function. These data highlight the complexity of the role of nutrition in aging

and the need to consider micronutrient quantity and quality when studying metabolic health outcomes.

As the world's population ages, the burden of age-related disease magnifies. In this Research Topic, important original research studies show that healthspan can be improved with specific nutritional interventions including a reduction in caloric or saturated fat intake, optimizing dietary macronutrient consumption, increasing intake of specific minerals, and ensuring nutritional requirements are met. Together, these excellent papers provide a major step forward into our understanding of how nutrition impacts health and paves a path for future research on the role of nutrition in metabolic aging.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

ZC was currently supported by NIH K99HL159241. DW was supported by NIH F32AG069361.

Acknowledgments

The authors acknowledge www.flaticon.com for graphical support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Niccoli T, Partridge L. Ageing as a risk factor for disease. *Curr Biol.* (2012) 22:R741–52. doi: 10.1016/j.cub.2012.07.024

2. Wahl D, Coogan SC, Solon-Biet SM, de Cabo R, Haran JB, Raubenheimer D, et al. Cognitive and behavioral evaluation of nutritional interventions in rodent models of brain aging and dementia. *Clin Interv Aging.* (2017) 12:1419–28. doi: 10.2147/cia.S145247

3. Mitchell SJ, Madrigal-Matute J, Scheibye-Knudsen M, Fang E, Aon M, González-Reyes JA, et al. Effects of sex, strain, and energy intake on hallmarks of aging in mice. *Cell Metab.* (2016) 23:1093–112. doi: 10.1016/j.cmet.2016.05.027

4. Torrens-Mas M, Navas-Enamorado C, Wahl D, Sanchez-Polo A, Picca A, Oliver J, et al. Sex specific differences in response to calorie restriction in skeletal muscle of young rats. *Nutrients.* (2022) 14:4535. doi: 10.3390/nu14214535

5. Gokarn R, Solon-Biet S, Youngson NA, Wahl D, Cogger VC, McMahon AC, et al. The relationship between dietary macronutrients and hepatic telomere length in aging mice. *J Gerontol A Biol Sci Med Sci.* (2018) 73:446–9. doi: 10.1093/gerona/glx186

6. Wahl D, LaRocca TJ. Transcriptomic effects of healthspan-promoting dietary interventions: current evidence and future directions. *Front Nutr.* (2021) 8:712129. doi: 10.3389/fnut.2021.712129

7. Lakatta EG, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: Part I: aging arteries: a "set up" for vascular disease. *Circulation.* (2003) 107:139–46. doi: 10.1161/01.cir.0000048892.83521.58

8. Rao SS, Disraeli P, McGregor T. Impaired glucose tolerance and impaired fasting glucose. *Am Fam Physician.* (2004) 69:1961–8. Available online at: https://www.aafp.org/pubs/afp/issues/2004/0415/p1961.html

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 05 May 2022
doi: 10.3389/fnut.2022.892675

Prevalence of Undernutrition and Risk of Undernutrition in Overweight and Obese Older People

Claire Sulmont-Rossé[1]*, *Virginie Van Wymelbeke-Delannoy*[1,2] *and Isabelle Maître*[3]

[1] Centre des Sciences du Goût et de l'Alimentation, INRAE, Université Bourgogne Franche-Comté, Institut Agro, CNRS, Dijon, France, [2] CHU Dijon Bourgogne F Mitterrand, Unité du Pôle Personnes Âgées, Dijon, France, [3] Ecole Supérieure d'Agricultures (ESA), USC 1422 GRAPPE, INRAE, SFR 4207 QUASAV, Angers, France

Older people with excess body weight are not spared from undernutrition. They may face appetite decline which may lead to insufficient nutrient intake. They also have a higher risk of developing chronic diseases which may have a negative impact on protein-anabolic pathways. The present study aimed to determine the prevalence of undernutrition in overweight and obese older people from a secondary analysis on data collected through two French surveys among people aged 65 or over (n = 782; 31% men; 65–103 years old). Undernutrition was assessed using the MNA screening tool (Mini-Nutritional Assessment). Results showed that 2% of the respondents with a BMI over 25 were undernourished (MNA score below 17/30) and 23% were at risk of undernutrition (MNA score of 17–23.5). Specifically, 18% of overweight and 29% of obese respondents were at risk of undernutrition. Taking into account the most recent French census data, it can be estimated that in France, around 1,7 million people aged over 65 with a BMI over 25 are undernourished or at risk of undernutrition. Given the worldwide increase in the number of overweight/obese individuals in the last few decades, further research will be needed to develop strategies to tackle nutritional risk in overweight/obese older adults.

Keywords: aged, body weight, body mass index, malnutrition, dependence

OPEN ACCESS

Edited by:
Devin Wahl,
Colorado State University,
United States

Reviewed by:
Pinar Soysal,
Bezmiâlem Vakıf Üniversitesi, Turkey
Zachary Clayton,
University of Colorado Boulder,
United States

***Correspondence:**
Claire Sulmont-Rossé
claire.sulmont-rosse@inrae.fr

Specialty section:
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

Received: *09 March 2022*
Accepted: *11 April 2022*
Published: *05 May 2022*

Citation:
Sulmont-Rossé C, Van Wymelbeke-Delannoy V and Maître I (2022) Prevalence of Undernutrition and Risk of Undernutrition in Overweight and Obese Older People. Front. Nutr. 9:892675. doi: 10.3389/fnut.2022.892675

INTRODUCTION

The issue of overweight and obesity in the older population is set to become a major public health issue in the near future given the increasing number of overweight and obese individuals worldwide. In the United States, 76% of men and 73% of women aged 60 years and over are classified as being overweight (Body Mass Index (BMI) $\geq$ 25 kg/m^2). Among them, 37% of men and 42% of women are classified as having obesity (BMI $\geq$ 30 kg/m^2) (1). In the EU-27, overweight affects 63% and obesity affects 20% of adults aged 65 and over (59 and 17% of adults aged 75 and over) (2).

Obesity increases the risk of developing a wide range of diseases such metabolic diseases, cardiovascular diseases and certain types of cancer which may affect quality of life and functional status in older people (3). However, some studies have suggested that obesity is associated with reduced mortality from cardiovascular diseases in older adults compared to younger adults (4, 5). For instance, in people with heart failure, those with a BMI between 30 and 35 had lower mortality than those who would normally be considered an ideal weight (6). Several biological mechanisms have been proposed to explain this "obesity paradox" during the course of aging, but this phenomenon remained controversial among scientists. Two recent reviews have highlighted

the fact that most of the studies on the "obesity paradox" have focused on the BMI, whereas other factors such as lean body mass, fat distribution or cardiac fitness may be gaining importance in determining survival in obese older adults (7, 8). Mezian et al. (9) noted that although obesity may be protective against metabolic diseases, it accelerates muscle loss and causes sarcopenia.

Although undernutrition is less common than obesity, this disorder is one of the biggest threats to the health and autonomy of older adults (10, 11). Undernutrition is caused by insufficient nutrient intake and/or compromised assimilation of nutrients (12). In the older population, undernutrition is associated with adverse functional and clinical outcomes. It leads to altered body composition (loss of muscle mass) and diminished biological function (impairment of immune function). It increases the risk of falls and fractures, and the risk of infectious episodes and hospital readmission (13, 14). Without care, undernutrition induces or worsens a state of frailty and dependence, and affects quality of life and life expectancy (15–17).

Older people with overweight or obesity are not spared from undernutrition. Like all older individuals, they are likely to face changes which may disrupt eating behavior and lead to a decline in appetite and food intake (18). These changes can be physiological (hormonal dysregulation, oral impairment, sensory decline…), psychological (depression, dementia…) or sociological (retirement, loss of income, loneliness…) (19). In addition, obese individuals have a higher risk of developing chronic diseases leading to organ failure, and a higher risk of cancer and infection. These health conditions often result in acute complications and hospitalization. Chronic and acute diseases have a negative impact on nutritional status, both through a negative impact on skeletal muscle protein-anabolic pathways and due to reduced appetite and nutrient intake (20). Finally, Fleury et al. (21) observed an average daily protein intake per kg body weight of 1.0 g for older people with normal weight status and 0.7 for obese older people when considering the recommendation of 1.2 g protein / kg body weight / day in older people (22). In the obese older people, insufficient protein intake may lead to sarcopenic obesity, which is characterized by skeletal-muscle atrophy and increased adiposity (23). Some evidence indicates that the coexistence of sarcopenia and obesity is associated with higher levels of metabolic disorders, functional decline and an increased risk of mortality compared with to sarcopenia or obesity alone (24, 25).

To the best of our knowledge, there are very few studies reporting prevalence data for undernutrition and risk of undernutrition in overweight and obese older adults. In Brazil, Klee Oehlschlaeger et al. (26) reported that 23% of seniors with a BMI over 25 kg/m^2 were identified as at risk or undernourished by the MNA. In this study, respondents were community-dwelling seniors aged 60 or older and attending recreational or physical activity groups ($n = 210$), and 83% of respondents had excess weight (overweight and obesity). In Turkey, in seniors aged 65 and older with a BMI over 25 kg/m^2 ($n = 1{,}205$), Özkaya and Gürbüz (27) reported that undernutrition and risk of malnutriton were respectively 19% and 31%. This study used the MNA-Short Form (28). Neither of these studies provided separate data for overweight and obese participants.

Consequently, the primary outcome of the present study was to provide prevalence data for undernutrition and risk of undernutrition for overweight and obese older people. The secondary outcome was to investigate the relationship between weight status and nutritional status, taking into account possible cofounders. We hypothesized that being underweight will be associated with a higher risk of undernutrition and that being overweight or obese will be associated with a lower risk of undernutrition compared to normal-weight respondents. However, given the worldwide increase in the number of overweight/obese individuals in the last few decades, we hypothesized that the number of overweight or obese older people being at risk of undernutrition will be significant. Data were derived from a secondary analysis on data collected through two French surveys conducted in 2011 and 2016 among people aged 65 years or over. In these surveys, undernutrition was assessed using the MNA, in line with the recommendations from the French Health Authority (29).

METHOD

Participants

The present study is a secondary analysis of data collected through two surveys conducted among French individuals aged 65 years or over: the AUPALESENS project in 2011 and the RENESSENS project in 2015. The primary outcome of the AUPALESENS survey was to compare the undernutrition risk between older people who delegated all or part of their meal-related activities (food purchasing, cooking) and older people who were still preparing their own meals. This survey included 559 people living at home without or with help and people living in nursing home. The primary outcome of the RENESSENS survey was to compare the undernutrition risk when meals were delegated to different third parties. Consequently, this second survey included 319 people with food help provided by a caregiver, a meals-on-wheels service or a nursing home. In both surveys, recruitment was achieved through advertisements in local newspapers, flyers in local senior associations and with support from local care facilities.

Sample characteristics and primary analyses are published in Van Wymelbeke-Delannoy et al. (30). For the present secondary analysis, we were able to combine the data from these two surveys seeing that the nutritional status, the weight status and the background variables were measured using the same methods. In this secondary analysis, four living arrangements were considered: (1) people living independently at home; (2) people living at home with help provided by a care-giver; (2) people living at home with a regular meals-on-wheels service; (4) people living in a nursing home. Inclusion criteria were the following: no acute illness at the time of the survey no food allergies; no prescribed diet and MMSE score of at least 21 to ensure reliable responses in the survey (Mini Mental State Examination; 31).

Procedure

Each participant underwent two 60 to 90-min face-to-face interviews to collect nutritional and background data. Nutritional status was assessed with the MNA which consists of 18 items including anthropometric measurements as well as question on diet, appetite, health and disabilities (31). The score ranges from 0 to 30. A score below 17 indicates undernutrition, a score of 17–23.5 indicates a risk of undernutrition, and a score of 24 or higher indicates a satisfactory nutritional status.

BMI was calculated as body weight (kg) divided by height (m) squared. Body weight was imputed by weight measurement completed during the interview for community-dwelling participants or by a measurement of weight within 3 months for institutionalized participants. At home, body weight was measured to the nearest 0.1 kg using an electronic scale (TERRAILLON®). Participants were weighed with their clothes on, and the weight was adjusted by subtracting the average weight for the type of clothing. Height was imputed by the height value estimated from the knee-heel length using the Chumléa formula (32). Knee-heel length of the non-dominant leg was measured using a baby-height gauge with the knee and ankle joints fixed at 90° angles. A BMI lower than 21 indicates underweight, a BMI of 21–25 indicates normal weight, a BMI of 25–30 indicates overweight and a BMI of 30 or higher indicates obesity, following the recommendation of French Health Authority (29).

Background variables included age, gender, marital status, main living place during childhood, education, self-perceived financial resources, functional capacities, cognitive status and comorbidities. Regarding functional capacities, respondents completed the Short Physical Performance Battery (SPPB) which combines the results of gait speed, chair stand, and balance tests (33). The SPPB score ranges from 0 to 12 (best functional performance). Regarding cognitive status, respondents completed the Mini Mental State Examination (MMSE). The MMSE is an 11-question test that assesses five areas of cognitive function: orientation, registration, attention, recall, and language. The score ranges from 0 to 30 (best cognitive performance). Scores that are greater than or equal to 25 points (out of 30) indicate normal cognition. Below this, scores can indicate mild (21–24 points), moderate (10–20 points), or severe (≤9 points) cognitive impairment (**34**). Regarding comorbidities, respondents were asked to describe any health problems and to provide their medical prescriptions. The responses and prescriptions were then analyzed by a physician, who determined the number of comorbidities and the presence or not of a metabolic disease (*e.g.* diabetes, high blood pressure).

Data Analysis

Descriptive data are presented as percentages or means (M) and standard errors (SE). The threshold for significance was set at 5%. For each variable, differences across weight status categories were tested using a univariate logistic regression analysis. The odds ratio (OR) and 95% confidence interval (CI) were computed for each variable associated with a significant effect. To evaluate the independence of the observed associations, the variables with a p value < 0.05 were simultaneously entered in a multivariate logistic regression analysis. Interaction effects were tested and removed as they were never significant. All statistical analyses were performed with SAS software (SAS Institute INC., Cary, NC, USA).

RESULTS

Characteristics of the Study Sample

Seven-hundred-eighty-two participants were included in the present secondary data analysis. The characteristics of the study sample are presented in **Table 1** for the whole population and for each weight status group. The respondents tended to be older, with 57% of respondents aged 80 or over. Women outnumbered men and widows outnumbered couples. Most of the respondents had a medium education level (only 20% achieved graduate school) and low to fair income (only 22% reported good income). Among the respondents, 8% were underweight, 39% overweight, and 26% obese.

According to univariate logistic analyses, underweight people were more likely to be single than in a couple (OR = 2.28; CI = 1.50–7.14) or widowed (OR = 2.28; CI = 1.17-4.46) compared to normal-weight individuals. In other words, the singles rate was higher in underweight people than in normal-weight people. Obese people were more likely to have a low income than a good income (OR = 2.27; Ci = 1.22–4.24) compared to normal-weight people. Obesity was also associated with a higher number of morbidities (OR = 1.15; CI = 1.04–1.27) compared to normal-weight status. Finally, people with underweight or obesity were more likely to suffer from a metabolic disease (OR = 1.56; CI = 1.09–2.23 and OR = 2.15; CI = 1.44–3.19, respectively) and they displayed a lower mobility score (SPPB) than normal-weight people (OR = 0.89; CI = 0.83–0.95 and OR = 0.94; CI = 0.90–0.99, respectively).

Weight Status and Nutritional Status

The prevalence of undernutrition was similar across the weight status group: 3% for underweight and normal-weight respondents, 2% for overweight and obese respondents. However, being underweight was associated with the highest prevalence for the risk of undernutrition (60%). In fact, in the MNA tool, a low BMI contributes to a decrease in the global score (BMI<19:−3 points; 19 ≥ BMI < 21:−2 points; 21 ≥ BMI < 23:−1 points) (**Table 1**). According to a univariate logistic analysis, underweight people were more likely to be at risk of undernutrition than well-nourished (OR = 3.61; CI = 1.98–6.58) compared to normal-weight people (*i.e.* the prevalence of people at risk of undernutrition was higher in underweight than in normal-weight people).

The risk of undernutrition ranged from 18% in overweight to 29% in obese and 27% in normal-weight participants (**Table 1**). According to the univariate logistic analysis, overweight people were more likely to be well-nourished than at risk of undernutrition (OR = 1.78; CI = 1.18–2.68) compared to normal-weight people (*i.e.* the prevalence of well-nourished people was higher in overweight than in normal-weight people).

To further decipher the relationship between weight status and nutritional status taking into account all the possible cofounders, a multivariate logistic analysis was performed in

TABLE 1 | Characteristics of the population according to BMI status (underweight: BMI < 21; normal: 21 ≥ BMI < 25; overweight: 25 ≥ BMI < 30; obese: BMI ≥ 30).

	Total	Underweight	Normal	Overweight	Obese	*p*-Value[b]
N	782	62	206	308	206	
Sex, % men	31%	32%	25%	36%	27%	0.0414
Age (yr)[a]	80.5 (0.3)	81.8 (1.1)	81.0 (0.6)	80.3 (0.5)	80.0 (0.6)	0.3618
65–80 yr	43%	42%	44%	44%	42%	0.9328
≥80 yr	57%	58%	56%	56%	52%	
Living arrangement						
At home, without help	37%	26%	39%	41%	32%	0.1004
At home, care-giver	27%	29%	25%	25%	31%	
At home, meals-on-wheels	17%	23%	21%	14%	16%	
Nursing home	19%	22%	15%	20%	21%	
Main living place during childhood						
France	97%	97%	97%	96%	98%	0.8206
Other country	3%	3%	3%	4%	2%	
Marital status						
Single	21%	39%	19%	21%	19%	0.0058
Couple	33%	21%	35%	37%	28%	
Widow	46%	40%	46%	42%	53%	
Education						
None	12%	7%	9%	12%	16%	0.0909
Primary	37%	44%	42%	32%	37%	
Secondary	31%	23%	30%	35%	31%	
Post-secondary	20%	26%	19%	21%	17%	
Income						
Low	26%	32%	23%	22%	34%	0.0596
Fair	52%	45%	54%	54%	51%	
Good	22%	23%	23%	24%	15%	
SPPB[a]	7.4 (0.1)	5.6 (0.6)	7.7 (0.3)	8.1 (0.2)	6.7 (0.3)	<0.001
MMSE[a]	27.0 (1.0)	27.4 (0.3)	27.2 (0.2)	27.1 (0.1)	26.7 (0.2)	0.1094
Comorbidities[a]	3.2 (0.1)	3.3 (0.3)	3.0 (0.1)	3.0 (0.1)	3.6 (0.2)	0.0098
Metabolic disease, %	57%	42%	48%	59%	66%	<0.001
MNA[a]						
Well-nourished (>23.5)	71%	37%	70%	80%	69%	<0.001
At risk (17–23.5)	27%	60%	27%	18%	29%	
Undernourished (<17)	2%	3%	3%	2%	2%	

SPPB, Short Physical Activity Battery; MMSE, Mini-Mental State Examination; MNA, Mini-Nutritional Assessment. [a]Mean ± Standard error; [b]p-value of the Wald Chi-Square derived from a univariate logistic analysis to assess differences between weight status.

which weight status was a dependent variable. All the variables proved to be significantly associated with weight status in univariate analyses (*i.e.*, sex, marital status, income, mobility, comorbidities, presence of a metabolic disease and nutritional status) as independent variables. Interactions were assessed and removed as none proved to be significant. When controlling for possible confounding factors, the nutritional status (MNA) remained significant (**Table 2**). The results confirmed that, in comparison with normal-weight respondents, being underweight was significantly associated with a higher risk of being at risk of undernutrition than well-nourished compared to normal-weight respondents. Conversely, being overweight or obese was significantly associated with a lower risk of being at risk of undernutrition than well-nourished compared to normal-weight respondents (**Table 3**). A closer examination of the link between BMI and metabolic disease showed that in underweight people, respondents suffering from a metabolic disease were more at risk of undernutrition than respondents without metabolic disease (OR = 4.20; CI = 1.30–13.58). However, no such difference was observed in people with overweight (OR = 0.82; CI = 0.47–1.43) or obesity (OR = 1.34; CI = 0.70–2.56).

DISCUSSION

In the older population recruited for the present experiment, 8% were underweight (8% for women and for men), 39% were overweight (36% for women, 46% for men) and 26% were obese (28% for women, 23% for men). These data are similar to those observed in the French INCA 3 study conducted in 2014–2015 among 727 people aged between 65 and 79 years old: 40% were

TABLE 2 | Results of the multivariate logistic regression analysis (dependent variable: weight status): Wald Chi-square and *p* value associated with each independent variable (Likelihood ratio = 166.41; $p < 0.001$).

	Wald Chi-square	*p*-value
Sex	4.81	0.1862
Matrimonial status	12.16	0.0585
Income	8.60	0.1973
Mobility (SPPB)	35.15	0.6459
Comorbidities	19.56	0.9275
Presence of a metabolic disease	13.79	0.0032
Nutritional status (MNA)	32.33	<0.0001

SPPB, Short Physical Activity Battery; MNA, Mini-Nutritional Assessment. All the variables associated with a p value < 0.05 in the univariate regression analyses were simultaneously entered in a multivariate logistic regression analysis as independent variables. Interactions were tested and removed as none proved to be significant.

TABLE 3 | Results of the multivariate logistic regression analysis (dependent variable: weight status): odds ratio estimates for the independent variable "nutritional status" (reference: normal weight).

	Odds ratio	95% CI
Underweight		
Undernourished versus well-nourished	1.62	0.16–16.59
At risk of undernutrition versus well-nourished	5.41	1.98–14.80
Overweight		
Undernourished versus well-nourished	0.15	0.02–0.96
At risk of undernutrition versus well-nourished	0.39	0.21–0.72
Obese		
Undernourished versus well-nourished	1.80	0.32–9.97
At risk of undernutrition versus well-nourished	0.46	0.23–0.90

overweight and 24% were obese (35). A study conducted in 2008 among 4 296 French older individuals aged 75 or over showed higher rate of underweight for women (20%) but not for men (8%), a similar rate of overweight (31% in women; 44% in men) and lower rates for obesity (15% in women; 14% in men) (36). Finally, according to the recent data from Eurostat (2) for France, 2% of people aged 65 or over are underweight, 38% are overweight and 17% are obese. In all of these studies, overweight was defined as a BMI between 25 and 30 kg and obesity as a BMI over 30, while the threshold for underweight varied [BMI lower than 21 in Vernay et al. (36) and in the present study; lower than 18 in Eurostat, 2021], which may explain the different rates of underweight.

Regarding nutritional status, the present study showed that 2% of the respondents with a BMI over 25 were undernourished (no difference was observed between overweight and obese people) and 23% were at risk of undernutrition (18% in overweight to 29% in obese). These features are similar to those reported by Klee Oehlschlaeger et al. (26) who observed that 23% of the seniors with a BMI over 25 were identified as at risk or undernourished by the MNA (Brazilian study), but lower than the ones reported by Özkaya and Gürbüz (27). In this Turkish study, it was observed that 19% of seniors with a BMI over 25 were undernourished and 31% were at risk of undernutrition.

According to recent census data (37), the French population has around 13,453 million people aged 65 or over. If the rates reported by Eurostat (2) for overweight and obesity in France are applied to this population, there are 5.112 million seniors are classified as overweight and 2.287 million seniors are classified as obese. Finally, when the undernutrition and risk of undernutrition rates observed in the present study are applied, there is a total 1.022 million overweight seniors and 0.709 million obese seniors are undernourished or at risk of undernutrition. In other words, it can be estimated that in France, around 1.7 million French people aged 65 or over with a BMI over 25 are undernourished or at risk of undernutrition according to the MNA. If the same iteration is applied for people with a BMI lower than 21, it can be estimated than there are around 300 000 underweight seniors who are undernourished or at risk of undernutrition in France (underweight rate was set at 8%, as observed in the present study).

According to the present results, underweight people were more likely to be at risk of undernutrition than well-nourished compared with normal weight status. In fact, a low BMI is a criterion that is used in several undernutrition screening tools including the Mini-Nutritional Assessment - MNA (31), the Nutritional Risk Screening - NRS (38) and the Undernutrition Universal Screening Tool - MUST (39), though with slightly different BMI thresholds [<19, (19–21), (21–23) for the MNA; < 20.5 for the NRS; <18.5; [18.5–20] for the MUST]. Recently, the European Society for Clinical Nutrition and Metabolism (ESPEN) agreed to consider a BMI <22 as a phenotypic criteria for the diagnosis of undernutrition in people older than 70 years (40). Conversely, overweight and obesity were associated with a lower undernutrition risk compared with normal weight status. However, older people are particularly susceptible to the adverse effects of excess body weight on physical function because of 1) a progressive loss of muscle mass and redistribution of body fat, which leads to sarcopenic obesity (41), and 2) a need to carry greater body mass due to obesity (42). In addition, obesity may result in undernutrition being overlooked in these patients (27), and the loss of muscle mass may go unnoticed until the individual begins to lose physical function (43). Stenholm et al. (44) underlined the importance of recognizing older obese persons with decreased muscle mass or strength, and suggested that clinicians and scientists needed "to identify new targets for prevention and cure of this important geriatric syndrome". However, future researches are needed to identify which factors lead to undernutrition in obese and overweight older people compared to normal-weight or underweight people, such as the impact of metabolic disorder on protein-anabolic pathways, the impact of gut microbiota or the BMI trajectories over the life course. In fact, studies have suggested that accelerated loss of muscle mass in older adults with diabetes is mediated by a direct effect of diabetes on skeletal muscle (45, 46). It is also acknowledged that obesity is associated with alterations in the composition and diversity of the gut microbiota, which may have an impact on the absorption, storage, and expenditure of energy obtained from dietary intake. However, the extent to

which this disruption in the microbial composition influences the nutritional status in obese older people has to be elucidated (47, 48). Finally, some authors have highlighted the importance of studying the life trajectory of the BMI on possible adverse outcomes (49–51). In those studies, higher frailty and mortality rates were observed in men who were overweight in midlife and in later age. However, higher frailty and mortality rates were also observed men who were overweight in midlife but were reclassified as normal-weight in later life because of weight loss, when compared to men who were constantly normal-weight. In the present experiment, it would have been of great interest to investigate nutritional status as a function of weight trajectories and not only as a function of weight status in old age.

Study Limitations

The first limitation of the present study lies in the exclusion of older people with severe cognitive impairments. In France, it is estimated that 4% of people aged 65 or over suffer from a neurological disorder (*e.g.* Alzheimer's disease, Parkinson's disease) (52). However, it was not possible to include these people in the surveys to ensure the reliability of data collected from participants. People suffering from cognitive disorders were included in the "nursing home" category from the RENESSENS survey, but their data were excluded from the present secondary analysis to homogenize the inclusion criteria between the two surveys. However, cognitive disorders are often associated with feeding difficulties and changes in eating behavior which may cause a decrease in food intake (53–55) and altered nutritional status (56, 57). Our decision to exclude individuals with severe cognitive impairments may thus have led to an underestimation in the prevalence of undernutrition and risk of undernutrition.

A second limitation lies in the use of the Mini-Nutritional Assessment questionnaire to assess nutritional status. Recently, Cederholm et al. (40) suggested that undernutrition could be diagnosed and graded using three phenotypic criteria (non-volitional weight loss, low body mass index, and reduced muscle mass) and two etiological criteria (reduced food intake or assimilation, and inflammation or disease burden). However, at the time of the surveys, this new approach had not been yet published, and the MNA was recognized as an appropriate tool to screen for undernutrition and risk of undernutrition (58). In addition, it should be noted that Cederholm et al. (40) agreed on the use of a validated questionnaire such the MNA as a first-line screening tool to identify "at risk" status (59).

A third limitation is the cross-sectional nature of the present experimental design and the absence of data regarding the weight of the respondent at midlife. Some authors have highlighted the importance of studying the life trajectory of the BMI on possible adverse outcomes (49–51). In those studies, higher frailty and mortality rates were observed in men who were overweight in midlife and in later age. However, higher frailty and mortality rates were also observed men who were overweight in midlife but were reclassified as normal-weight in later life because of weight loss, when compared to men who were constantly normal-weight. In the present experiment, it would have been of great interest to investigate nutritional status as a function of weight trajectories and not only as a function of weight status in old age.

CONCLUSION

This secondary analysis of two datasets collected from French old respondents showed that 2% of the respondents with a BMI over 25 were undernourished (no difference was observed between overweight and obese people) and 23% were at risk of undernutrition (18% in overweight to 29% in obese). Taking into account recent French census data, it can be estimated that in France, around 1.7 million people aged 65 or over with a BMI over 25 are undernourished or at risk of undernutrition. Given the growing number of overweight and obese individuals worldwide, the issue of undernutrition in the overweight/obese older population is set to become a major public health issue in the near future.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://doi.org/10.15454/MJTRXM.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by CPP Est 1 - AUPALESENS: #2010-A01079-30; RENESSENS: #2014-A00775-42. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

CS-R, VV, and IM: conceptualization, research, resource provision, and data collection. CS-R: data analysis and writing original version. VV and IM: review and correction. All authors contributed to the article and approved the submitted version.

FUNDING

The surveys were funded by the French National Research Agency: [ANR-09-ALIA-011-02 and ANR-13-ALID-0006-02]. The present work also received funding from ANR (ANR-20-HDHL-0003 FORTIPHY), Research Council Norway (RCN 321819), BBSRC (BB/V018329/1) under the umbrella of the European Joint Programming Initiative A Healthy Diet for a Healthy Life (JPI HDHL) and of the ERA-NET Cofund ERA-HDHL (GA N°696295 of the EU Horizon 2020 Research and Innovation Programme).

ACKNOWLEDGMENTS

The authors thanks Abla Atmani, Elodie Caumon, Géraldine Chaillot, Célia Créma, Julia Ménard, Marion Provost (AUPALESENS), Caroline Barast, Valérie Feyen, Elina Houde, Albane Guyonnet, Caroline Laval, Ioanna Meintani, Virginia Quintas, Elisabeth Rubeillon, and Adeline Tinet (RENESSENS). The authors also thanks Suzanne Rankin for English correction.

REFERENCES

1. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among uS adults, 1999–2010. *Jama*. (2012) 307:491–7. doi: 10.1001/jama.2012.39
2. Eurostat. Body Mass Index (BMI) By Sex, Age and Educational Attainment Level. (2021). Available online at: https://ec.europa.eu/eurostat/databrowser/view/HLTH_EHIS_BM1E__custom_1162105/bookmark/table?lang=en&bookmarkId=6f21bd9e--cbe6--4467--9821--2050435af363 (Accessed: 16 February 16, 2022).
3. Chen H, Guo X. Obesity and functional disability in elderly Americans. *J Am Geriatr Soc*. (2008) 56:689–94. doi: 10.1111/j.1532-5415.2007.01624.x
4. Wang Z, Hoy WE. Age–dependent decline of association between obesity and coronary heart disease: a cohort study in a remote Australian aboriginal community. *BMJ Open*. (2013) 3:e004042. doi: 10.1136/bmjopen-2013-004042
5. Stevens J, Cai J, Pamuk ER, Williamson DF, Thun MJ, Wood JL. The effect of age on the association between body–mass index and mortality. *N Engl J Med*. (1998) 338:1–7. doi: 10.1056/NEJM199801013380101
6. Habbu A, Lakkis NM, Dokainish H. The obesity paradox: fact or fiction? *Am J Cardiol*. (2006) 98:944–8. doi: 10.1016/j.amjcard.2006.04.039
7. Bosello O, Vanzo A. Obesity paradox and aging. *Eat Weight Disord*. (2021) 26:27–35. doi: 10.1007/s40519-019-00815-4
8. Donini LM, Pinto A, Giusti AM, Lenzi A, Poggiogalle E. Obesity or bMI paradox? *Beneath the Tip of the Iceberg. Front. Nutr*. (2020) 39:578–84. doi: 10.3389/fnut.2020.00053
9. Mezian K, Angerová Y, Kara M, Özçakar L. Obesity paradox in sarcopenia and knee osteoarthritis: comment on the article by andrews. *ACR Open Rheumatol*. (2021) 3:812–3. doi: 10.1002/acr2.11330
10. Leslie WS. Improving the dietary intake of frail older people. *Proc Nutr Soc*. (2011) 70:263–7. doi: 10.1017/S0029665111000036
11. Di Francesco V, Fantin F, Omizzolo F, Residori L, Bissoli L, Bosello O, et al. The anorexia of aging. *Dig Dis*. (2007) 25:129–37. doi: 10.1159/000099477
12. Cederholm T, Barazzoni R, Austin P, Ballmer P, Biolo G, Bischoff SC, et al. ESPEN guidelines on definitions and terminology of clinical nutrition. *Clin Nutr*. (2017) 36:49–64. doi: 10.1016/j.clnu.2016.09.004
13. Raynaud–Simon A, Revel–Delhom C, Hebuterne X, French N, Health Program FHHA. Clinical practice guidelines from the french health high authority: nutritional support strategy in protein–energy malnutrition in the elderly. *Clin Nutr*. (2011) 30:312–9. doi: 10.1016/j.clnu.2010.12.003
14. Hickson M. Malnutrition and Aging. *Postgrad Med J*. (2006) 82:2–8. doi: 10.1136/pgmj.2005.037564
15. Rasheed S, Woods T. Malnutrition and quality of life in older people: A systematic review and meta–analysis. *Aging Res Rev*. (2013) 12:561–6. doi: 10.1016/j.arr.2012.11.003
16. Hiesmayr M, Schindler K, Pernicka E, Schuh C, Schoeniger–Hekele A, Bauer P, et al. Decreased food intake is a risk factor for mortality in hospitalized patients: the nutrition day survey (2006) *Clin Nutr*. (2009) 28:484–91. doi: 10.1016/j.clnu.2009.05.013
17. Wallace JI, Schwartz RS, Lacroix AZ, Uhlmann RF, Pearlman RA. Involuntary weight–loss in older outpatients – incidence and clinical–significance. *Journal of the American Geriatrics Society*. (1995) 43:329–37. doi: 10.1111/j.1532-5415.1995.tb05803.x
18. van der Pols–Vijlbrief R, Wijnhoven HAH, Schaap LA, Terwee CB, Visser M. Determinants of protein–energy malnutrition in community–dwelling older adults: a systematic review of observational studies. *Aging Res Rev*. (2014) 18:112–31. doi: 10.1016/j.arr.2014.09.001
19. Sulmont–Rossé C. Eating in the elderly. In: Meiselman HL, editor. *Handbook of Eating and Drinking: Interdisciplinary Perspectives. Cham: Springer International* Publishing (2020). p. 433–57
20. Barazzoni R, Gortan Cappellari G. Double burden of malnutrition in persons with obesity. *Rev Endocr Metab Dis*. (2020) 21:307–13. doi: 10.1007/s11154-020-09578-1
21. Fleury S, Van Wymelbeke–Delannoy V, Lesourd B, Tronchon P, Maître I, Sulmont–Rossé C. Home–Delivered meals: characterization of food intake in elderly beneficiaries. *Nutrients*. (2021) 13:2064. doi: 10.3390/nu13062064
22. Deutz NE, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy–Westphal A, et al. Protein intake and exercise for optimal muscle function with aging: recommendations from the eSPEN expert group. *Clin Nutr*. (2014) 33:929–36. doi: 10.1016/j.clnu.2014.04.007
23. Choi K. Sarcopenia sarcopenic obesity. *Korean J Intern Med*. (2016) 31:1054–60. doi: 10.3904/kjim.2016.193
24. Wannamethee SG, Atkins JL. Muscle loss and obesity: the health implications of sarcopenia and sarcopenic obesity. *Proc Nutr Soc*. (2015) 74:405–12. doi: 10.1017/S002966511500169X
25. Kohara K. Sarcopenic obesity in aging population: current status and future directions for research. *Endocrine*. (2014) 45:15–25. doi: 10.1007/s12020-013-9992-0
26. Klee Oehlschlaeger MH, Alberici Pastore C, Schüler Cavalli A, Gonzalez MC. Nutritional status, muscle mass and strength of elderly in southern brazil. *Nutr Hosp*. (2014) 31:363–70. doi: 10.3305/nh.2015.31.1.7264
27. Özkaya I, Gürbüz M. Malnourishment in the overweight and obese elderly. *Nutr Hosp*. (2019) 36:39–42. doi: 10.20960/nh.02062
28. Rubenstein LZ, Harker JO, Salvà A, Guigoz Y, Vellas B. Screening for undernutrition in geriatric practice: developing the short–form mini–nutritional assessment (MNA–SF). *J Gerontol A Biol Sci Med Sci*. (2001) 56:6 doi: 10.1093/gerona/56.6.M366
29. HAS. *Stratégies de Prise en Charge en Cas de Dénutrition Protéino–Énergétique Chez les Personnes Âgées. Haute Autorité de Santé*. (2007). Available online at: https://www.has--sante.fr/upload/docs/application/pdf/denutrition_personne_agee_2007_--_recommandations.pdf (accessed February 16, 2022).
30. Van Wymelbeke–Delannoy V, Maître I, Salle A, Lesourd B, Bailly N, Sulmont Rossé C. Prevalence of malnutrition risk among older french adults with culinary dependence. *Age Aging*. (2022) 51:afab208. doi: 10.1093/ageing/afab208
31. Guigoz Y, Lauque S, Vellas BJ. Identifying the elderly at risk for malnutrition – the mini nutritional assessment. *Clin Geriatr Med*. (2002) 18:737–57. doi: 10.1016/S0749-0690(02)00059-9
32. Chumléa W, Roche A, Steinbaugh M. Estimating stature from the knee height for 60 to 90 years of age. *J Am Geriatr Soc*. (1985) 33:116–20. doi: 10.1111/j.1532-5415.1985.tb02276.x
33. Guralnik JM, Simonsick EM, Ferrucci L, Glynn RJ, Berkman LF, Blazer DG, et al. A short physical performance battery assessing lower extremity function: association with self–reported disability and prediction of mortality and nursing home admission. *J Gerontol*. (1994) 49:M85–94. doi: 10.1093/geronj/49.2.M85
34. Folstein M, Folstein S, McHugh P. Mini–mental–state: a practical method for grading cognitive state of patients for the clinician. *J Psychiatr Res*. (1975) 12:189–98. doi: 10.1016/0022-3956(75)90026-6
35. ANSES. *Etude Individuelle Nationale des Consommations Alimentaires 3 (INCA 3) Paris, France: ANSES*. (2017). Available online at: https://www.anses.fr/fr/system/files/NUT2014SA0234Ra.pdf (accessed August 6, 2019).
36. Vernay M, Chan–Chee C, Szego E, Castetbon K. Underweight, obesity and disability in elderly community dwellers in france:The french national survey on disability and health in households (HSM, 2008) *Bull Epidemiol Hebd*. (2013) 33–34:425–32. Available online at: http://beh.santepubliquefrance.fr/beh/2013/33-34/pdf/2013_33-34_3.pdf
37. INSEE. *Tableaux de L'économie Française. Montrouge, France: INSEE*. (2020). Available online at: https://www.insee.fr/fr/statistiques/4277619?sommaire=

4318291#:~:text=Tableaux%20et%20graphiques--,Pr%C3%A9sentation,7%20points%20en%20vingt%20ans (accessed March 11, 2021).
38. Reber E, Gomes F, Vasiloglou MF, Schuetz P, Stanga Z. Nutritional risk screening and assessment. *J Clin Med*. (2019) 8:1065. doi: 10.3390/jcm8071065
39. Elia M. *Screening for Malnutrition: a Multidisciplinary Responsibility. Development and Use of the "Malnutrition Universal Screening Tool" ("MUST") for Adults. MAG, a Standing Committee of BAPEN*. (2003). Available online at: https://www.bapen.org.uk/pdfs/must/must--report.pdf (accessed January 2021).
40. Cederholm T, Jensen GL, Correia M, Gonzalez MC, Fukushima R, Higashiguchi T, et al. GLIM criteria for the diagnosis of malnutrition – a consensus report from the global clinical nutrition community. *Clin Nutr*. (2019) 38:1–9. doi: 10.1016/j.clnu.2019.02.033
41. Roubenoff R. Sarcopenic obesity: the confluence of two epidemics. *Obes Res*. (2004) 12:887–8. doi: 10.1038/oby.2004.107
42. Bouchonville MF, Villareal DT. Sarcopenic obesity: how do we treat it? *Curr Opin Endocrinol Diabetes Obes*. (2013) 20:412–9. doi: 10.1097/01.med.0000433071.11466.7f
43. Cetin DC, Nasr G. Obesity in the elderly: more complicated than you think. *Cleve Clin J Med*. (2014) 81:51–61. doi: 10.3949/ccjm.81a.12165
44. Stenholm S, Harris TB, Rantanen T, Visser M, Kritchevsky SB, Ferrucci L. Sarcopenic obesity: definition, cause and consequences. *Curr Opin Clin Nutr Metab Care*. (2008) 11:693–700. doi: 10.1097/MCO.0b013e328312c37d
45. Kalyani RR, Saudek CD, Brancati FL, Selvin E. Association of diabetes, comorbidities, and a1C with functional disability in older adults: results from the national health and nutrition examination survey (NHANES), 1999–2006. *Diabetes Care*. (2010) 33:1055–60. doi: 10.2337/dc09-1597
46. Park SW, Goodpaster BH, Lee JS, Kuller LH, Boudreau R, de Rekeneire N, et al. Excessive loss of skeletal muscle mass in older adults with type 2 diabetes. *Diabetes Care*. (2009) 32:1993–7. doi: 10.2337/dc09-0264
47. Prokopidis K, Cervo MM, Gandham A, Scott D. Impact of protein intake in older adults with sarcopenia and obesity: a gut microbiota perspective. *Nutrients*. (2020) 12(8) doi: 10.3390/nu12082285
48. de Clercq NC, Groen AK, Romijn JA, Nieuwdorp M. Gut microbiota in obesity and undernutrition. *Adv Nutr*. (2016) 7:1080–9. doi: 10.3945/an.116.012914
49. Holme I, Tonstad S. Survival in elderly men in relation to midlife and current bMI. *Age Aging*. (2015) 44:434–9. doi: 10.1093/ageing/afu202
50. Strandberg TE, Stenholm S, Strandberg AY, Salomaa VV, Pitkälä KH, Tilvis RS. The "Obesity paradox," frailty, disability, and mortality in older men: a prospective, longitudinal cohort study. *Am J Epidemiol*. (2013) 178:1452–60. doi: 10.1093/aje/kwt157
51. Rejeski WJ, Marsh AP, Chmelo E, Rejeski JJ. Obesity, intentional weight loss and physical disability in older adults. *Obes Rev*. (2010) 11:671–85. doi: 10.1111/j.1467-789X.2009.00679.x
52. Carrère A, Dubost C–L. *Etat de Santé et Dépendance des Seniors. INSEE*. (2018). Available from: https://www.insee.fr/fr/statistiques/fichier/3646032/FPORSOC18e_EC5_sante-seniors.pdf (Accessed February 16, 2022).
53. Correia SD, Morillo LS, Jacob W, Mansur LL. Swallowing in moderate and severe phases of alzheimer's disease. *Arquivos De Neuro–Psiquiatria*. (2010) 68:855–61. doi: 10.1590/S0004-282X2010000600005
54. Cullen P, Abid F, Patel A, Coope B, Ballard CG. Eating disorders in dementia. *Int J Geriatr Psychiatry*. (1997) 12:559–62. doi: 10.1002/(SICI)1099-1166(199705)12:5<559::AID-GPS550>3.0.CO;2-W
55. Wasson K, Tate H, Hayes C. Food refusal and dysphagia in older people with dementia: ethical and practical issues. *Int J Palliat Nurs*. (2001) 7:465–71. doi: 10.12968/ijpn.2001.7.10.9902
56. Gillette–Guyonnet S, Lauque S, Ousset PJ. Nutrition et maladie d'Alzheimer. *Psychologie et Neuropsychiatrie du Vieillissement*. (2005) 3:S35–41. doi: 10.3917/gs.134.0225
57. Smith KL, Greenwood CE. Weight loss and nutritional considerations in alzheimer disease. *J Nutr for the Elderly*. (2008) 27:381–403. doi: 10.1080/01639360802265939
58. HAS. *Diagnostic de la Dénutrition Chez la Personne Âgée de 70 ans et Plus. Haute Autorité de Santé*. (2021). Available online at: https://www.has--sante.fr/upload/docs/application/pdf/2021--11/reco368_recommandations_denutrition_pa_cd_20211110_v1.pdf (accessed January 14, 2022).
59. van Bokhorst–de van der Schueren MAE, Guaitoli PR, Jansma EP, de Vet HCW. A systematic review of malnutrition screening tools for the nursing home setting. *J Am Med Dir Assoc*. (2014) 15:171–84. doi: 10.1016/j.jamda.2013.10.006

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 23 May 2022
doi: 10.3389/fnut.2022.899255

Late-Onset Calorie Restriction Improves Lipid Metabolism and Aggravates Inflammation in the Liver of Old Wistar Rats

Ana Teofilović[1], Miloš Vratarić[1], Nataša Veličković[1], Danijela Vojnović Milutinović[1], Aleksandra Mladenovic[2], Milica Prvulovic[2] and Ana Djordjevic[1]*

[1] Department of Biochemistry, Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia, [2] Department of Neurobiology, Institute for Biological Research "Siniša Stanković" – National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia

Aging is a progressive process that could disturb metabolic homeostasis in the liver *via* ectopic lipid accumulation, oxidative stress, and deterioration of inflammatory response. Although calorie restriction (CR) is recognized as beneficial for life span and health span prolongation, it is still unclear how late-onset CR, characterized by late beginning and short duration, affects age-related processes. The aim of this study was to examine how late-onset CR-induced metabolic adjustments impact lipid status and inflammation in the liver of old rats. The experiments were conducted on aging male Wistar rats fed *ad libitum* (AL) or exposed to late-onset CR (60% of AL daily intake) from 21st to 24th month. The results showed that late-onset CR reduces body weight, visceral adipose tissue and liver mass, and triglyceride levels when compared to old animals on AL diet. The ameliorating effects of CR on lipid metabolism include increased activity of AMP-activated protein kinase, suppressed *de novo* fatty acid synthesis, stimulated β-oxidation, decreased lipotoxicity, and limited triglyceride synthesis and packaging in the liver. Restricted diet regime, however, does not improve expression of antioxidant enzymes, although it leads to progression of age-related inflammation in the liver, partially through lower corticosterone concentration and decreased activation of glucocorticoid receptor. In conclusion, late-onset CR is able to restore age-related imbalance of lipid metabolism in the liver, but has a negative impact on hepatic inflammatory status, implying that the type of diet for older individuals must be balanced and chosen carefully with appropriate duration and start point.

Keywords: calorie restriction, lipid metabolism, inflammation, liver, aging

OPEN ACCESS

Edited by:
Zachary Clayton,
University of Colorado, Boulder,
United States

Reviewed by:
Devin Wahl,
Colorado State University,
United States
Jingqi Fang,
Buck Institute for Research on Aging,
United States

****Correspondence:***
Ana Teofilović
avasiljevic@ibiss.bg.ac.rs

Specialty section:
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

Received: *18 March 2022*
Accepted: *25 April 2022*
Published: *23 May 2022*

Citation:
Teofilović A, Vratarić M, Veličković N, Vojnović Milutinović D, Mladenovic A, Prvulovic M and Djordjevic A (2022) Late-Onset Calorie Restriction Improves Lipid Metabolism and Aggravates Inflammation in the Liver of Old Wistar Rats. Front. Nutr. 9:899255. doi: 10.3389/fnut.2022.899255

INTRODUCTION

Aging is a natural and progressive process and a major risk factor for development of neurodegenerative and cardiovascular diseases, type 2 diabetes, metabolic syndrome, and tumors (1, 2). One of the most commonly investigated experimental approaches for life span and health span increase and attenuation of age-associated disorders is calorie restriction (CR). At the molecular level, CR activates lipolysis in adipose tissue, stimulates mitochondrial oxidation of fatty acids (FA), reduces dyslipidemia, increases insulin sensitivity, and regulates immune response (3). A key role in mediating the effects of CR on age-related diseases is played by the liver, as it is crucial for the maintenance of energy homeostasis (4).

The aging process is commonly accompanied by visceral adiposity and disturbed lipid homeostasis in the liver. Elevated influx of FA from adipose tissue, stimulation of *de novo* lipogenesis, increased production of very-low-density lipoproteins (VLDL), and lower rate of β-oxidation lead to the progression of hepatic lipotoxicity, steatosis, and dyslipidemia. A well-characterized sensor of disrupted energy balance, whose regulatory potential declines with aging, is the AMP-activated protein kinase (AMPK) (5, 6). Increased AMPK activity induced by CR could be a relevant mediator in lipid metabolism restoration through stimulation of ATP-producing processes (7). AMPK inhibits the activity of one of the key enzymes of *de novo* lipogenesis, acetyl-CoA carboxylase (ACC) by its phosphorylation at Serine 79. Reduced availability of ACC product, malonyl-CoA, results in activation of carnitine palmitoyl-transferase 1 (CPT1), which leads to increased transport of FA into the mitochondria and elevated intensity of β-oxidation (8). AMPK can also phosphorylate and stimulate peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α), a transcriptional activator of CPT1, which additionally contributes to FA elimination (9).

Disturbed metabolic balance and lipid accumulation during aging are both potent triggers for the development of chronic inflammation in the liver. Excess of FA could directly stimulate proinflammatory Toll-like receptor 4 (TLR4), while both FA and lipid species can activate proinflammatory transcriptional regulator nuclear factor kappa B (NFκB), which upregulates the expression of tumor necrosis factor α (TNFα), interleukins, and chemokines (10, 11). Age-associated oxidative stress, a result of increased production of reactive oxygen species (ROS) and decreased expression and activity of antioxidant enzymes, is also a well-described activator of NFκB and downstream inflammatory signaling. Although the early initiated CR is recognized as a potent factor in improving oxidative stress, its effect on redox balance during aging is complex and depends on species, sex, tissue, and duration of limited calorie intake (12, 13).

The age-induced inflammation is usually counterbalanced by increased systemic concentrations of glucocorticoid hormones (14). It was shown that energy depletion related to CR additionally elevates the level of glucocorticoids that could override inflammatory processes and suppress immune response (15). The anti-inflammatory properties of glucocorticoids are achieved by activating the glucocorticoid receptor (GR), a transcriptional factor that negatively regulates the expression of NFκB and other proinflammatory genes. Tissue-specific action of glucocorticoids is more complex, as their intracellular concentration depends on hormone regeneration by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and glucocorticoid clearance regulated by 5α and 5β-reductases (16, 17).

Although a large number of studies point out the beneficial effects of CR, its onset, duration, and percentage of restriction are recognized as important determinants of restrictive diet welfare. Late-onset and short duration of CR provoke significantly lower beneficial cognitive response and even worsen age-related frailty as compared to lifelong restriction started at young age (18, 19). As it is not clear how CR started at old age affects disturbed metabolic balance caused by aging, the aim of this study was to examine the impact of metabolic alterations induced by late-onset CR on lipid status and inflammation in the liver of old male Wistar rats. To that end, we analyzed the level of stimulatory phosphorylation of the energy sensor AMPK, expression of enzymes and transporters involved in *de novo* lipogenesis, FA oxidation, and triglyceride packaging. The inflammatory status was examined through gene expression of *Tlr4* and *Tnf*α, and through protein level and subcellular distribution of NFκB. Protein levels of antioxidant enzymes, glucocorticoid prereceptor metabolism, and GR expression in the liver of old rats upon CR were also assessed.

MATERIALS AND METHODS

Animals and Treatment

In the beginning of the experiment, male Wistar rats were divided into three groups (n = 8 per group). Two groups had *ad libitum* (AL) access to the standard commercial food until they were 6 (6 mAL) or 24 months old (24 mAL). The third group (24 mCR) had unlimited access to food till the 21st month of age, which was followed by a restricted diet regime from 21 to 24 month (late-onset CR) when the animals received 60% of average AL daily food intake. Animals have been acclimated to CR, as restricted diet regime was gradually introduced by reducing the daily average food intake by 10% every 2 days; so after 7 days, food consumption decreased by 40%. The detailed composition of used commercial food (Veterinary Institute Subotica, Subotica, Serbia) was previously published (20). The animals were housed under standard conditions (22°C, 12 h light and dark cycles, constant humidity, free access to water, housed two-three per cage). All the procedures were in compliance with the European Communities Council Directive (2010/63/EU) and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research Siniša Stanković – The National Institute of Republic of Serbia, University of Belgrade (reference number 02-12/17).

Tissue Collection, Blood Plasma Preparation, and Determination of Blood Parameters

After overnight fasting, animals were anesthetized with an intraperitoneal injection of Zoletil 100 (75 mg/kg) and sacrificed. Blood was rapidly collected from the trunk into polypropylene tubes. Serum was isolated after 30 min of coagulation at room temperature and centrifuged at 2,000 × g for 15 min. Collected sera were stored at −70°C for later processing. Glucose concentrations in serum were measured by using a commercially available reagent (OSR6121, Beckman Coulter, Brea, CA, United States) on Olympus AU400 (BLOCK Scientific, North Bellport, NY, United States). The concentrations of serum free fatty acids (FFAs) were assessed by a commercially available kit (FA115, Randox Laboratories Ltd., Crumlin, United Kingdom) and serum triglycerides (TG) were analyzed with triglycerides reagent (Code 12528, Biosystem,

Barcelona, Spain). Both measurements were performed on the semi-automatic biochemistry analyzer Rayto 1904-C (Rayto, Shenzhen, China).

After blood collection and transcardial perfusion, livers and retroperitoneal and perirenal depots of visceral adipose tissue (VAT) were carefully excised, weighed, and stored in liquid nitrogen until further analysis.

Preparation of Whole-Cell Extract

Frozen livers from individual animals were weighed and homogenized with a glass–Teflon homogenizer in five volumes (w/v) of ice-cold RIPA buffer [50 mM Tris-HCl, pH 7.2, 1 mM EDTA-Na_2, 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 2 mM dithiothreitol (DTT), protease and phosphatase inhibitors]. Homogenates were sonicated (5 s/30 s pause/5 s/30 s pause/5 s, 1A, 50/60 Hz, Hielscher Ultrasound Processor, Teltow, Germany), incubated for 60 min on ice with continuous agitation and vortexing, and centrifuged (16,000 × *g*, 20 min, 4°C, Eppendorf 5804/R, Hamburg, Germany). The supernatants were used as the whole-cell extracts.

Preparation of Cytoplasmic, Nuclear, Microsomal, and Mitochondrial Fractions of the Liver

After thawing, livers from individual animals were weighed and homogenized with Janke-Kunkel Ultra Turax (30 s/30 s pause/30 s) in four volumes (w/v) of ice-cold homogenization buffer (20 mM Tris-HCl pH 7.2, 10% glycerol, 50 mM NaCl, 1 mM EDTA-disodium, 1 mM EGTA Na_2, 2 mM DTT, protease and phosphatase inhibitors). The homogenates were filtered through gauze and centrifuged (2,000 × *g*, 15 min, 4°C). The resulting supernatants (S1) were further processed to generate cytoplasmic, microsomal, and mitochondrial fractions, while the pellets (P1) were used to obtain nuclear fractions. The S1 were centrifuged (14,000 × *g*, 30 min, 4°C, Eppendorf 5804/R, Hamburg, Germany). Resulting supernatants (S2) were further processed to generate cytoplasmic and microsomal fractions, while the pellets (P2) were resuspended in one volume of homogenization buffer, which contain 0.05% Triton X-100 and were used as a mitochondrial fraction. The S2 were ultracentrifuged (200,000 × *g*, 90 min, 4°C, Beckman L7-55, Brea, CA, United States) and the final supernatants were used as cytoplasmic fractions. The final pellets were resuspended in 100 mM Na-pyrophosphate buffer (pH 7.4) and centrifuged (200,000 × *g*, 60 min, 4°C, Beckman L7-55, Brea, CA, United States) for the preparation of microsomal fractions. The microsomal pellets were suspended in a storage buffer (50 mM KPO_4 pH 7.4, 0.1 mM EDTA-disodium, 10% glycerol, and 0.1 mM DTT Hielscher Ultrasound Processor, Teltow, Germany), sonicated (5 s/30 s pause/5 s/30 s pause/5 s, 1A, 50/60 Hz, Hielscher Ultrasound Processor, Teltow, Germany), and used as microsomal fractions. For nuclear fractions preparation, P1 from the first centrifugation were washed twice in HEPES buffer (25 mM HEPES pH 7.6, 1 mM EDTA-disodium, 1 mM EGTA-disodium, 10% glycerol, 50 mM NaCl, 2 mM DTT, protease and phosphatase inhibitors like in homogenization buffer, Eppendorf 5804/R, Hamburg, Germany, Eppendorf 5804/R, Hamburg, Germany) by centrifugation (4,000 × *g*, 10 min, 4°C). The resulting pellets were suspended in one volume of NUN buffer (25 mM HEPES, pH 7.6, 1 M Urea, 300 mM NaCl, 1% Nonidet P-40, protease and phosphatase inhibitors). After incubation on ice for 90 min with continuous agitation and frequent vortexing, suspensions were centrifuged (8,000 × *g*, 10 min, 4°C, Eppendorf 5804/R, Hamburg, Germany). The resulting supernatants were used as nuclear fractions. All steps were conducted at 0–4°C and all samples were aliquoted and stored at −70°C.

Preparation of Membrane Fraction of the Liver

Parts of frozen livers from individual animals were weighed and homogenized with a glass-Teflon homogenizer in ten volumes (w/v) of ice-cold DEA buffer (0.25% diethyl amine, 100 mM NaCl, protease and phosphatase inhibitors) and centrifuged (100,000 × *g*, 30 min, 4°C, Beckman L7-55, Brea, CA, United States). The pellets were homogenized in the same volume of 1% Triton buffer (1% Triton, 150 mM NaCl, 50 mM Tris-HCl pH 7.4, 2 mM EDTA, protease and phosphatase inhibitors) with a glass-Teflon homogenizer. Homogenates were put through a 23-gauge needle, incubated for 30 min on ice, and centrifuged (100,000 × *g*, 30 min, 4°C, Beckman L7-55, Brea, CA, United States). The supernatants were used as membrane fractions. Protein content of all fractions was determined by the method of Spector (21) using bovine serum albumin as a standard.

Corticosterone Assay

Liver CORT concentrations were determined in the cytoplasmic fractions by a Corticosterone High Sensitivity EIA kit (Corticosterone HSEIA, AC-15F1, Immunodiagnostic Systems Ltd., Boldon Colliery, United Kingdom), according to the manufacturer's instructions. The standards and samples were measured in duplicate. The plates were read at 450 and 650 nm on the Multiskan Spectrum (Thermo Electron Corporation, Waltham, MA, United States). The hormone concentrations were determined using the 4PL curve-fitting method (Graph-Pad Prism 5, GraphPad Software, Inc., San Diego, CA, United States) and are given as ng/mg of proteins.

Western Blot Analysis

The samples were boiled in Laemmli's buffer and 40 μg proteins of cytoplasmic, microsomal, and whole cell extracts and 60 μg proteins of nuclear, mitochondrial, and membrane fractions were resolved on 8 or 10% sodium dodecyl sulfate-polyacrylamide gels. After protein transfer from the gels to polyvinylidenedifluoride membranes (Immobilon-FL, Merck Millipore, Burlington, MA, United States), membranes were incubated overnight at 4°C with the following primary polyclonal antibodies: anti-AMPKα1/2 (sc-25792, 1:500), anti-CD36 (sc-7309, 1:1,000), anti-CPT1 (sc-139482, 1:500), anti-IκB-α (sc-371, 1:250), anti-phospho-IκB-α (Ser32) (sc-2859s, 1:250), anti-NFκB/p65 (sc-372, 1:1,000), anti-Hexose-6-phosphate dehydrogenase (H6PDH) (sc-67394, 1:500), and anti-GR (sc- 8992, 1:250), all purchased from Santa

Cruz Biotechnology (Dallas, TX, United States). Anti-phospho-AMPKα1/2 (Thr 172) (2535, 1:1,000), anti-ACC (3676, 1:1,000), and anti-phospho-ACC (Ser70), (1:1,000) were purchased from Cell Signaling Technology (Danvers, TX, United States), while anti-PGC1α (ab54481, 1:250), anti-Catalase (ab16731, 1:2,000), anti-SOD1 (ab13498, 1:2,000), anti-SOD2 (ab13533, 1:2,000), and anti-11β-HSD1 (ab393364, 1:1,000) were obtained from Abcam (Cambridge, United Kingdom).

As the proteins of interest were detected in the different fractions of the hepatocytes, constitutively expressed proteins specific for each fraction were used as equal load controls. Namely, anti-β-actin (ab8227, Abcam, 1:10,000) was used as the loading control for cytoplasmatic fraction and whole cell extract, while anti-Hsp60 (sc-13115, Santa Cruz, 1:1,000), anti-Lamin B1 (sc-374015, Santa Cruz, 1:1,000), and anti-Calnexin (ab22595, Abcam, 1:2,000) were used for mitochondrial, nuclear, and microsomal fraction, respectively. Ponceau S was used as the loading control for plasma membrane fraction. Membranes were subsequently washed and incubated for 90 min with horseradish peroxidase conjugated secondary antibodies, goat anti-rabbit IgG (ab6721, Abcam, 1:20,000), or rabbit anti-mouse IgG (ab97046, Abcam, 1:30,000). The immunoreactive protein bands were visualized by chemiluminiscent (ECL) method using iBright CL1500 (Thermo Fisher Scientific, Waltham, MA, United States) and quantitative analysis was performed by iBright software.

RNA Isolation and Reverse Transcription

Total RNA was isolated from the liver using TRIreagent® (AmBion, Life Technologies, Austin, TX, United States). Concentrations of isolated RNA were quantified on NanoPhotometer N60 (Implen, Munich, Germany) by reading the optical density at 260 nm, while the quality and integrity of RNA was confirmed on 2% agarose gel. For the synthesis of complementary DNA (cDNA), Applied Biosystems High-Capacity cDNA reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, United States) was used according to the manufacturer's instructions. The cDNA was stored at −70°C until analysis.

Real-Time Polymerase Chain Reaction

The expression of glucokinase (*Gck*), *Tlr4*, stearoyl-CoA desaturase 1 (*Scd1*), microsomal triglyceride transfer protein (*Mttp*), and apolipoprotein B-100 (*Apo B-100*) genes was analyzed using Power SYBR® Green PCR Master Mix (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, United States). Specific primers (Metabion, Planegg, Germany) for *Gck* (forward 5′-AGA TGC TAT CAA GAG GAG AG-3′, reverse 5′-ACA ATC ATG CCG ACC TCA CAT-3′), *Tlr4* (forward 5′-ATC ATC CAG GAA GGC TTC CA-3′, reverse 5′-GCT AAG AGG GCG ATA CAA TTC-3′), *Scd1* (forward 5′-TGG TGC TCT TTC CCT GTT TGC-3′, reverse 5′-TGG GCT TTG GAA GGT GGA CA-3′), *Mttp* (forward 5′-AAG GCC AAT ATG GAC ATC CAG GTT-3′, reverse 5′-TGG TTA TTA CCA CAG CCA CCC GAT-3′), and *Apo B-100* (forward 5′-AGT CTA CTG GAA GCC ATG AAG GG-3′, reverse 5′-AAT CTG CTG AGG AAG CCT GCT CA-3′) were used for selective amplification. Quantitative normalization of cDNA in each sample was performed using β-*actin* (forward 5′-GAC CCA GAT CAT GTT TGA GAC C-3′, reverse 5′-AGG CAT ACA GGG ACA ACA CA-3′) as the endogenous control. Thermal cycling conditions were 2 min incubation at 50°C, 10 min at 95°C followed by 40 cycles of 95°C for 15 s, and 60°C for 60 s. Quantification of *Tnf*α (Rn01525859_g1) and *5α-reductase* (Rn00567064_m1) genes expression was performed using TaqMan® gene expression FAM-labeled probe set (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, United States). Quantitative normalization of cDNA in each sample was performed using β-*actin* (Rn00667869_m1) as the endogenous control. Thermal cycling conditions were 2 min incubation at 50°C, 10 min at 95°C followed by 60 cycles of 95°C for 15 s, and 60°C for 60 s. All real-time PCR reactions were performed in duplicate using Quant Studio^{TM}3 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, United States). Relative quantification of gene expression was performed using the comparative $2^{-\Delta\Delta Ct}$ method. The same cDNA simple was used as the calibrator on each plate. The results were analyzed by Quant Studio^{TM} Design & Analysis v1.3.1 (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, United States) with a confidence level of 95% ($p \leq 0.05$).

Statistical Analysis

All data are given as means ± SEM (n = 8). The data were tested for normality by Shapiro–Wilk test. Normally distributed data were analyzed by one-way ANOVA followed by Bonferroni *post-hoc* test. Data with deviation from normal distribution were analyzed by Kruskal–Wallis H test followed by Mann–Whitney U *post-hoc* test with Bonferroni correction. A p-value < 0.05 was considered statistically significant. Statistical analyses were performed by the STATISTICA 12 software (StatSoft, Inc., Tulsa, OK, United States).

RESULTS

The Effects of Late-Onset Calorie Restriction on Morphological and Biochemical Parameters

Body weight, liver, and VAT mass were significantly increased in aged rats on AL diet as compared to adult rats (24 mAL vs. 6 mAL, $^{*}p < 0.05$ for body and liver mass; $^{***}p < 0.001$ for VAT mass), whereas CR treatment reduced these parameters to control levels (24 mCR vs. 24 mAL, $^{\#}p < 0.05$ for body and liver mass; $^{\#\#\#}p < 0.001$ for VAT mass). The VAT-to-body ratio was changed in the same manner (increased, 24 mAL vs. 6 mAL, $^{***}p < 0.001$; decreased, 24 mCR vs. 24 mAL, $^{\#\#\#}p < 0.001$), but liver-to-body ratio did not show any changes (**Table 1**).

Serum glucose concentration was decreased in aged rats regardless of the applied diet in comparison with adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$; 24 mCR vs. 6 mAL, $^{\$}p < 0.05$; **Table 1**). This was in agreement with elevated *Gck* mRNA levels in both groups of older rats compared to adults (24 mAL vs. 6 mAL, $^{**}p < 0.01$; 24 mCR vs. 6 mAL, $^{\$\$\$}p < 0.001$; **Table 1**). FFAs in the serum were elevated in aged rats on AL diet as compared

with adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$; **Table 1**), while CR treatment did not provoke any differences. Serum triglyceride level was also significantly increased in aged rats (24 mAL vs. 6 mAL, $^{*}p < 0.05$), whereas CR treatment reduced this parameter (24 mCR vs. 24 mAL, $^{\#}p < 0.05$).

Late-Onset Calorie Restriction Improves Lipid Status in the Liver of Old Rats

In the liver of aged rats on AL diet regime, neither energy sensor AMPK protein expression, nor its stimulatory phosphorylation on Threonine 172 were changed compared with adults. The level of phosphorylated form of AMPK, as well as phospho-AMPK/total AMPK ratio, were elevated in rats after CR treatment compared with animals fed AL (24 mCR vs. 24 mAL, $^{\#}p < 0.05$; 24 mCR vs. 6 mAL, $^{\$\$\$}p < 0.001$; **Figure 1A**), implying that reduced caloric intake led to increased AMPK activity.

The aging process resulted in enhanced expression of total ACC protein level (24 mAL vs. 6 mAL, $^{**}p < 0.01$) and its phosphorylated form on Serine 79 (24 mAL vs. 6 mAL, $^{*}p < 0.05$; **Figure 1B**). Late-onset CR reduced the level of total and phosphorylated ACC as compared with AL diet regime regardless of age (24 mCR vs. 24 mAL,$^{\#\#\#}p < 0.001$; 24 mCR vs. 6 mAL, $^{\$}p < 0.05$). The ratio of inhibitory phosphorylation to total ACC was decreased in the liver of aged rats in comparison to adult animals (24 mAL *vs.* 6 mAL, $^{*}p < 0.05$), while attenuation of *de novo* lipogenesis after CR was supported by increment of this ratio in 24 mCR group in regard to 24 mAL group (24 mCR vs. 24 mAL, $^{\#}p < 0.05$; **Figure 1B**). Examination of further steps of FA metabolism showed the reduction of *Scd1* mRNA level in aged rats on AL diet as compared to adults (24 mAL vs. 6 mAL, $^{**}p < 0.01$; **Figure 1C**), while CR treatment significantly raised the level of *Scd1* (24 mCR vs. 24 mAL, $^{\#\#\#}p < 0.001$; 24 mCR vs. 6 mAL, $^{\$\$}p < 0.01$). On the contrary, the gene expression of *Mttp* was increased in aged rats as compared with adults (24 mAL *vs.* 6 mAL, $^{**}p < 0.01$) and decreased in CR group in regard to aged rats on AL (24 mCR *vs.* 24 mAL, $^{\#\#}p < 0.01$; **Figure 1C**). This result was in agreement with reduction of *Apo B-100* mRNA level in rats after CR treatment in comparison with old rats on AL diet (24 mCR *vs.* 24 mAL, $^{\#\#}p < 0.01$; **Figure 1C**) and implied that late-onset CR prevented triglycerides packaging in the liver. As expected, triglycerides in serum were elevated in aged rats as compared with adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$) and decreased in CR group in regard to aged rats on AL diet (24 mCR vs. 24 mAL, $^{\#}p < 0.05$; **Table 1**).

In accordance with the concentration of FFAs in the serum, the protein level of CD36 transporter in the plasma membrane was increased in old rats on AL diet as compared with adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$; **Figure 2A**), while restricted diet regime lowered and equalized its level with adults (24 mCR vs. 24 mAL, $^{\#}p < 0.05$; **Figure 2A**). The intensity of FA β-oxidation was reduced during aging because the CPT1 protein level in the hepatic mitochondrial fraction was reduced in both groups of aged rats as compared to adult animals (24 mAL vs. 6 mAL, $^{***}p < 0.001$; 24 mCR vs. 6 mAL $^{\$\$\$}p < 0.001$; **Figure 2B**). However, the applied CR regime partially raised the CPT1 level in comparison with aged animals on a standard diet (24 mCR vs. 24 mAL, $^{\#}p < 0.05$; **Figure 2B**). This finding was supported by increased level of PGC-1α in CR group in regard to groups with AL diet regime (24 mCR vs. 24 mAL, $^{\#}p < 0.05$; 24 mCR vs. 6 mAL, $^{\$}p < 0.05$; **Figure 2C**).

TABLE 1 | The effects of late-onset calorie restriction on morphological and biochemical parameters.

	6 mAL	24 mAL	24 mCR
Body mass (g)	403.75 ± 5.96	510.00 ± 28.45 *	423.75 ± 19.36 #
Mass of liver (g)	9.88 ± 0.28	11.66 ± 0.61 *	9.74 ± 0.45 #
Liver to body mass ratio (x1,000)	24.48 ± 0.69	23.88 ± 1.55	23.04 ± 0.64
Mass of VAT (g)	5.40 ± 0.36	13.25 ± 1.70 ***	5.97 ± 0.82 ###
VAT to body mass ratio (x1,000)	13.37 ± 0.84	26.08 ± 2.24 ***	13.77 ± 1.35 ###
Glucose (mmol/l)	8.51 ± 0.28	6.51 ± 0.58 *	6.70 ± 0.45 $
Gck mRNA (fold change)	1.00 ± 0.19	6.00 ± 1.51**	9.53 ± 1.77$$$
FFA (mmol/l)	1.25 ± 0.20	5.33 ± 1.61 *	2.91 ± 1.10
TG (mmol/l)	0.73 ± 0.09	1.35 ± 0.14 *	0.85 ± 0.11 #
Liver CORT (ng/mg)	0.33 ± 0.02	0.57 ± 0.09 *	0.35 ± 0.03 #

Body weight, liver and visceral adipose tissue (VAT) mass, concentration of blood glucose, free fatty acids (FFA) and triglycerides (TG), liver corticosterone (CORT), and glucokinase (Gck) mRNA level were measured in 24th-month-old male Wistar rats who were on ad libitum (AL) diet or on calorie restriction (CR) from 21st to 24th months. All data are presented as means ± SEM (n = 8). Different symbols denote significant differences between aged rats on AL diet and adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$), old rats on CR and on AL diet (24 mCR vs. 24 mAL, $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$), and old rats on CR and adults fed AL (24 mAL vs. 6 mAL, $^{\$}p < 0.05$, $^{\$\$\$}p < 0.001$).

Aging Process Decreases Antioxidant Enzyme Expression, While Late-Onset Calorie Restriction Exacerbates Inflammatory Status in the Liver

The aging process, as expected, resulted in a decrease in protein levels of all examined antioxidant enzymes, SOD1, SOD2, and catalase, regardless of the diet regime (24 mAL vs. 6 mAL, $^{***}p < 0.001$; 24 mCR vs. 6 mAL, $^{\$\$}p < 0.01$ for SOD1; 24 mCR vs. 6 mAL, $^{\$}p < 0.05$ for SOD2; 24 mAL vs. 6 mAL, $^{*}p < 0.05$; 24 mCR vs. 6 mAL, $^{\$}p < 0.05$ for catalase; **Figure 3**), which implies that late-onset CR did not ameliorate the effects of aging on antioxidant enzymes.

Regarding the inflammation, increased ratio between phosphorylated (at Ser^{32}) and total form of NFκB inhibitor (IκB) was observed in both groups of old rats, but with a greater significance after CR treatment (24 mAL vs. 6 mAL, $^{*}p < 0.05$; 24 mCR *vs.* 6 mAL, $^{\$\$}p < 0.01$; **Figure 4A**). Increment in IκB phosphorylation leads to its degradation and consequent activation of NFκB. Although the aging and CR treatment had no effect on NFκB protein level in the cytoplasm, its level was increased in nuclear fraction of old rats regardless of the diet regime (24 mAL vs. 6 mAL, $^{*}p < 0.05$; 24 mCR vs. 6 mAL, $^{\$\$\$}p < 0.001$), with a more severe increment of inflammatory response after CR compared with aged rats on AL diet (24 mCR vs. 24 mAL, $^{\#}p < 0.05$; **Figure 4B**). The level of proinflammatory

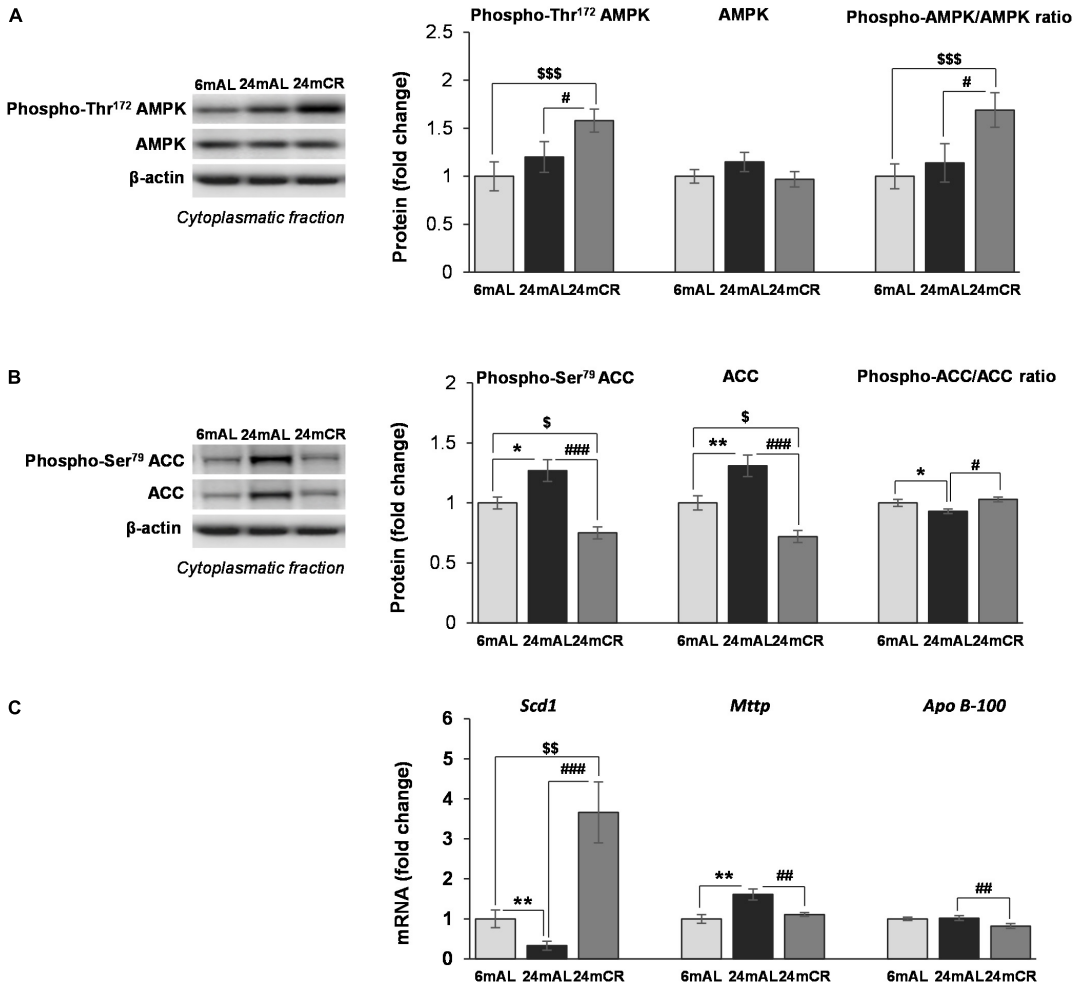


FIGURE 1 | The levels of phosphorylated and total AMPK and ACC proteins and other mediators of lipid synthesis in the liver. Representative Western blots and relative quantification of phospho (Thr172) and total AMPK **(A)** and phospho (Ser79) and total ACC **(B)** in the cytoplasmatic fraction of the liver of adults (6 mAL), aged rats on AL diet (24 mAL), and old rats on CR (24 mCR). β-Actin was used as the loading control for cytoplasmatic fraction. Quantification of *Scd1*, *Mttp*, and *Apo B-100* mRNA levels **(C)** in the liver was done relative to the amount of β-actin. All values are given as means ± SEM ($n = 8$). Asterisk indicates a significant difference between aged rats on AL diet and adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$, $^{**}p < 0.01$), hashtag indicates a difference between old rats on different diet regimes (24 mCR vs. 24 mAL, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$), and symbol $ denotes a difference between old rats on CR and adults fed AL (24 mCR vs. 6 mAL, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$).

cytokine *Tnf*α was also significantly increased in aged rats on AL diet and after restricted diet regime (24 mAL vs. 6 mAL, $^{*}p < 0.05$; 24 mCR vs. 24 mAL, $^{\#}p < 0.05$; 24 mCR vs. 6 mAL, $^{\$}p < 0.05$; **Figure 4D**). Interestingly, only the interaction between CR and aging led to increased *Tlr4* gene expression in the liver of old rats after late-onset CR (24 mCR vs. 24 mAL, $^{\#\#}p < 0.01$; 24 mCR vs. 6 mAL, $^{\$\$}p < 0.01$; **Figure 4C**).

Late-Onset Calorie Restriction Lowers the Level of Corticosterone and Its Nuclear Receptor in the Liver

Protein level of 11β-HSD1 and the level of enzyme that provides its cofactor, H6PDH, were unchanged in all the examined groups (**Figure 5A**). However, the level of corticosterone in the liver was increased in aged rats on AL diet as compared with adults, while CR treatment significantly lowered its level (24 mAL vs. 6 mAL, $^{*}p < 0.05$; 24 mCR vs. 24 mAL, $^{\#}p < 0.05$; **Table 1**). Aging process stimulated glucocorticoid clearance in the liver, as *5α-reductase* mRNA level was increased in both groups of old animals (24 mAL vs. 6 mAL, $^{*}p < 0.05$; 24 mCR vs. 6mAL, $^{\$}p < 0.05$; **Figure 5B**). As a result of *5α-reductase* overexpression and decreased corticosterone concentration, the protein level of GR in the nuclear fraction of the liver was decreased in aged rats on CR regime as compared to old animals on AL diet (24 mCR vs. 24 mAL, $^{\#}p < 0.05$; **Figure 5C**).

DISCUSSION

On the one hand, the results from this study showed that late-onset CR has some positive effects on age-related homeostasis

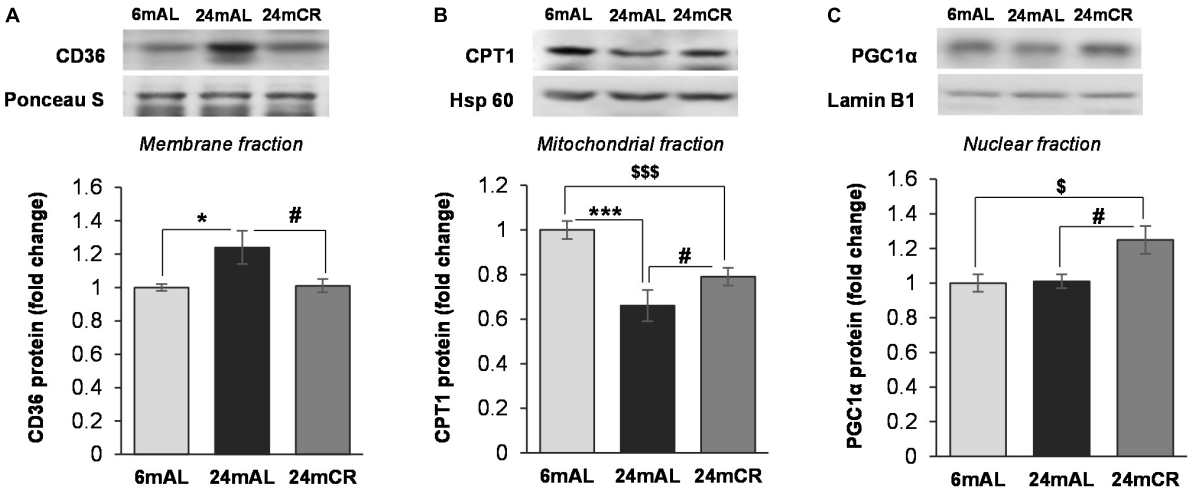


FIGURE 2 | The levels of proteins involved in FA transport and β-oxidation. Representative Western blots and relative quantification of CD36 level in membrane fraction **(A)**, CPT1 level in mitochondrial **(B)**, and PGC1α level in nuclear fraction **(C)** of the liver of adults (6 mAL), aged rats on AL diet (24 mAL) and old rats on CR (24 mCR). Ponceau S, Hsp 60, and Lamin B1 were used as loading controls for membrane, mitochondrial, and nuclear fraction, respectively. All values are given as means ± SEM (n = 8). Asterisk indicates a significant difference between aged rats on AL diet and adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$, $^{***}p < 0.001$), hashtag indicates a significant difference between old rats on different diet regimes (24 mCR vs. 24 mAL, $^{\#}p < 0.05$), and symbol $ denotes a difference between old rats on CR and adults fed AL (24 mCR *vs.* 6 mAL, $^{\$}p < 0.05$, $^{\$\$\$}p < 0.001$).

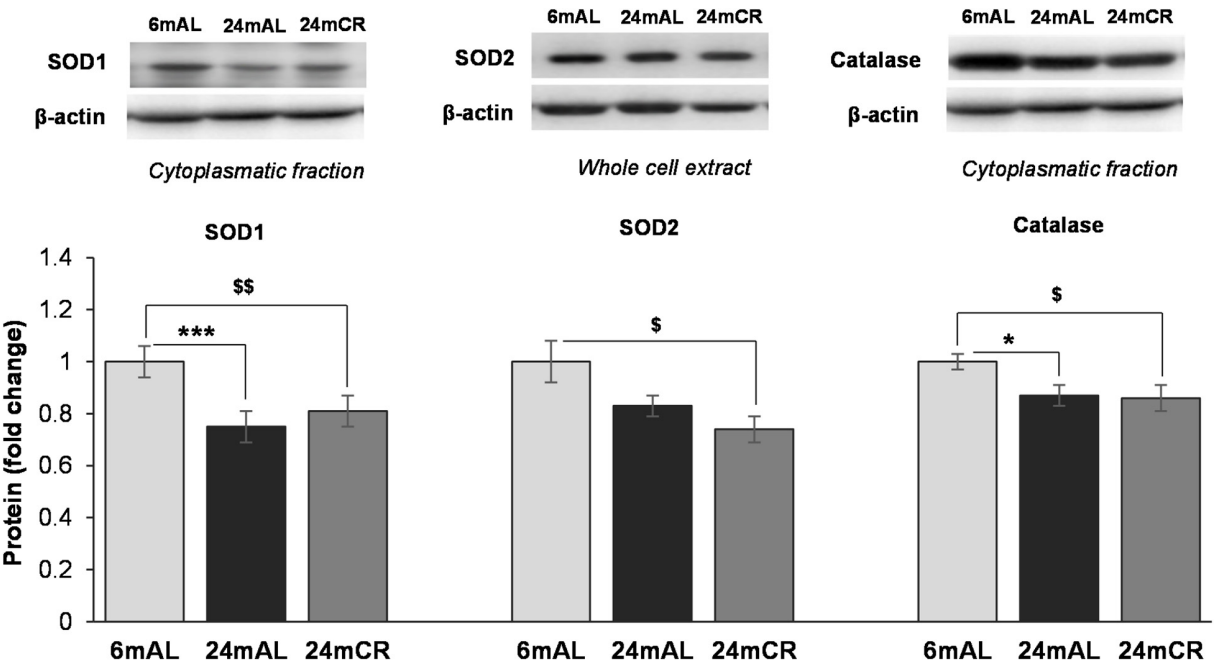


FIGURE 3 | Protein levels of antioxidant enzymes in the liver. Representative Western blots and relative quantification of SOD1 and Catalase in cytoplasmatic and SOD2 protein level in whole cell extract of the liver of adults (6 mAL), aged rats on AL diet (24 mAL), and old rats on CR (24 mCR). β-actin was used as the loading control for cytoplasmatic fraction and whole cell extract. All values are given as means ± SEM (n = 8). Asterisk indicates a significant difference between aged rats on AL diet and adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$, $^{***}p < 0.001$) and symbol $ indicates a significant difference between old rats on CR and adults on AL diet (24 mCR vs. 6 mAL, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$).

disturbances, as it leads to reduced body weight, VAT and liver mass, and serum triglyceride level. In addition, restrictive diet regime induces the activity of AMPK, suppresses *de novo* FA synthesis, stimulates β-oxidation, decreases lipotoxicity, and limits triglyceride synthesis and packaging in the liver. On the other hand, inflammatory response associated with aging becomes more severe after late-onset CR, which could be ascribed to inability of old rats to restore the potential for

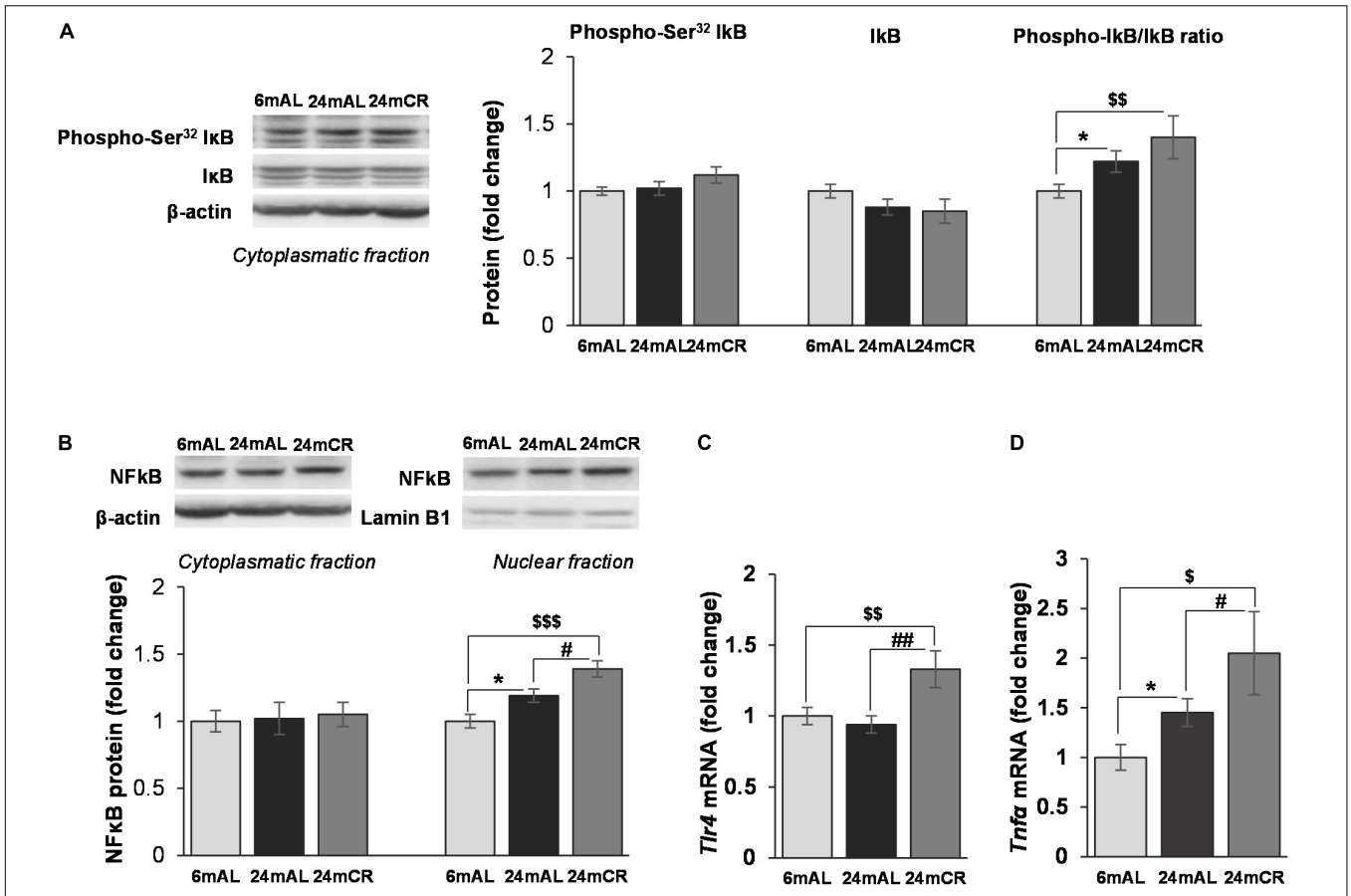


FIGURE 4 | The levels of phosphorylated and total IκB and protein and gene expression of proinflammatory mediators in the liver. Representative Western blots and relative quantification of phospho (Ser32) and total IκB in cytoplasmatic **(A)** and NFκB protein level in citoplasmatic and nuclear fractions **(B)** of the liver of adults (6 mAL), aged rats on AL diet (24 mAL), and old rats on CR (24 mCR). β-actin and Lamin B1 were used as loading controls for cytoplasmatic and nuclear fraction, respectively. Quantification of *Tlr4* **(C)** and *Tnf*α **(D)** mRNA levels in the liver was done relative to the amount of β-actin. All values are given as means ± SEM ($n = 8$). Asterisk indicates a significant difference between aged rats on AL diet and adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$), hashtag indicates a significant difference between old rats on different diet regimes (24 mCR vs. 24 mAL, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$), and symbol $ denotes a difference between old rats on CR and adults on AL diet (24 mCR vs. 6 mAL, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$).

antioxidant protection in the setting of stimulated FA oxidation. Late-onset CR also reduced corticosterone concentration and increased *5α-reductase* expression in the livers of aged rats limiting the hormone's ability to activate the GR. This probably results in diminution of anti-inflammatory properties of glucocorticoids after late-onset CR and additionally contributes to the deterioration of immune response in the liver of old rats.

One of the most evident age-induced alterations of standard-fed old animals from this study is weight gain. Increased body and adipose tissue mass could be referred to as an anorexigenic response caused by age-related leptin resistance and progressive imbalance between fat storage and lipolytic processes in adipocytes (22, 23). Age-induced adiposity increases the level of circulating FFAs and limits glucose utilization that burdens liver's efforts to maintain glucose tolerance and insulin sensitivity (24, 25). This is in agreement with the increased hepatic *Gck* expression and decreased blood glucose level observed in old animals in our study. Long-term *Gck* overexpression and hepatic glucose excess also observed in older mice and humans are associated with an elevated rate of *de novo* lipogenesis, increased hepatic triglyceride content, and circulating lipid levels, which contribute to the ectopic lipid accumulation and hypertriglyceridemia (26, 27). In this study, age-related shift of fat metabolism toward lipogenesis in old rats was confirmed by several findings: (1) increased ACC protein content, which implies stimulated initial step of *de novo* FA synthesis, (2) *Mttp* overexpression, which indicates enhanced triglyceride packaging, and (3) decreased level of CPT1 protein, as a hallmark of lower rate of FA β-oxidation. Observed increased FA influx *via* the CD36 transporter and lowered *Scd1* expression in old rats have further worsened liver lipid status due to accumulation of saturated FA that promotes hepatocellular lipotoxicity, injury, and apoptosis (28).

Reduction of body fat in older individuals is one of the essential benefits of energy-depleting conditions caused by a restricted diet regime (29). Old rats from the present study had a preserved ability to adequately respond to reduced caloric intake, as stimulatory phosphorylation of AMPK, crucial energy sensor

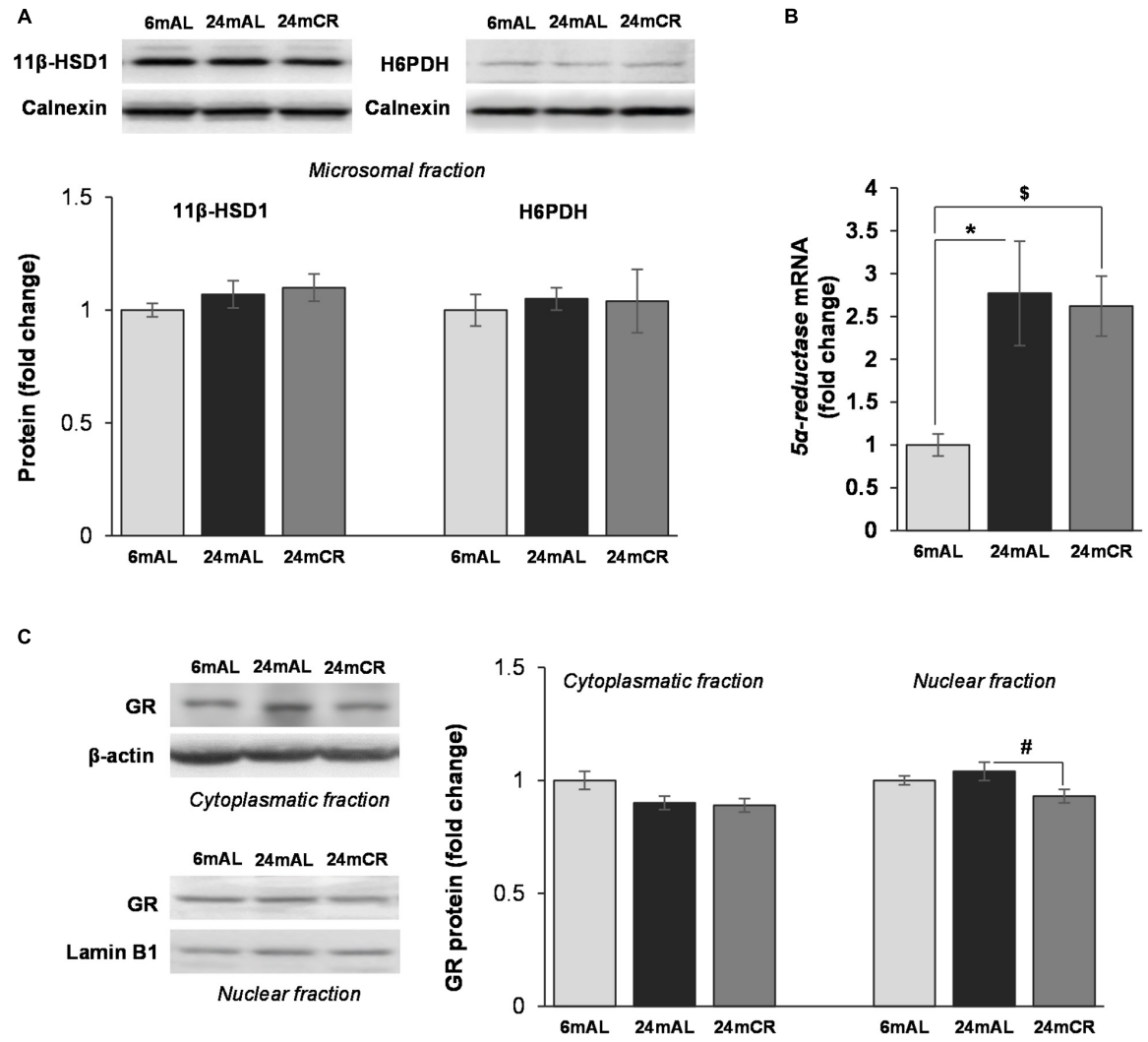


FIGURE 5 | Glucocorticoid prereceptor metabolism and GR protein level in the liver. Representative Western blots and relative quantification of 11β-HSD1 and H6PDH protein levels in microsomal fraction **(A)** and GR protein levels in cytoplasmatic and nuclear fraction **(C)** of the liver of adults (6 mAL), aged rats on AL diet (24 mAL), and old rats on CR (24 mCR). Calnexin, β-actin and Lamin B1 were used as loading controls for microsomal, cytoplasmatic, and nuclear fraction, respectively. Quantification of *5α-reductase* mRNA **(B)** level in the liver was done relative to the amount of β-actin. All values are given as means ± SEM ($n = 8$). Asterisk indicates significant difference between aged rats on AL diet and adults (24 mAL vs. 6 mAL, $^{*}p < 0.05$) and symbol $ indicates a significant difference between old rats on CR and adults fed AL (24 mCR *vs*. 6 mAL, $^{\$}p < 0.05$).

in the liver, was increased. In this line, stimulated AMPK activity raised the inhibitory phosphorylation of ACC, thus leading to its repression, which resulted in the inhibition of FA *de novo* synthesis and stimulation of energy-producing processes such as β-oxidation (30). Indeed, it has been previously shown that ACC knockout mice are characterized by intensified β-oxidation in the muscle, heart, and liver, reduced body weight, and decreased fat content, even after significant caloric overload (31). In our study, redirection of lipid metabolism toward FA oxidation in aged rats after CR was demonstrated by an increased level of transcriptional coactivator PGC1α followed by partial increment of CPT1. Although the CPT1 expression was increased after CR, it could not reach protein level observed in adult rats. This could be explained by reduced oxidative capacity, previously observed in older people (32). Utilization of FA in β-oxidation after CR decreased the abundance of substrate for triglyceride synthesis and packing. It has been previously shown that increased AMPK activity is accompanied with inhibition of glycerol-3-phosphate acyltransferase, the first enzyme in the formation of glycerolipids (33). After CR, triglyceride packaging and secretion in the form of VLDL were most probably diminished, as transcription of genes encoding *Mttp* and *Apo B-100* was decreased and restored to the level observed in adult animals. The beneficial effects of CR are additionally proven by the significantly higher expression of *Scd1*, even in regard to adults, leading to the removal of saturated FA and its transformation into neutral lipid droplets in the liver (34). Hence, late-onset CR not only inhibits lipogenesis and stimulates β-oxidation but also leads to the reduction of hepatic lipotoxity,

implying that applied diet regime was able to restore age-related imbalance of fat metabolism.

Impairment of lipid metabolism during aging is associated with increased FFAs and accumulation of cytotoxic lipids, which could act as ligands and second messengers that activate several protein kinases and trigger downstream proinflammatory signaling cascade (35). Although the level of FFAs was increased in standard-fed aged rats from this study, the expression of *Tlr4* receptor was unchanged. It was previously shown in macrophages that the aging process could even reduce the level of most TLRs and hence weaken their function (36, 37). However, FFAs can stimulate proinflammatory transcriptional regulator NFκB through initiation of Bax protein translocation into lysosomes resulting in release of cysteine protease and cathepsin B, potent activators of IκB kinase (38). In this study, increased ratio between phosphorylated and total IκB, which implies IκB ubiquitination and its subsequent degradation, was observed in aged animals and was more prominent after CR treatment. The lower level of NFκB inhibitor leads to its activation and translocation to the nucleus, which is in agreement with a higher level of NFκB protein observed in nuclear fraction and enhanced transcription of *Tnf*α gene in older rats. Interestingly, the activation of NFκB proinflammatory signaling pathways in aged rats on standard diet was further enhanced in rats exposed to late-onset CR. Other authors reported that a different percentage of restricted calorie regime: mild (15%), moderate (40%), and even higher (50%), lowered cytokine expression and led to the alleviation of inflammatory status (39–41). However, these CR treatments started earlier and had different duration implying that aggravation of inflammatory response observed in our study could rather be a consequence of postponed CR start point. An important factor in NFκB activation during aging could also be oxidative stress caused by increased availability of ROS, potent activators of redox-sensitive transcription factors, cytokines, and inflammasomes (42). Decreased expression of hepatic antioxidant enzymes SOD and catalase in our study confirmed impaired ability of old rats to maintain redox balance. One of the most expected outcomes of restricted diet regime is oxidative stress reduction. However, many studies reported that, despite limited oxidative damage, such dietary approach had a poor effect on mitochondrial ROS production or antioxidant enzyme activity (12). Similarly, in our study, the level of antioxidant enzymes in aged rats after late-onset CR remained decreased, also implying that their expression was not affected by the change in dietary regime. The inability of aged rats to adapt the expression of antioxidant enzymes to increased mitochondrial β-oxidation caused by CR could lead to excess of ROS. This can be, at least partially, the reason for significantly deteriorated inflammatory reaction observed in old rats on restrictive diet regime. Furthermore, increased expression of *Tlr4* receptor after late-onset CR could provoke additional proinflammatory signaling pathway. It has been previously shown that limited food access upregulated TLR4 in colonic cells and macrophages, thus leading to elevated production of TNFα (43). However, aggravated inflammatory response after late-onset CR found herein is examined only in male rats and represents a limitation of the present study. Indeed, previous literature data points to important sex-related alterations in rodent response to CR (44). Namely, it has been shown that different types of CR initiate modified proinflammatory response in female rats (45), implying that additional studies on female rats should be performed in order to adjust dietary protocols and therapeutic strategies.

Stressful conditions induced by aging and reduced caloric intake are suitable for action of glucocorticoid hormones. As the level of proteins involved in tissue-specific hormone regeneration remains unchanged, increased corticosterone concentration in the liver of standard-fed old rats was probably caused by hormone overflow from circulation. Age-induced chronic cytokine production could trigger corticosterone secretion and maintain its elevated level in the circulation due to dysfunction of negative feedback of the hypothalamic–pituitary–adrenal (HPA) axis (46, 47). In spite of increased corticosterone, the level of cytoplasmic GR and its translocation to the nucleus were not changed by aging. The lack of GR activation should be attributed to the increased expression of *5α-reductase* with aging that inactivates corticosterone to its tetrahydro metabolites, which can bind to GR but cannot lead to its dimerization and nuclear translocation (48). Although many studies have demonstrated a stimulatory effect of energy restriction on HPA axis activity in obese humans and animals (49), in this study, we observed a decrease of liver corticosterone and its restoration to the control level. This is in accordance with studies that showed a decrease in circulating corticosterone concentrations following 6 weeks of food deprivation in obese rats or following a 4-week severely energy-restricted diet in obese male and female rats (50, 51). The lower corticosterone level in the liver and retention of increased expression of *5α-reductase* resultantly lead to decreased GR level in the nuclear fraction, which implies the reduced GR signaling in the liver of old animals after CR. This can lead to the attenuation of anti-inflammatory properties of glucocorticoids and further progression of inflammatory response induced by aging. This assumption is confirmed by profound activation of NFκB and significantly increased *Trl4* and *Tnf*α gene expression in the liver of old animals on a restrictive diet regime. The possible mechanism could lay in diminished interaction between GR and NFκB and reduced expression of glucocorticoid-induced leucine zipper (GILZ) that attenuates NFκB translocation to the nucleus (52).

CONCLUSION

In conclusion, short duration and late-onset CR exerts beneficial effect on lipid content, but has a negative impact on hepatic inflammatory status. This implies that the type of the diet could contribute to age-related chronic diseases progression in older individuals and must be chosen carefully. Reduction of lipogenic nutrients and supplementation with antioxidants and vitamins and minerals that have anti-inflammatory properties are recommended. Consumption of foods that promote gut microbiome diversity and composition could be especially beneficial because it reduces the level

of bacterial lipopolysaccharides that provoke inflammatory response *via* TLR4 receptor. Only targeted, balanced, and mild regimens of CR with an appropriate duration and start point could promote longevity and optimal conditions for healthy aging.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković" - The National Institute of Republic of Serbia, University of Belgrade (reference number 02-12/17).

AUTHOR CONTRIBUTIONS

AT, NV, and AD planned the experimental research. AM designed animal model. MV, AT, and MP carried out the experiments. AT, NV, and DVM analyzed the data. AT and MV wrote the manuscript with support from NV, AD, DVM, and AM. All authors reviewed and approved the final manuscript.

FUNDING

This study was supported by the Ministry of Education, Science, and Technological Development of Republic of Serbia under Grant 451-03-68/2022-14/200007.

REFERENCES

1. Niccoli T, Partridge L. Ageing as a risk factor for disease. *Curr Biol.* (2012) 22:R741–52. doi: 10.1016/j.cub.2012.07.024
2. Barzilai N, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in aging. *Diabetes.* (2012) 61:1315–22. doi: 10.2337/db11-1300
3. Anderson RM, Weindruch R. Metabolic reprogramming, caloric restriction and aging. *Trends Endocrinol Metab.* (2010) 21:134–41. doi: 10.1016/j.tem.2009.11.005
4. Kim IH, Kisseleva T, Brenner DA. Aging and liver disease. *Curr Opin Gastroenterol.* (2015) 31:184–91. doi: 10.1097/MOG.0000000000000176
5. Salminen A, Kaarniranta K, Kauppinen A. Age-related changes in AMPK activation: role for AMPK phosphatases and inhibitory phosphorylation by upstream signaling pathways. *Ageing Res Rev.* (2016) 28:15–26. doi: 10.1016/j.arr.2016.04.003
6. Boudaba N, Marion A, Huet C, Pierre R, Viollet B, Foretz M. AMPK re-activation suppresses hepatic steatosis but its downregulation does not promote fatty liver development. *EBioMedicine.* (2018) 28:194–209. doi: 10.1016/j.ebiom.2018.01.008
7. Ma L, Wang R, Wang H, Zhang Y, Zhao Z. Long-term caloric restriction activates the myocardial SIRT1/AMPK/PGC-1α pathway in C57BL/6J male mice. *Food Nutr Res.* (2020) 64:1–7. doi: 10.29219/fnr.v64.3668
8. Jeon S-M. Regulation and function of AMPK in physiology and diseases. *Exp Mol Med.* (2016) 48:e245–245. doi: 10.1038/emm.2016.81
9. Cantó C, Auwerx J. PGC-1alpha, SIRT1 and AMPK, an energy sensing network that controls energy expenditure. *Curr Opin Lipidol.* (2009) 20:98–105. doi: 10.1097/MOL.0b013e328328d0a4
10. Rogero MM, Calder PC. Obesity, inflammation, toll-like receptor 4 and fatty acids. *Nutrients.* (2018) 10:432. doi: 10.3390/nu10040432
11. Boden G. Fatty acid-induced inflammation and insulin resistance in skeletal muscle and liver. *Curr Diab Rep.* (2006) 6:177–81. doi: 10.1007/s11892-006-0031-x
12. Walsh ME, Shi Y, Van Remmen H. The effects of dietary restriction on oxidative stress in rodents. *Free Radic Biol Med.* (2014) 66:88–99. doi: 10.1016/j.freeradbiomed.2013.05.037
13. Rao G, Xia E, Nadakavukaren MJ, Richardson A. Effect of dietary restriction on the age-dependent changes in the expression of antioxidant enzymes in rat liver. *J Nutr.* (1990) 120:602–9. doi: 10.1093/jn/120.6.602
14. Kharwanlang B, Sharma R. Glucocorticoid hormones in aging. In: Rattan S, Sharma R editors. *Hormones in Ageing and Longevity. Healthy Ageing and Longevity*. (Vol. 6), Cham: Springer (2017). doi: 10.1007/978-3-319-63001-4_3
15. Dallman MF, la Fleur SE, Pecoraro NC, Gomez F, Houshyar H, Akana SF. Minireview: glucocorticoids—food intake, abdominal obesity, and wealthy nations in 2004. *Endocrinology.* (2004) 145:2633–8. doi: 10.1210/en.2004-0037
16. White PC, Rogoff D, McMillan DR, Lavery GG. Hexose 6-phosphate dehydrogenase (H6PD) and corticosteroid metabolism. *Mol Cell Endocrinol.* (2007) 265–266:89–92. doi: 10.1016/j.mce.2006.12.022
17. Stewart MJ, Parikh S, Xiao G, Tonge PJ, Kisker C. Structural basis and mechanism of enoyl reductase inhibition by triclosan. *J Mol Biol.* (1999) 290:859–65. doi: 10.1006/jmbi.1999.2907
18. Todorovic ST, Smiljanic KR, Ruzdijic SD, Djordjevic ANM, Kanazir SD. Effects of different dietary protocols on general activity and frailty of male wistar rats during aging. *J Gerontol A Biol Sci Med Sci.* (2018) 73:1036–44. doi: 10.1093/gerona/gly015
19. Prvulovic MR, Milanovic DJ, Vujovic PZ, Jovic MS, Kanazir SD, Todorovic ST, et al. Late-onset calorie restriction worsens cognitive performances and increases frailty level in female wistar rats. *J Gerontol A Biol Sci Med Sci.* (2021) 77:glab353. doi: 10.1093/gerona/glab353
20. Teofiloviæ A, Brkljaèiæ J, Djordjevic A, VojnoviæMilutinoviæ D, Tappy L, Matiæ G, et al. Impact of insulin and glucocorticoid signalling on hepatic glucose homeostasis in the rat exposed to high-fructose diet and chronic stress. *Int J Food Sci Nutr.* (2020) 71:815–25. doi: 10.1080/09637486.2020.1728236
21. Spector T. Refinement of the coomassie blue method of protein quantitation. A simple and linear spectrophotometric assay for less than or equal to 0.5 to 50 microgram of protein. *Anal Biochem.* (1978) 86:142–6. doi: 10.1016/0003-2697(78)90327-5
22. Izquierdo AG, Crujeiras AB, Casanueva FF, Carreira MC. Leptin, obesity, and leptin resistance: where are we 25 years later? *Nutrients.* (2019) 11:2704. doi: 10.3390/nu11112704
23. Bonzón-Kulichenko E, Moltó E, Pintado C, Fernández A, Arribas C, Schwudke D, et al. Changes in visceral adipose tissue plasma membrane lipid composition in old rats are associated with adipocyte hypertrophy with aging. *J Gerontol Ser A.* (2018) 73:1139–46. doi: 10.1093/gerona/gly081
24. Frohnert BI, Jacobs DR, Steinberger J, Moran A, Steffen LM, Sinaiko AR. Relation between serum free fatty acids and adiposity, insulin resistance, and cardiovascular risk factors from adolescence to adulthood. *Diabetes.* (2013) 62:3163–9. doi: 10.2337/db12-1122
25. Escrivá F, Gavete ML, Fermín Y, Pérez C, Gallardo N, Alvarez C, et al. Effect of age and moderate food restriction on insulin sensitivity in Wistar rats: role of adiposity. *J Endocrinol.* (2007) 194:131–41. doi: 10.1677/joe.1.07043
26. Ferre T, Riu E, Franckhauser S, Agudo J, Bosch F. Long-term overexpression of glucokinase in the liver of transgenic mice leads to insulin resistance. *Diabetologia.* (2003) 46:1662–8. doi: 10.1007/s00125-003-1244-z
27. Flannery C, Dufour S, Rabøl R, Shulman GI, Petersen KF. Skeletal muscle insulin resistance promotes increased hepatic *de novo* lipogenesis,

hyperlipidemia, and hepatic steatosis in the elderly. *Diabetes.* (2012) 61:2711–7. doi: 10.2337/db12-0206
28. Alkhouri N, Dixon LJ, Feldstein AE. Lipotoxicity in nonalcoholic fatty liver disease: not all lipids are created equal. *Expert Rev Gastroenterol Hepatol.* (2009) 3:445–51. doi: 10.1586/egh.09.32
29. Bales CW, Porter Starr KN. Obesity interventions for older adults: diet as a determinant of physical function. *Adv Nutr.* (2018) 9:151–9. doi: 10.1093/advances/nmx016
30. Hardie DG, Pan DA. Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase. *Biochem Soc Trans.* (2002) 30:1064–70. doi: 10.1042/bst0301064
31. Srivastava RAK, Pinkosky SL, Filippov S, Hanselman JC, Cramer CT, Newton RS. AMP-activated protein kinase: an emerging drug target to regulate imbalances in lipid and carbohydrate metabolism to treat cardio-metabolic diseases. *J Lipid Res.* (2012) 53:2490–514. doi: 10.1194/jlr.R025882
32. Layec G, Haseler LJ, Richardson RS. Reduced muscle oxidative capacity is independent of O2 availability in elderly people. *Age.* (2013) 35:1183–92. doi: 10.1007/s11357-012-9442-6
33. Ruderman N, Prentki M. AMP kinase and malonyl-CoA: targets for therapy of the metabolic syndrome. *Nat Rev Drug Discov.* (2004) 3:340–51. doi: 10.1038/nrd1344
34. Turyn J, Stojek M, Swierczynski J. Up-regulation of stearoyl-CoA desaturase 1 and elongase 6 genes expression in rat lipogenic tissues by chronic food restriction and chronic food restriction/refeeding. *Mol Cell Biochem.* (2010) 345:181–8. doi: 10.1007/s11010-010-0571-x
35. Geng Y, Faber KN, de Meijer VE, Blokzijl H, Moshage H. How does hepatic lipid accumulation lead to lipotoxicity in non-alcoholic fatty liver disease? *Hepatol Int.* (2021) 15:21–35. doi: 10.1007/s12072-020-10121-2
36. Renshaw M, Rockwell J, Engleman C, Gewirtz A, Katz J, Sambhara S. Cutting edge: impaired toll-like receptor expression and function in aging. *J Immunol.* (2002) 169:4697–701. doi: 10.4049/jimmunol.169.9.4697
37. Boehmer ED, Goral J, Faunce DE, Kovacs EJ. Age-dependent decrease in Toll-like receptor 4-mediated proinflammatory cytokine production and mitogen-activated protein kinase expression. *J Leukoc Biol.* (2004) 75:342–9. doi: 10.1189/jlb.0803389
38. Feldstein AE, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, et al. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. *Hepatol Baltim Md.* (2004) 40:185–94. doi: 10.1002/hep.20283
39. Park CY, Park S, Kim MS, Kim H-K, Han SN. Effects of mild calorie restriction on lipid metabolism and inflammation in liver and adipose tissue. *Biochem Biophys Res Commun.* (2017) 490:636–42. doi: 10.1016/j.bbrc.2017.06.090
40. Allen BD, Liao C-Y, Shu J, Muglia LJ, Majzoub JA, Diaz V, et al. Hyperadrenocorticism of calorie restriction contributes to its anti-inflammatory action in mice. *Aging Cell.* (2019) 18:e12944. doi: 10.1111/acel.12944
41. MacDonald L, Hazi A, Paolini AG, Kent S. Calorie restriction dose-dependently abates lipopolysaccharide-induced fever, sickness behavior, and circulating interleukin-6 while increasing corticosterone. *Brain Behav Immun.* (2014) 40:18–26. doi: 10.1016/j.bbi.2014.01.005
42. Chung HY, Kim DH, Lee EK, Chung KW, Chung S, Lee B, et al. Redefining chronic inflammation in aging and age-related diseases: proposal of the senoinflammation concept. *Aging Dis.* (2019) 10:367–82. doi: 10.14336/AD.2018.0324
43. Belmonte L, Achamrah N, Nobis S, Guérin C, Riou G, Bôle-Feysot C, et al. A role for intestinal TLR4-driven inflammatory response during activity-based anorexia. *Sci Rep.* (2016) 6:35813. doi: 10.1038/srep35813
44. Kane AE, Sinclair DA, Mitchell JR, Mitchell SJ. Sex differences in the response to dietary restriction in rodents. *Curr Opin Physiol.* (2018) 6:28–34. doi: 10.1016/j.cophys.2018.03.008
45. Dogan S, Ray A, Cleary MP. The influence of different calorie restriction protocols on serum pro-inflammatory cytokines, adipokines and IGF-I levels in female C57BL6 mice: short term and long term diet effects. *Meta Gene.* (2017) 12:22–32. doi: 10.1016/j.mgene.2016.12.013
46. Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, et al. Regulation of the hypothalamic-pituitary-adrenocortical stress response. *Compr Physiol.* (2016) 6:603–21. doi: 10.1002/cphy.c150015
47. Gupta D, Morley JE. Hypothalamic-pituitary-adrenal (HPA) axis and aging. *Compr Physiol.* (2014) 4:1495–510. doi: 10.1002/cphy.c130049
48. McInnes KJ, Kenyon CJ, Chapman KE, Livingstone DEW, Macdonald LJ, Walker BR, et al. 5alpha-reduced glucocorticoids, novel endogenous activators of the glucocorticoid receptor. *J Biol Chem.* (2004) 279:22908–12. doi: 10.1074/jbc.M402822200
49. Seimon RV, Hostland N, Silveira SL, Gibson AA, Sainsbury A. Effects of energy restriction on activity of the hypothalamo-pituitary-adrenal axis in obese humans and rodents: implications for diet-induced changes in body composition. *Horm Mol Biol Clin Investig.* (2013) 15:71–80. doi: 10.1515/hmbci-2013-0038
50. Dubuc PU, Carlisle HJ. Food restriction normalizes somatic growth and diabetes in adrenalectomized ob/ob mice. *Am J Physiol.* (1988) 255:R787–93. doi: 10.1152/ajpregu.1988.255.5.R787
51. Hainer V, Stich V, Kunesová M, Parízková J, Zák A, Wernischová V, et al. Effect of 4-wk treatment of obesity by very-low-calorie diet on anthropometric, metabolic, and hormonal indexes. *Am J Clin Nutr.* (1992) 56:281S–2S. doi: 10.1093/ajcn/56.1.281S
52. Ronchetti S, Migliorati G, Riccardi C. GILZ as a mediator of the anti-inflammatory effects of glucocorticoids. *Front Endocrinol.* (2015) 6:170. doi: 10.3389/fendo.2015.00170

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 07 July 2022
doi: 10.3389/fnut.2022.925908

Citrate Synthase Insufficiency Leads to Specific Metabolic Adaptations in the Heart and Skeletal Muscles Upon Low-Carbohydrate Diet Feeding in Mice

*Kanako Sumi[1], Yuiko Hatanaka[1], Reina Takahashi[1], Naoko Wada[1], Chihiro Ono[1], Yuri Sakamoto[2], Hirohito Sone[3,4] and Kaoruko Iida[1,4,5]**

[1] Department of Food and Nutrition Science, Graduate School of Humanities and Sciences, Ochanomizu University, Bunkyo, Japan, [2] Department of Clinical Dietetics and Human Nutrition, Faculty of Pharmacy and Pharmaceutical Sciences, Josai University, Sakado, Japan, [3] Department of Hematology, Endocrinology and Metabolism, Faculty of Medicine, Niigata University, Niigata, Japan, [4] Department of Endocrinology and Metabolism, Faculty of Medicine, University of Tsukuba, Tsukuba, Japan, [5] The Institute for Human Life Innovation, Ochanomizu University, Bunkyo, Japan

OPEN ACCESS

Edited by:
Zachary Clayton,
University of Colorado Boulder,
United States

Reviewed by:
Thomas LaRocca,
Colorado State University,
United States
Jingqi Fang,
Buck Institute for Research on Aging,
United States
Keenan Thomas Greyslak,
University of Oregon, United States

***Correspondence:**
Kaoruko Iida
iida.kaoruko@ocha.ac.jp

Specialty section:
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

Received: 22 April 2022
Accepted: 14 June 2022
Published: 07 July 2022

Citation:
Sumi K, Hatanaka Y, Takahashi R, Wada N, Ono C, Sakamoto Y, Sone H and Iida K (2022) Citrate Synthase Insufficiency Leads to Specific Metabolic Adaptations in the Heart and Skeletal Muscles Upon Low-Carbohydrate Diet Feeding in Mice. Front. Nutr. 9:925908. doi: 10.3389/fnut.2022.925908

A decrease in TCA cycle activity may lead to impaired nutrition metabolism and cellular energy shortage. Herein, we aimed to characterize the detailed metabolic changes that compensate for energy shortages in energy-consuming organs (heart and skeletal muscles) in mice with knockout of citrate synthase (CS), an important enzyme in the TCA cycle. CS hetero knockout (CS+/−) mice and wild-type mice were fed a low-carbohydrate ketogenic diet (LCKD) or high-fat, high-carbohydrate diet (HFHCD) to induce metabolic changes. Body weight, blood serum parameters, metabolic gene expression, and adenosine triphosphate (ATP) levels were measured in the heart and skeletal muscles. Glycogen content, anabolic and catabolic biomarkers, and morphological changes were also assessed in the skeletal muscles. After diet feeding, there were no differences observed in the body weight and blood serum parameters between wild-type and CS+/− mice. The cardiac expression of genes related to the utilization of fatty acids, monocarboxylates, and branched amino acids increased in LCKD-fed CS+/− mice. In contrast, no significant differences in gene expression were observed in the muscles of LCKD-fed mice or the heart and muscles of HFHCD-fed mice. ATP levels decreased only in the skeletal muscles of LCKD-fed CS+/− mice. Additionally, the decrease in glycogen content, suppression of p70 S6 kinase, and presence of type I fiber atrophy were observed in the muscles of LCKD-fed CS+/− mice. These results suggest that the energy-consuming organs with CS insufficiency may undergo tissue-specific adaption to compensate for energy shortages when the carbohydrate supply is limited.

Keywords: citrate synthase, heart, knockout mice, skeletal muscle, TCA cycle

Abbreviations: Akt, protein kinase B; AMPK, AMP-activated protein kinase; Atg1, atrogin-1; ATP, adenosine triphosphate; BCAA, branched-chain amino acid; CS, citrate synthase; CSA, cross-sectional area; ERRα, estrogen-related receptor α; FA, fatty acid; Gys-1, glycogen synthase-1; HFHCD, high-fat high-carbohydrate diet; LCKD, low-carbohydrate ketogenic diet; KO, knockout; LC3, light chain 3; MCT-1, monocarboxylate transporter-1; MCT-1, monocarboxylate transporter-1; MuRF-1, muscle RING finger-1; NRF1, nuclear respiratory factor 1; PGC1α, peroxisome proliferator-activated receptor γ coactivator 1α; S6K, p70 S6 kinase; TCA, tricarboxylic acid; WT, wild-type.

INTRODUCTION

The production of adenosine triphosphate (ATP) is essential for organ function, particularly for the mechanical and electrical functions of high energy-consuming organs such as the heart and skeletal muscles (1). In most cells, the mitochondrial tricarboxylic acid (TCA) cycle acts as the central driver of ATP biosynthesis. In eukaryotes, the TCA cycle operates in the matrix of the mitochondria following the biosynthesis of acetyl-CoA *via* the oxidation of mainly fat and carbohydrates (2, 3). Although fat is the main substrate for cardiac ATP production, the heart can also use other energy sources through multiple mechanisms, such as the control of enzyme activity, signal transduction events, and gene regulation (4, 5). In contrast, the skeletal muscles use glucose as their main fuel source and can switch to the use of fatty acids (FAs) under certain conditions (6).

Aging and metabolic diseases such as type 2 diabetes are closely associated with a decrease in the mitochondrial capacity including the TCA cycle activities (7–10), which could lead to the disruption of nutrient metabolism and a shortage of cellular energy. Recent studies have reported that the dysfunction of the TCA cycle along with a loss or a decrease in the activity of its related enzymes is involved in the etiology of various diseases (11). However, the effects of such TCA cycle impairment on the flexibility and capacity of substrate metabolism in the heart and skeletal muscles have not yet been thoroughly investigated. Tissue-specific adaptive responses to the impairments of this pathway should be determined to understand the mechanisms involved in the development of geriatric changes and metabolic disorders.

Citrate synthase (CS) is the rate-limiting enzyme of TCA cycle and plays a pivotal in regulating energy production through mitochondrial respiration. CS converts acetyl-CoA and oxaloacetate into citrate, and its activity is sometimes used as a biomarker of mitochondrial content and function (12, 13). CS activity in the muscles is increased by physical activities (14) and decreases with aging (15, 16) or in metabolic disorders such as type 2 diabetes (17). Animal studies revealed that in aged hearts, the gene expression and catalytic activity of CS were significantly decreased among TCA cycle enzymes (18, 19). Therefore, we considered CS knockout mice to be useful to investigate the effects of TCA cycle dysfunction on metabolic adaptations of the heart and skeletal muscles.

Homozygote CS knockout (CS–/–) mice present with embryonic lethality; therefore, heterozygous CS knockout (CS+/−) mice are preferred. The gene and protein expression and activity of CS in CS–/+ mice are approximately half of those in their wild-type (WT) (+/+) siblings. However, unexpectedly, we found that there were almost no differences in the growth, morphology, blood parameters, insulin sensitivity, ATP levels, and metabolic gene expression in the organs (including in the heart and skeletal muscles) between WT and CS+/− mice fed a regular diet (20). In contrast, previous reports showed that the H55N polymorphism in the mouse *Cs* gene was linked to low enzyme activity and the mice having this polymorphism exhibited decreased glucose tolerance during high-fat diet feeding (21, 22). Thus, we predicted that metabolic differences could be observed between WT and CS+/− mice under specific nutritional conditions.

Thus, we aimed to examine whether CS insufficiency affects the metabolic response of the heart and skeletal muscles in mice fed a special diet: a low-carbohydrate ketogenic diet (LCKD) that mimics a fasting state or a high-fat, high-carbohydrate (HFHCD) obesogenic diet that induces glucose intolerance. In this study, we investigated how chronic impairment of the mitochondrial CS activity affects the adaptive metabolic response to a ketogenic or obesogenic environment. Decrease in CS activity may cause energy deficiency, forcing the cell to rely on the most primitive route of energy production: cytosolic glycolysis. However, LCKD contains almost no glucose, which is the only source of the glycolytic pathway, and HFHCD induces insulin resistance (23), which disrupts glucose uptake. Therefore, it is interesting to assess how CS-insufficient organisms control their metabolism when consuming an LCKD or HFHCD.

MATERIALS AND METHODS

Animals and Rearing Conditions

Heterozygous CS+/− founder mice were created at Lexicon Genetics from their OmniBank library of knockout embryonic stem cell clones. We crossed this CS+/− founder mice more than ten times to transfer the null mutation onto the C57BL6/J genetic background. We used only male mice for the present studies. Male CS+/− mice and WT littermates at 8–11 weeks of age were used in this study. The mice were housed with 2–4 animals per cage in a temperature- and humidity-controlled facility with a 12-h light/dark cycle and free access to food and water.

Experimental Design and Diets

In the first experiment, the CS+/− ($n = 7$) and WT ($n = 7$) mice were fed a low-carbohydrate diet (88% fat, 11% protein, and 1% carbohydrate; 7.2 kcal/g), also called ketogenic diet because it induced ketosis in the subject consuming it, for 8 weeks. In the second experiment, the CS+/− ($n = 7$) and WT mice ($n = 10$) were fed a high-fat, high-carbohydrate (42% fat, 18% protein, and 40% carbohydrate; 4.7 kcal/g) for 8 weeks to induce obesity. The ingredients of each diet were purchased from Oriental Yeast Co. (Tokyo, Japan), and the composition of each diet is shown in **Supplementary Table 1**. Apart from these two main experiments, WT mice (10 weeks old, $n = 6$) were reared on a normal rodent diet (AIN-93G, Oriental Yeast Co.) for 8 weeks to confirm whether each experimental diet was effective at inducing metabolic changes.

The body weight of the mice was measured every 2 weeks throughout the experimental period. At the end of the experimental period, the mice were fasted for 4 h and anesthetized with isoflurane to collect blood samples into 1.5 mL microcentrifuge tubes. The mice were euthanized by cervical dislocation under anesthesia and immediately dissected to collect tissue samples from the heart and hindlimb muscles. All animal procedures were approved by the Animal Ethics Committee of Ochanomizu University (approval number: 19013 and 20018).

Biochemical Measurements of Blood Serum

The blood samples were centrifuged at 10,000 × *g* for 5 min in a microcentrifuge (Model 3500, Kubota, Tokyo, Japan), and the supernatant was transferred to a new tube and stored at –20°C until analysis. Blood glucose and non-esterified FA levels were measured using the biochemical colorimetric assay kits LabAssay™ Glucose (Cat. #298-65701, Wako, Osaka, Japan), LabAssay™ Triglyceride (Cat. #290-63701, Wako) and LabAssay™ NEFA (Cat. #294-63601, Wako) according to the manufacturer's protocols. The ketone body (3-hydroxybutyrate) concentration was measured using a Ketone Test Sanwa kit (Cat. #877434, Sanwa Kagaku, Tokyo, Japan), following the enzymatic protocol. Insulin concentration was evaluated using a Mouse Insulin ELISA kit (Morinaga, Yokohama, Japan), according to the manufacturer's instructions. All absorbance measurements were performed using an EnSpire® microplate reader (PerkinElmer, Waltham, MA, United States).

Gene Expression Profiling by Quantitative RT-PCR

The total mRNA was extracted from snap-frozen tissue using Sepasol RNA I reagent (Nacalai Tesque, Kyoto, Japan), according to the manufacturer's protocol. The RNA was treated with DNase and reverse-transcribed using ReverTra Ace™ qPCR RT Master Mix (Toyobo, Osaka, Japan). Diluted cDNA was used as a template to quantify the relative mRNA concentration. SYBR® Premix Ex Taq™ (Takara Bio, Shiga, Japan) was used to prepare the quantitative RT-PCR mixtures, which were evaluated on a Thermal Cycler Dice® Real Time System (Takara Bio), according to the manufacturer's instructions. The relative gene expression values were normalized with that of the β-actin gene. The sequences of the primers used to amplify each gene are shown in **Supplementary Table 2**.

Measurement of Adenosine Triphosphate Content

The ATP levels in each tissue were measured using a Tissue ATP assay kit (Toyo B-Net Co., Tokyo, Japan). Briefly, frozen tissues were weighed using a microanalytical balance, homogenized with 10 mL homogenate buffer (0.25 M sucrose, 10 mM HEPES-NaOH, pH 7.4) on ice and centrifuged at 1,000 × *g* for 10 min at 4°C. The supernatant was diluted 1:8 in ATP extraction reagent, left to stand for extraction (30 min), and then used for ATP measurement. ATP levels were quantified based on the luciferin and luciferase chemiluminescence measured in an EnSpire® microplate reader (PerkinElmer) according to the manufacturer's protocol and expressed per mg of tissue weight.

Quantification of Glycogen in the Muscle Tissue

Glycogen levels in the muscles collected from the mice were measured using a method based on that of Chan et al. (24). Briefly, the tissues were weighed using a microanalytical balance, dissolved in 30% KOH at 95°C, 2% Na_2SO_4, and 66% ethanol were then added, and the final solution was centrifuged (13,000 × *g*, 5 min at 4°C) to obtain a pellet. The pellet was washed twice with 66% ethanol, 0.2 M acetate buffer (pH 4.5) and amyloglucosidase (Sigma-Aldrich, Tokyo, Japan) were added, and the solution was incubated at 37°C for 30 min. The glucose content was measured using the LabAssay™ Glucose kit (Wako, Tokyo, Japan) and the amount of glycogen per gram of tissue (wet weight) was calculated.

Western Blotting Assays

Tissue samples of the gastrocnemius muscle were crushed in liquid nitrogen and homogenized in lysis buffer containing a protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, United States). After centrifugation at 14,000 × *g* for 20 min at 4°C, the supernatant was aliquoted and stored at –20°C. The samples containing 40 μg of total proteins were loaded onto a 10% SDS-PAGE gel, separated using Tris-glycine buffer containing 0.1% SDS, and transferred onto polyvinylidene fluoride membranes (GE Healthcare, Uppsala, Sweden).

The membranes were incubated overnight at 4°C in Blocking One-P solution (Nacalai Tesque, Kyoto Japan) with the following antibodies: AMPK (1:1,000, #2532), phospho-AMPK (1:1,000, #2535), Akt (1:1,000, #9272), phosphor-Akt (Ser473) (1:1,000, #9271), S6K (1:1,000, #9202), phosphor-S6K (1:250, #9205), and LC3B (1:500, #2775) (all from Cell Signaling Technology, Danvers, MA, United States). The membranes were then incubated with a secondary anti-rabbit IgG antibody (1:7,000, Cell Signaling Technology; #7074) in Tris-buffered saline containing 0.1% Tween 20 for 1 h at room temperature (20–25°C). The blots were developed using ECL™ prime western blotting detection reagent (GE Healthcare), and images were captured with an ImageQuant™ LAS 4000 (GE Healthcare) detection system. Normalization was performed using an anti-β-actin antibody (1:1,000, sc-47778; Santa Cruz Biotechnology, Dallas, TX, United States) and a corresponding secondary anti-mouse IgG antibody (1:7,000, #7076; Cell Signaling Technology).

Histological Analysis

Morphological changes in the muscles of LCKD-fed animals were examined. Transverse cryosections of the gastrocnemius muscles were used to assess the general tissue architecture and measure the cross-sectional area (CSA) of each muscle fiber.

Muscle sections (8 μm thick) were fixed with 10% formalin and then stained with hematoxylin and eosin. For immunofluorescence staining, the sections were air-dried for 10 min and then incubated with blocking solution [phosphate-buffered saline containing 0.1% Tween 20 (PBS-T), 5% bovine serum albumin (BSA), and 0.1% fish gelatin] for 1 h at room temperature. The sections were incubated overnight at 4°C with the mouse monoclonal antibodies directed to the anti-myosin heavy chain, BA-F8 (1:100) specific for myosin heavy chain I predominant in type I fiber or SC-71 (1:100) specific for myosin heavy chain II predominant in type II fiber (Development Studies Hybridoma Bank, Iowa City, IA, United States), diluted in PBS-T containing 0.8% BSA and 0.1% fish gelatin. After washing three times with PBS-T, the sections were incubated for 2 h at room temperature with the following secondary antibodies: FITC-conjugated anti-mouse IgG antibody (1:250, Thermo Fisher

Scientific, Waltham, MA, United States) for BA-F8 or rhodamine (TRITC)-conjugated anti-mouse IgG antibody (1:200, Jackson ImmunoResearch, West Grove, PA, United States) for SC-71. After washing three times with PBS-T, the sections were mounted with Fluoromount-G® (SouthernBiotech, Birmingham, AL, United States). For each staining technique, 12 representative fields per animal (four fields from three sections) were chosen, and the myofiber areas in the samples were analyzed using a digital microscope (BZ-X700; Keyence, Osaka, Japan) with its associated software.

Statistical Analyses

The data were expressed as the mean ± standard error of mean (SEM). All statistical analyses were performed using SPSS Statistics for Windows software (version 24, SPSS, Inc., Chicago, IL, United States). The normality of the variables was assessed using the Shapiro–Wilk test. Unpaired Student's *t*-test was used to identify significant differences between two groups. Two-way repeated analysis of variance was used to evaluate the differences in body weight between the groups over time. A non-parametric test (Mann–Whitney *U*-test) was used to compare the fiber areas in the muscle samples. Statistical significance was set at $p < 0.05$.

RESULTS

Body Weight and Blood Parameters of Citrate Synthase +/− and Wild-Type Mice

The experimental design and body weight of mice during the study are shown in **Figure 1**. There were no significant differences in body weight between WT and CS+/− mice on both LCKD and HCHFD feeding. The body weight of the LCKD-fed mice decreased after 2 weeks, after which the mice gained weight as they grew. In contrast, all mice fed a HCHFD gained weight throughout the 8-week experimental period (**Figure 1**). After 8 weeks, the average body weight of LCKD-fed mice was 28.9 ± 1.0 g in the WT group and 28.7 ± 0.8 g in the CS+/− group, and that of HFHCD-fed mice was 39.8 ± 1.1 g for WT mice and 40.5 ± 1.5 g for CS+/− mice (**Table 1**). Similarly, there were no significant differences in blood glucose, insulin, triglyceride, non-esterified FA, or 3-hydroxybutyrate levels between WT and CS+/− mice in both experiments (**Table 1**).

The results of comparison with the reference WT mice fed a regular diet are shown in **Supplementary Figure 1**. Compared with that of WT mice fed a regular diet, the body weight of HFHCD-fed mice significantly increased, whereas the body weight changes did not differ in LCKD-fed mice during the 8 weeks of experiment. In the blood parameters, the levels of serum 3-hydroxybutyrate and non-esterified FA in LCKD-fed mice and the levels of serum glucose and triglyceride in HCHFD-fed WT mice were significantly higher than those in the reference mice fed a regular diet. These data indicate that both the experimental diets were effective at inducing metabolic changes.

Expression Profile of Metabolic Genes

To assess the changes in metabolic fuel selection, the expression levels of genes related to substrate utilization in the heart and gastrocnemius muscles at the end of the 8-week experiment were compared between WT and CS+/− mice using quantitative RT-PCR. The tested genes and roles of their encoded proteins in substrate metabolism are summarized in **Figure 2**. In both the heart and gastrocnemius muscles, *Cs* mRNA expression was significantly lower in CS+/− mice than that in WT mice, as expected (**Figure 3**). In LCKD-fed mice, significant increases in the expression of genes related to utilization of various substrates, such as FAs, glucose, lactate, ketone bodies, and amino acids, were observed in the heart of CS+/− mice as compared to that in WT mice; however, there were no significant differences in the expression of these genes in the gastrocnemius muscles (**Figure 3A**). Among the genes related to mitochondrial electron transport chain, the gene expression of cytochrome c oxidase 1 (*Cox1*) encoded on mitochondrial DNA and ATP synthase F1 subunit beta (*Atp-5b*) encoded on genomic DNA were also upregulated in the heart but not in the muscles of LCKD-fed mice (**Figure 3C**). In contrast, in HFHCD-fed mice, the expression of only the *Cs* gene was significantly different between CS+/− and WT mice in both the heart and gastrocnemius muscles (**Figures 3B,D**).

Next, the expression of transcription factors that regulate the expression of substrate metabolism-related genes was measured in the hearts of CS+/− and WT mice. The mRNA expression levels of peroxisome proliferator-activated receptor γ coactivator 1α (*Pgc1α*) and estrogen-related receptor α (*Errα*), which mainly regulate genes involved in lipid metabolism and mitochondrial electron transport, and that of nuclear respiratory factor 1 (*Nrf1*), which is related to mitochondrial biogenesis, were significantly higher in CS+/− mice than those in WT mice fed an LCKD (**Figure 4A**). These differences were not observed in the hearts of HFHCD-fed mice (**Figure 4B**).

Adenosine Triphosphate Content in the Heart and Gastrocnemius Muscle

ATP levels were measured in the heart and gastrocnemius muscles of CS+/− and WT mice fed an LCKD or HFHCD. The ATP content in the heart was similar between WT and CS+/− mice in both experiments. However, in the gastrocnemius muscles of LCKD-fed mice, ATP levels were significantly lower in CS+/− mice than that in WT mice (**Figure 5**). In HFHCD-fed mice, the ATP levels of the gastrocnemius muscle were also lower in CS+/− mice than that in WT mice, but the difference was not significant (**Figure 5**).

Glycogen Metabolism in the Muscles of Low-Carbohydrate Ketogenic Diet-Fed Mice

Because the muscle ATP levels decreased in LCKD-fed CS+/− mice, we measured the glycogen content, an important energy source for the muscles, in the muscle tissues of LCKD-fed mice. At the end of the experimental period, muscle glycogen

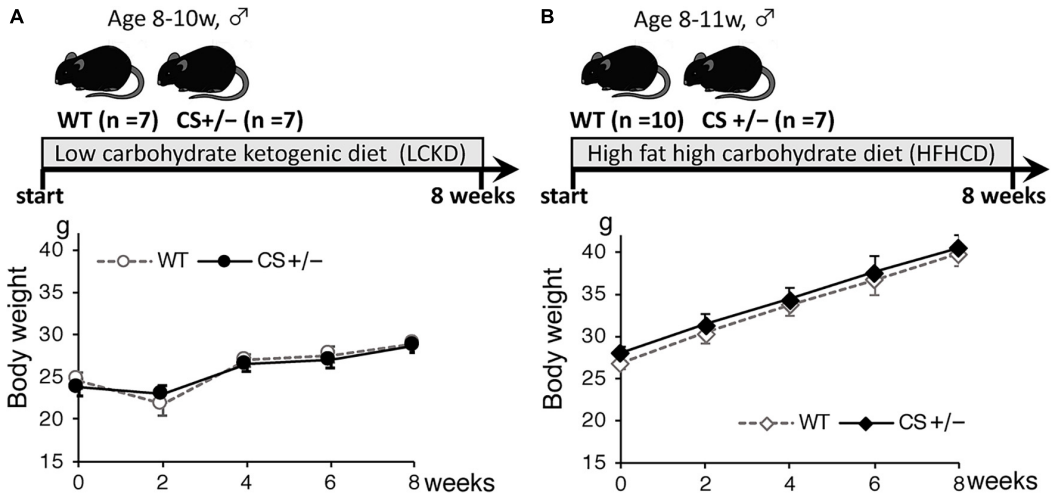


FIGURE 1 | Timeline of the experimental protocol (upper panel) and body weight of mice (lower panel) during the 8-week experimental period. Citrate synthase hetero knockout mice (CS+/−) and their wild-type siblings (WT) were fed **(A)** a low-carbohydrate ketogenic diet (LCKD), or **(B)** a high-fat, high-carbohydrate diet (HFHCD). Data are shown as the mean ± SEM (n = 7–10 per group).

TABLE 1 | Body weight and blood parameters.

	LCKD		HFHCD	
	WT	**CS+/−**	**WT**	**CS+/−**
Initial body weight [g]	24.6 ± 0.9	23.8 ± 1.0	26.9 ± 0.4	28.1 ± 0.7
Final body weight [g]	28.9 ± 1.0	28.7 ± 0.8	39.8 ± 1.1	40.5 ± 1.5
Glucose [mg/dL]	234.3 ± 13.4	204.9 ± 14.4	299.0 ± 14.9	268.9 ± 11.2
Insulin [ng/ml]	0.86 ± 0.16	1.38 ± 0.37	3.86 ± 1.34	3.69 ± 0.81
Triglyceride [mg/ml]	43.6 ± 2.5	45.0 ± 6.6	51.2 ± 3.2	56.9 ± 4.9
NEFA [mEq/L]	1.05 ± 0.08	1.12 ± 0.11	0.63 ± 0.08	0.80 ± 0.08
3-HB [μmol/L]	1387.4 ± 292.5	1320.5 ± 265.6	235.9 ± 16.3	211.2 ± 24.9

LCKD, low-carbohydrate ketogenic diet; HFHCD, high-fat high-carbohydrate diet; WT, wild type mice; CS+/−, citrate synthase-knockout mice; NEFA, non-esterified fatty acid; 3-HB, 3-hydroxybutyrate; n.d., not determined. The data are shown as mean ± SEM.

storage was significantly decreased in CS+/− mice compared to that in WT mice (**Figure 6A**). The mRNA levels of glycogen synthase-1 (*Gys-1*), a key enzyme in glycogen synthesis, tended to be lower (p = 0.069) in the muscles of CS+/− mice than that of WT mice.

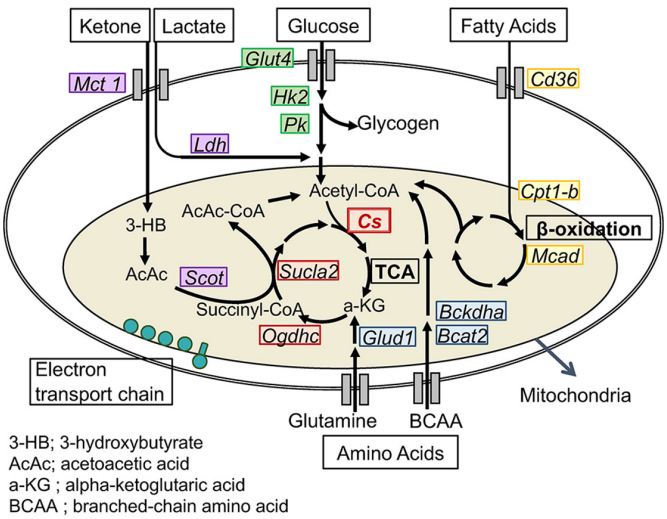
Anabolic and Catabolic Signaling in the Muscles of Low-Carbohydrate Ketogenic Diet-Fed Mice

We also evaluated the responses of anabolic and catabolic signaling in the muscles of LCKD-fed mice. The phosphorylation of AMP-activated protein kinase (AMPK), a critical cellular ATP sensor, as well as that of protein kinase B (Akt) and p70 S6 kinase (S6K), which are anabolic signaling molecules, was assessed. AMPK phosphorylation was significantly increased in the muscles of CS+/− mice, which is consistent with the decrease in the tissue ATP content (**Figure 6B**). Phosphorylation of p70 S6K was significantly suppressed in the muscles of CS+/− mice compared with that in WT mice, whereas the levels of phosphorylated Akt did not significantly differ between the two mouse groups (**Figure 6B**).

To evaluate the catabolic signaling, mRNA levels of the muscle-specific ubiquitin ligases atrogin-1 (*Atg1*), and muscle RING finger-1 (MuRF-1) as well as the levels of light chain 3 proteins LC3-I and LC3-II were determined. CS+/− and WT mice showed no significant differences in the protein ratio of LC3-II to LC3-I (a marker of autophagy) (**Figure 6C**) or in the mRNA levels of Atg1 and MuRF-1 (**Figure 6D**), which mediate protein degradation through autophagy-lysosomal and the ubiquitin-proteasome systems, respectively, under conditions of ATP shortage.

Muscle Morphology of Low-Carbohydrate Ketogenic Diet-Fed Mice

In the morphological analysis, CSA of the total myofibers did not differ between WT and CS+/− mice (**Figure 7A**). However, in LCKD-fed mice, further analysis of different muscle fiber types indicated that the CSA of type I



TCA cycle	
Cs	: citrate synthase
Ogdh	: oxoglutarate dehydrogenase
Sucla2	: succinyl-CoA ligase, subunit β
Glucose	
Glut4	: glucose transporter 4
Hk2	: hexokinase 2
Pk	: pyruvate kinase
Free fatty acids	
Cd36	: cluster of differentiation 36
Cpt1-b	: carnitine palmitoyltransferase1-b
Mcad	: medium-chain acyl-CoA dehydrogenase
Lactate and ketone body	
Ldh	: lactate dehydrogenase
Mct1	: monocarboxylic acid transporter 1
Scot	: Succinyl CoA 3-oxoacid CoA transferase
Amino acids	
Glud1	: glutamate dehydrogenase 1
Bcat2	: branched-chain amino acid transaminase 2
Bckdha	: branched-chain keto acid dehydrogenase e1, α
Electron transport chain	
Cox 1	: cytochrome c oxidase subunit 1
Cyt b	: cytochrome b
Cyt c;	: cytochrome c
Atp5b	: ATP synthase F1 subunit beta

FIGURE 2 | Metabolic genes analyzed in this study. The names and abbreviations of the genes and roles of their encoding proteins in fuel metabolism are summarized.

oxidative fibers was significantly lower in CS+/− mice than in WT mice, whereas the CSA of type II glycolytic fibers did not differ between WT and CS+/− mice (**Figure 7B**).

DISCUSSION

In this study, we compared the metabolic and physiological responses between WT and CS+/− mice fed with two types of diets: a low-carbohydrate ketogenic diet (LCKD) and a high-fat, high-carbohydrate (HFHCD) diet.

The body weight of mice decreased after starting the LCKD feeding but increased steadily during HFHCD feeding. LCKD consumption mimics the metabolic conditions of long-term starvation, and leads to body weight reduction in both humans and animals (25, 26). The possible causes of this weight loss include appetite loss due to the appetite-suppressant actions of ketosis or changes in appetite control hormones (27, 28), reduced lipogenesis, and increased lipolysis (29, 30). In contrast, HFHCD consumption induces obesity, leading to metabolic conditions such as insulin resistance in rodents (23). However, neither of these diets led to significant differences in body weight changes between CS+/− and WT mice in this study, suggesting that CS insufficiency did not affect hormonal or metabolic factors involved in weight control.

Our results revealed an increase in the cardiac expression levels of genes related to the utilization of energy substrates, including FAs, glucose, lactate, ketone bodies, and amino acids in LCKD-fed CS+/− mice. FAs are the main fuel source for ATP in the heart; the adult myocardium utilizes FAs to supply approximately 60–70% of the total ATP (5). The heart increasingly relies on FA oxidation during intracellular glucose shortages, which occur under certain conditions such as diabetes (31). Thus, in the fasting-mimicking LCKD-fed mice, the increased expression of genes related to FA oxidation in CS+/− mouse hearts may be an adaptive response to energy shortages caused by CS insufficiency.

We also observed increased gene expression of the PGC1α coactivator and its transcription factor binding partners, ERRα and NRF1, in the hearts of CS+/− mice. These molecules amplify mitochondrial FA oxidation and respiration by regulating the expression of genes related to lipid metabolism and oxidative phosphorylation (32, 33). In fact, the cardiac expressions of genes involved in not only FA oxidation but also oxidative phosphorylation were increased in LCKD-fed mice. Therefore, the increase in their expression levels may be part of a compensatory response to CS insufficiency.

It is well known that the shift of substrate utilization from FAs to glucose in the myocardium has been observed in cardiac disorders (34, 35). Nevertheless, unexpectedly, the expression levels of the genes related to cytosolic glycolysis were not altered in the hearts of animals with CS insufficiency. Similarly, recent studies showed that glycolysis and glucose oxidation did not increase in failing hearts (36, 37) and that the heart consumes small amounts of glucose as a fuel source, whether healthy or failing (38). In agreement with these findings, our results suggest that cardiac tissues do not enhance glycolysis as an adaptive strategy to compensate for energy shortages.

Lactate and ketone bodies have not received much attention as oxidative fuel for the heart. However, in humans and rodents, both fuels enter the cardiomyocytes mainly through monocarboxylate transporter-1 (MCT-1) (39) and contribute to energy production as an efficient energy substrate in the heart (40). Our results showed that the expression levels of genes related to lactate and ketone oxidation were significantly increased in the heart of LCKD-fed CS+/− mice. As important

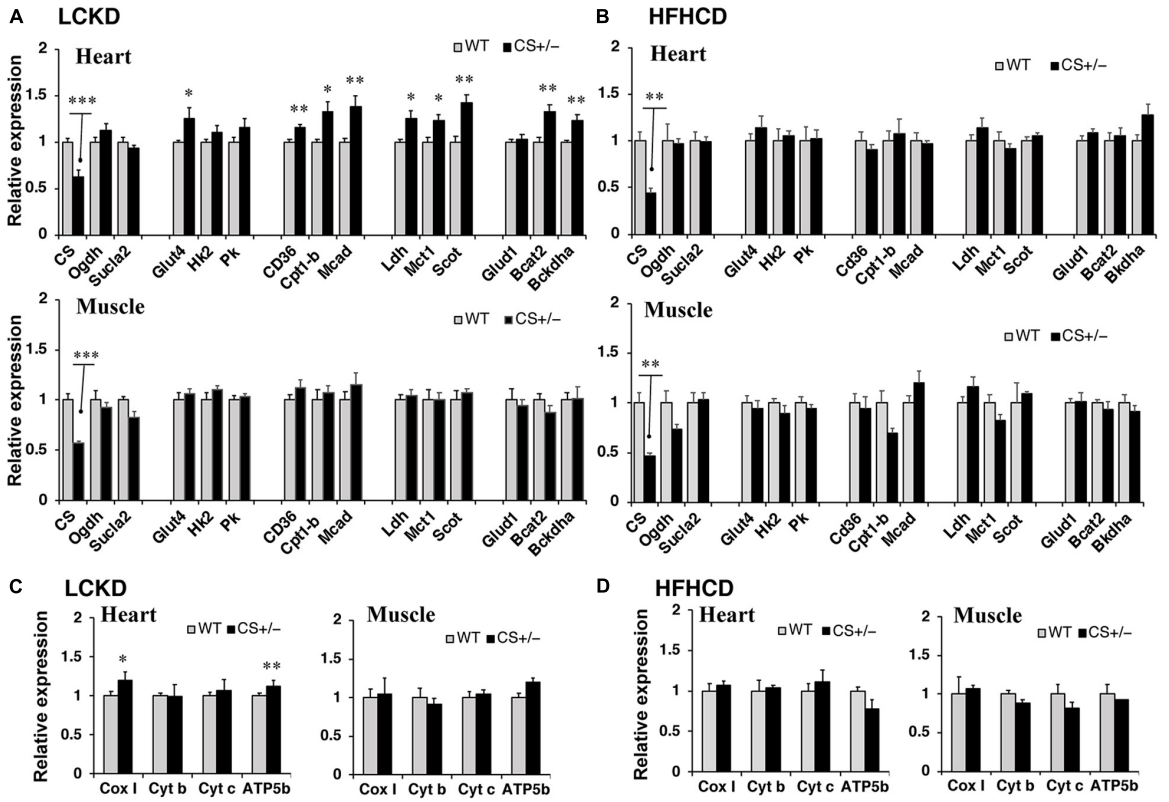


FIGURE 3 | Effects of each diet exposure on metabolic gene expression in the heart and gastrocnemius muscles of citrate synthase-knockout mice (CS+/−, black bars) and their wild-type siblings (WT, gray bars). The mRNA abundance was quantified and normalized to that of β-actin in **(A,C)** mice fed a LCKD and **(B,D)** mice fed a HFHCKD. Values are expressed as the fold-change compared with expression levels in WT mice, which were arbitrarily set to 1 (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ vs. WT).

energy substrates, the consumption of lactate and ketone bodies increases by nearly two and threefold in failing hearts, respectively (38), and the cardiac expression levels of *Mct1* and *Scot* are upregulated under conditions of energy deficiency such as during exercise training (41) and in the failing heart (42). Therefore, the oxidation of lactate and ketone bodies may be an alternative pathway of energy production in the CS-insufficient heart.

The major amino acids consumed by the heart are glutamate and branched amino acids (BCAAs), which contribute to approximately 5% of total ATP generation (38, 43). Our results revealed increased expression levels of genes related to amino acid catabolism, particularly BCAAs, in the hearts of LCKD-fed CS+/− mice. The contribution of amino acids to energy production in the heart has not been widely examined; however, a recent study revealed that the levels of BCAAs were increased significantly in the heart a few days after pressure overload or surgical infarction (44). Hence, BCAAs may be alternative fuels in energy-deficient hearts. Increased expression levels of genes encoding BCAA catabolic enzymes may be an adaptive compensation in CS-deprived hearts under conditions of a limited glucose supply.

In contrast to the results observed in the heart, the metabolic gene expression levels in the muscles showed no significant differences between CS+/− and WT mice, even in those consuming a LCKD. Thus, the ATP content decreased, and AMPK was activated in the muscles of LCKD-fed CS+/− mice.

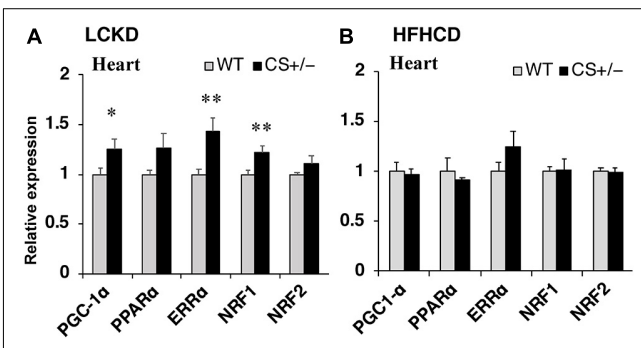


FIGURE 4 | Effects of each diet exposure on the gene expression of transcription factors and transcriptional co-activators related to fuel metabolism in the hearts of citrate synthase-knockout mice (CS+/−, black bars) and their wild-type siblings (WT, gray bars). The mRNA abundance was quantified and normalized to that of β-actin in **(A)** mice fed a LCKD and **(B)** mice fed a HFHCKD. Values are expressed as the fold-change compared with the expression levels in WT mice, arbitrarily set to 1 (*$p < 0.05$; **$p < 0.01$ vs. WT).

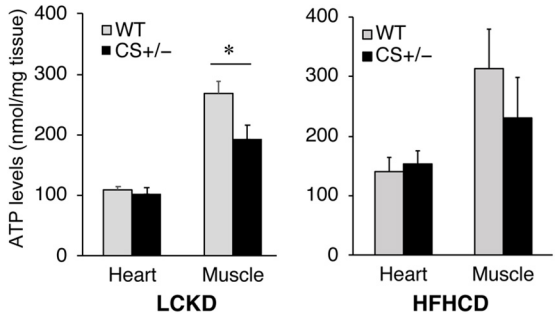


FIGURE 5 | Effects of each diet exposure on the ATP content of the hearts and gastrocnemius muscles of citrate synthase-knockout mice (CS+/−, black bars) and their wild-type siblings (WT, gray bars) fed a LCKD (left panel) or a HFHCKD (right panel). Values are expressed on a wet weight basis (*$p < 0.05$).

These findings suggest that the metabolic gene expression in the muscle tissues is not increased as a compensatory response to decreased energy production. The mechanism by which the different adaptive responses to insufficient CS are induced in the heart and the muscles remains unclear. Nonetheless, our findings are consistent with those of a previous study revealing that the ATP content and production were decreased by approximately 50% during aging in the muscle but not in the heart mitochondria (45).

Under restricted carbohydrate intake, we observed that the storage of glycogen, an important fuel for muscle contraction, and the activation of S6K, a key anabolic signaling molecule, were significantly suppressed in the muscles of CS+/− mice. Further, the muscles presented fiber atrophy predominantly in mitochondria-rich type I fiber. Under ATP deprivation, the cells shift from a state of growth to that of survival mode. A previous study reported that increased AMPK activity inhibited mTOR and its downstream targets S6K, resulting in reduced cell sizes and growth rates to protect cells from energy deprivation-induced apoptosis (46). Furthermore, leg muscles release large amounts of amino acids, which act as alternative energy sources for the heart (38). Therefore, the inhibition of glycogen storage and signaling suppression of protein synthesis in the muscles of CS+/− mice may help ensure an adequate fuel supply to the heart when dietary carbohydrates are restricted.

In previous studies, mice fed an HFHCD for 4 months developed heart failure as well as cardiometabolic alterations associated with mitochondrial protein modification (47,

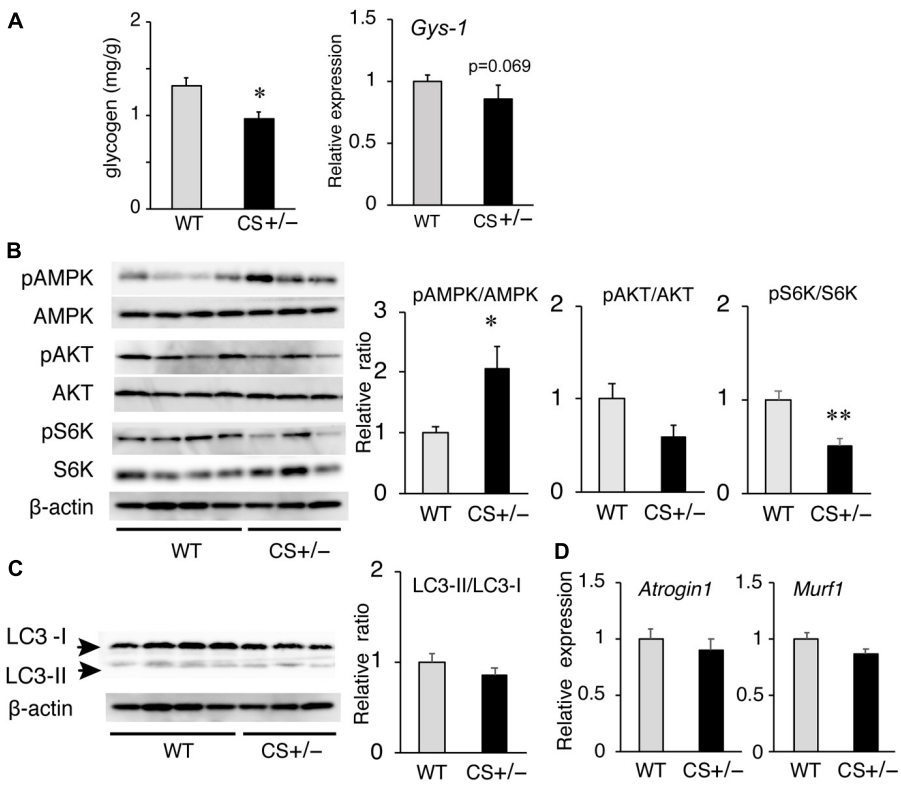


FIGURE 6 | Effects of a ketogenic diet exposure on muscle glycogen, and anabolic and catabolic signaling in the gastrocnemius muscles of citrate synthase-knockout mice (CS+/−) and their wild-type siblings (WT). **(A)** Muscle glycogen content (left panel) and mRNA levels of glycogen synthase 1 (*Gys1*) (right panel). **(B)** Activation of AMP-activated kinase (AMPK) and anabolic signaling molecules Akt and p70 S6 kinase (S6K). β-Actin was used as a loading control. **(C)** Protein levels of autophagy-related proteins LC3-I and LC3-II and the LC3-II/LC3-I ratio. **(D)** mRNA levels of atrogin-1 (*Atg1*) and muscle RING finger-1 (*MuRF-1*). In mRNA and protein measurements, values are expressed as the fold-change compared with the values in WT mice, arbitrarily set to 1 (*$p < 0.05$; **$p < 0.01$ vs. WT). The images show representative results of two independent experiments.

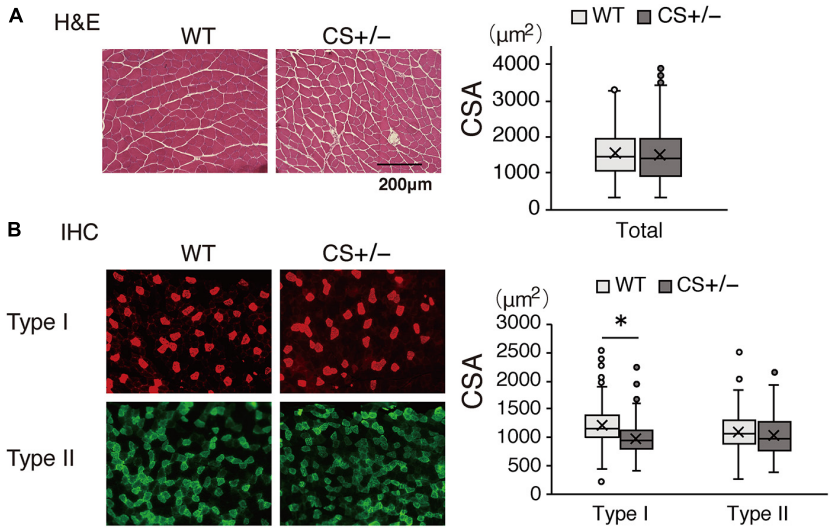


FIGURE 7 | Effects of a ketogenic diet exposure on muscle fiber size. The individual cross-sectional area of **(A)** total fibers stained with hematoxylin and eosin and **(B)** type I and type II fibers stained with immunohistochemistry. Left panel, representative images of the stained sections are shown; right panel, values of the cross-sectional area (CSA) are presented in a box-and-whisker plot. Boxes are constructed with the intervals between the first and third quartiles of the data distribution; lines in the boxes are the median values; positive and negative bars are the 5th and 95th percentile individual values, respectively (*$p < 0.05$).

48). Another study showed that HFHCD-feeding led to decreased glucose tolerance in female mice with genetically lower CS activity compared to that in wild-type mice (22). Therefore, we expected that HFHCD-fed CS+/− mice would develop metabolic disarrangement, at least in the heart. However, no significant differences were observed in metabolic parameters in both the heart and gastrocnemius muscles between CS+/− and WT mice. Thus, further studies with longer exposure to HFHCD or using female animals are needed to clarify whether HFHCD consumption affects energy metabolism in the heart and skeletal muscles of CS+/− mice.

This study has some limitations. First, we assessed mRNA expression related to mitochondrial capacity but did not measure mitochondrial content. As the expression of the mitochondrial DNA transcript *Cox-1* was increased in the heart of LCKD-fed mice, CS insufficiency might lead to an increase in not only gene expression but also mitochondrial copy number. This requires further investigation. Second, we did not evaluate the functional changes in the heart of LCKD-fed mice. Considering the increase in the expression of genes related to fuel utilization in these mice, it is necessary to investigate whether the cardiac functions are preserved.

In conclusion, in mice under ketogenic conditions and with low CS activity, the heart showed increased expression of genes related to fuel utilization. In contrast, suppression of anabolic signaling leading to oxidative fiber atrophy was observed in the muscles. These findings suggest that the heart switches between different fuel sources by regulating gene expression to adapt to chronic energy shortages. In contrast, the skeletal muscles may sacrifice their growth to secure fuel sources for the heart. Our data provide an overview of fuel use in organs under conditions of energy shortage and a basis for understanding the etiology of metabolic disorders related to impaired TCA cycle metabolism.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by the Animal Ethics Committee of Ochanomizu University.

AUTHOR CONTRIBUTIONS

KS, YH, RT, NW, CO, and YS: investigation and data curation. KS, RT, and KI: formal analysis. KS and KI: writing the manuscript. HS: review and editing the manuscript. KI: conceptualization and project administration. YS and KI: funding acquisition. All authors contributed to the article and approved the submitted version.

FUNDING

This study was supported by a Grant-in-Aid for Scientific Research (grant no. JP21H00805) from the Ministry of

Education, Culture, Sports, Science, and Technology of Japan (MEXT).

ACKNOWLEDGMENTS

We thank Prof. Hitoshi Shimano and the late Prof. Nobuhiro Yamada at the University of Tsukuba for their contribution to the conceptualization of the entire project, creating CS knockout mice, continuous technical assistance, numerous valuable advices, and generous financial support. We also thank our colleagues at the University of Tsukuba for a lot of helpful comments in conducting this research.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.925908/full#supplementary-material

REFERENCES

1. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. *Cell.* (2012) 148:1145–59. doi: 10.1016/j.cell.2012.02.035
2. Akram M. Citric acid cycle and role of its intermediates in metabolism. *Cell Biochem Biophys.* (2014) 68:475–8. doi: 10.1007/s12013-013-9750-1
3. Judge A, Dodd MS. Metabolism. *Essays Biochem.* (2020) 64:607–47. doi: 10.1042/EBC20190041
4. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev.* (2005) 85:1093–129. doi: 10.1152/physrev.00006.2004
5. Shi X, Qiu H. New insights into energy substrate utilization and metabolic remodeling in cardiac physiological adaption. *Front Physiol.* (2022) 13:831829. doi: 10.3389/fphys.2022.831829
6. Goodpaster BH, Sparks LM. Metabolic flexibility in health and disease. *Cell Metab.* (2017) 25:1027–36. doi: 10.1016/j.cmet.2017.04.015
7. Hepple RT. Impact of aging on mitochondrial function in cardiac and skeletal muscle. *Free Radic Biol Med.* (2016) 98:177–86. doi: 10.1016/j.freeradbiomed.2016.03.017
8. Boengler K, Kosiol M, Mayr M, Schulz R, Rohrbach S. Mitochondria and ageing: role in heart, skeletal muscle and adipose tissue. *J Cachexia Sarcopenia Muscle.* (2017) 8:349–69. doi: 10.1002/jcsm.12178
9. Schrauwen-Hinderling VB, Kooi ME, Schrauwen P. Mitochondrial function and diabetes: consequences for skeletal and cardiac muscle metabolism. *Antioxid Redox Signal.* (2016) 24:39–51. doi: 10.1089/ars.2015.6291
10. Pinti MV, Fink GK, Hathaway QA, Durr AJ, Kunovac A, Hollander JM. Mitochondrial dysfunction in type 2 diabetes mellitus: an organ-based analysis. *Am J Physiol Endocrinol Metab.* (2019) 316:E268–85. doi: 10.1152/ajpendo.00314.2018
11. Kang W, Suzuki M, Saito T, Miyado K. Emerging role of TCA cycle-related enzymes in human diseases. *Int J Mol Sci.* (2021) 22:13057. doi: 10.3390/ijms222313057
12. Larsen S, Nielsen J, Hansen CN, Nielsen LB, Wibrand F, Stride N, et al. Biomarkers of mitochondrial content in skeletal muscle of healthy young human subjects. *J Physiol.* (2012) 590:3349–60. doi: 10.1113/jphysiol.2012.230185
13. Jacobs RA, Diaz V, Meinild AK, Gassmann M, Lundby C. The C57BL/6 mouse serves as a suitable model of human skeletal muscle mitochondrial function. *Exp Physiol.* (2013) 98:908–21. doi: 10.1113/expphysiol.2012.070037
14. Carter SL, Rennie CD, Hamilton SJ. Tarnopolsky. Changes in skeletal muscle in males and females following endurance training. *Can J Physiol Pharmacol.* (2001) 79:386–92. doi: 10.1139/y01-008
15. Houmard JA, Weidner ML, Gavigan KE, Tyndall GL, Hickey MS, Alshami A. Fiber type and citrate synthase activity in the human gastrocnemius and vastus lateralis with aging. *J Appl Physiol.* (1998) 85:1337–41. doi: 10.1152/jappl.1998.85.4.1337
16. Crane JD, Devries MC, Safdar A, Hamadeh MJ, Tarnopolsky MA. The effect of aging on human skeletal muscle mitochondrial and intramyocellular lipid ultrastructure. *J Gerontol A Biol Sci Med Sci.* (2010) 65:119–28. doi: 10.1093/gerona/glp179
17. Ortenblad N, Mogensen M, Petersen I, Hojlund K, Levin K, Sahlin K, et al. Reduced insulin-mediated citrate synthase activity in cultured skeletal muscle cells from patients with type 2 diabetes: evidence for an intrinsic oxidative enzyme defect. *Biochim Biophys Acta.* (2005) 1741:206–14. doi: 10.1016/j.bbadis.2005.04.001
18. LeMoine CM, McClelland GB, Lyons CN, Mathieu-Costello O, Moyes CD. Control of mitochondrial gene expression in the aging rat myocardium. *Biochem Cell Biol.* (2006) 84:191–8. doi: 10.1139/o05-169
19. Preston CC, Oberlin AS, Holmuhamedov EL, Gupta A, Sagar S, Syed RH, et al. Aging-induced alterations in gene transcripts and functional activity of mitochondrial oxidative phosphorylation complexes in the heart. *Mech Ageing Dev.* (2008) 129:304–12. doi: 10.1016/j.mad.2008.02.010
20. Sone H, Shimano H, Jo S, Kodama H, Taguchi M, Takahashi A, et al. Metabolic analysis of citrate synthase knock-out mice. *J Jpn Diabetic Soc.* (2004) 47:S165.
21. Gabriel BM, Al-Tarrah M, Alhindi Y, Kilikevicius A, Venckunas T, Gray SR, et al. H55n polymorphism is associated with low citrate synthase activity which regulates lipid metabolism in mouse muscle cells. *PLoS One.* (2017) 12:e0185789. doi: 10.1371/journal.pone.0185789
22. Alhindi Y, Vaanholt LM, Al-Tarrah M, Gray SR, Speakman JR, Hambly C, et al. Low citrate synthase activity is associated with glucose intolerance and lipotoxicity. *J Nutr Metab.* (2019) 2019:8594825. doi: 10.1155/2019/8594825
23. Panchal SK, Brown L. Rodent models for metabolic syndrome research. *J Biomed Biotechnol.* (2011) 2011:351982. doi: 10.1155/2011/351982
24. Chan TM, Exton JH. A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes. *Anal Biochem.* (1976) 71:96–105.
25. Kosinski C, Jornayvaz FR. Effects of ketogenic diets on cardiovascular risk factors: evidence from animal and human studies. *Nutrients.* (2017) 9:517. doi: 10.3390/nu9050517
26. Kumar S, Behl T, Sachdeva M, Sehgal A, Kumari S, Kumar A, et al. Implicating the effect of ketogenic diet as a preventive measure to obesity and diabetes mellitus. *Life Sci.* (2021) 264:118661. doi: 10.1016/j.lfs.2020.118661
27. Sumithran P, Prendergast LA, Delbridge E, Purcell K, Shulkes A, Kriketos A, et al. Ketosis and appetite-mediating nutrients and hormones after weight loss. *Eur J Clin Nutr.* (2013) 67:759–64. doi: 10.1038/ejcn.2013.90
28. Gibson AA, Seimon RV, Lee CM, Ayre J, Franklin J, Markovic TP, et al. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obes Rev.* (2015) 16:64–76. doi: 10.1111/obr.12230
29. Cahill GF Jr. Fuel metabolism in starvation. *Annu Rev Nutr.* (2006) 26:1–22. doi: 10.1146/annurev.nutr.26.061505.111258
30. Veldhorst MA, Westerterp-Plantenga MS, Westerterp KR. Gluconeogenesis and energy expenditure after a high-protein, carbohydrate-free diet. *Am J Clin Nutr.* (2009) 90:519–26. doi: 10.3945/ajcn.2009.27834
31. Chong CR, Clarke K, Levelt E. Metabolic remodeling in diabetic cardiomyopathy. *Cardiovasc Res.* (2017) 113:422–30. doi: 10.1093/cvr/cvx018
32. Scarpulla RC. Nuclear control of respiratory gene expression in mammalian cells. *J Cell Biochem.* (2006) 97:673–83. doi: 10.1002/jcb.20743
33. Villena JA, Kralli A. Erralpha: a metabolic function for the oldest orphan. *Trends Endocrinol Metab.* (2008) 19:269–76. doi: 10.1016/j.tem.2008.07.005
34. van Bilsen M, van Nieuwenhoven FA, van der Vusse GJ. Metabolic remodelling of the failing heart: beneficial or detrimental?. *Cardiovasc Res.* (2009) 81:420–8. doi: 10.1093/cvr/cvn282
35. Bertero E, Maack C. Metabolic remodelling in heart failure. *Nat Rev Cardiol.* (2018) 15:457–70. doi: 10.1038/s41569-018-0044-6
36. Zhabyeyev P, Gandhi M, Mori J, Basu R, Kassiri Z, Clanachan A, et al. Pressure-overload-induced heart failure induces a selective reduction in

glucose oxidation at physiological afterload. *Cardiovasc Res.* (2013) 97:676–85. doi: 10.1093/cvr/cvs424
37. Zhang L, Jaswal JS, Ussher JR, Sankaralingam S, Wagg C, Zaugg M, et al. Cardiac insulin-resistance and decreased mitochondrial energy production precede the development of systolic heart failure after pressure-overload hypertrophy. *Circ Heart Fail.* (2013) 6:1039–48. doi: 10.1161/CIRCHEARTFAILURE.112.000228
38. Murashige D, Jang C, Neinast M, Edwards JJ, Cowan A, Hyman MC, et al. Comprehensive quantification of fuel use by the failing and nonfailing human heart. *Science.* (2020) 370:364–8. doi: 10.1126/science.abc8861
39. Halestrap AP. The monocarboxylate transporter family–structure and functional characterization. *IUBMB Life.* (2012) 64:1–9. doi: 10.1002/iub.573
40. Lopaschuk GD, Karwi QG, Ho KL, Pherwani S, Ketema EB. Ketone metabolism in the failing heart. *Biochim Biophys Acta Mol Cell Biol Lipids.* (2020) 1865:158813. doi: 10.1016/j.bbalip.2020.158813
41. De Araujo GG, Gobatto CA, de Barros Manchado-Gobatto F, Teixeira LF, Dos Reis IG, Caperuto LC. Mct1 and Mct4 kinetic of mRNA expression in different tissues after aerobic exercise at maximal lactate steady state workload. *Physiol Res.* (2015) 64:513–22. doi: 10.33549/physiolres.932695
42. Bedi KC Jr., Snyder NW, Brandimarto J, Aziz M, Mesaros C, Worth AJ, et al. Evidence for intramyocardial disruption of lipid metabolism and increased myocardial ketone utilization in advanced human heart failure. *Circulation.* (2016) 133:706–16. doi: 10.1161/CIRCULATIONAHA.115.017545
43. Neinast MD, Jang C, Hui S, Murashige DS, Chu Q, Morscher RJ, et al. Quantitative analysis of the whole-body metabolic fate of branched-chain amino acids. *Cell Metab.* (2019) 29:417–29.e4. doi: 10.1016/j.cmet.2018.10.013
44. Sansbury BE, DeMartino AM, Xie Z, Brooks AC, Brainard RE, Watson LJ, et al. Metabolomic analysis of pressure-overloaded and infarcted mouse hearts. *Circ Heart Fail.* (2014) 7:634–42. doi: 10.1161/CIRCHEARTFAILURE.114.001151
45. Drew B, Phaneuf S, Dirks A, Selman C, Gredilla R, Lezza A, et al. Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. *Am J Physiol Regul Integr Comp Physiol.* (2003) 284:R474–80. doi: 10.1152/ajpregu.00455.2002
46. Inoki K, Zhu T, Guan KL. Tsc2 mediates cellular energy response to control cell growth and survival. *Cell.* (2003) 115:577–90. doi: 10.1016/s0092-8674(03)00929-2
47. Abdesselam I, Pepino P, Troalen T, Macia M, Ancel P, Masi B, et al. Time course of cardiometabolic alterations in a high fat high sucrose diet mice model and improvement after glp-1 analog treatment using multimodal cardiovascular magnetic resonance. *J Cardiovasc Magn Reson.* (2015) 17:95. doi: 10.1186/s12968-015-0198-x
48. Sverdlov AL, Elezaby A, Qin F, Behring JB, Luptak I, Calamaras TD, et al. Mitochondrial reactive oxygen species mediate cardiac structural, functional, and mitochondrial consequences of diet-induced metabolic heart disease. *J Am Heart Assoc.* (2016) 5:e002555. doi: 10.1161/JAHA.115.002555

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 08 July 2022
doi: 10.3389/fnut.2022.955101

Effects of Variability in Glycemic Indices on Longevity in Chinese Centenarians

Sheng-han Ji[1,2†], Chen Dong[3†], Rou Chen[1,2], Chen-chen Shen[4], Jing Xiao[5], Yun-juan Gu[1,6] and Jian-lin Gao[3*]*

[1] Department of Endocrinology and Metabolism, Affiliated Hospital of Nantong University, Nantong, China, [2] Medical School of Nantong University, Nantong University, Nantong, China, [3] Research Center of Gerontology and Longevity, Affiliated Hospital of Nantong University, Nantong, China, [4] Department of Cardiology, Rugao Bo'ai Branch of Nantong University Affiliated Hospital, Nantong, China, [5] Department of Epidemiology and Medical Statistics, School of Public Health, Nantong University, Nantong, China, [6] Department of Health Medicine, Affiliated Hospital of Nantong University, Nantong, China

OPEN ACCESS

Edited by:
Devin Wahl,
Colorado State University,
United States

Reviewed by:
Jian-bin Su,
Nantong First People's Hospital, China
Giovanni Vitale,
Italian Auxological Institute (IRCCS), Italy

***Correspondence:**
Yun-juan Gu
desette@ntu.edu.cn
Jian-lin Gao
gjl4486@163.com

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

Received: 28 May 2022
Accepted: 20 June 2022
Published: 08 July 2022

Citation:
Ji S-h, Dong C, Chen R, Shen C-c, Xiao J, Gu Y-j and Gao J-l (2022) Effects of Variability in Glycemic Indices on Longevity in Chinese Centenarians. Front. Nutr. 9:955101. doi: 10.3389/fnut.2022.955101

Background: Large fluctuations in blood glucose levels greatly impact the health and life span of elderly individuals. This study describes the characteristics of variability in glycemic indices in centenarians with the aim of emphasizing the importance of glycemic variability in elderly people.

Methods: We recruited individuals from Rugao City, Jiangsu Province, China from April 2020 to May 2021. The study cohort included 60 centenarians and 60 first-generation offspring, as well as 20 randomly selected non-cohabitant control individuals aged 60–80 years. A FreeStyle Libre H (hospital version) continuous glucose monitoring (CGM) device (Abbott Ireland UK) was used to measure glycemic variability. The indices measured included the time in target glucose range (TIR), time below target glucose range (TBR), time above target glucose range (TAR), mean amplitude of glycemic excursions (MAGE), mean of daily differences (MODD), coefficient of variation (CV), standard deviation of blood glucose (SDBG), continuous overlapping net glycemic action (CONGA), glucose management indicator (GMI) and estimated glycated hemoglobin (eHbA1c). Logistic regression was used to analyze the association between glycemic variability and longevity.

Results: Mean blood glucose (MBG), eHbA1c, GMI, mean fasting plasma glucose (M-FPG) and CONGA were lower in the centenarian group (p all < 0.05). PPGE-2 was higher in the control group than that measured in the centenarian and first-generation offspring groups ($p < 0.05$). There were no differences between the groups in MAGE, MODD, MAG, or TIR ($p > 0.05$). The risk of not achieving longevity increased with each one unit increase in MBG by 126% [2.26 (1.05–4.91)], eHbA1c by 67% [1.67 (1.03–2.72)], GMI by 568% [6.68 (1.11–40.30)], M-FPG by 365% [4.65 (1.57–13.75)], M-PPG1h by 98% [1.98 (1.18–3.31)], CONGA1 by 102% [2.02 (1.01–4.06)], Li by 200% [3.00 (1.04–8.61)], and PPGE-2 by 150% [2.50 (1.39–4.50)]. However, the risk of achieving longevity decreased with each unit increase of LBGI by 53% [0.47 (0.28–0.80)], ADRR by 60% [0.40 (0.18–0.86)], and TBR by 11% [0.89 (0.80–0.98)].

Conclusion: Fluctuation in blood glucose levels in centenarians is relatively small. Maintaining an average blood glucose level and keeping blood glucose fluctuations in the normal range is conducive to longevity.

Keywords: centenarians, continuous glucose monitoring, glucose variability, hypoglycemia, longevity

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disease characterized by a long course of disease and health complications, which can shorten the lifespan of middle-aged and elderly individuals (1). The World Health Organization (WHO) has reported that DM is one of the first ten major causes of death and disability globally (2). In China, ∼1.4 million people die of DM every year, representing one death every 22 seconds (2). DM is therefore an important determinant of reduced longevity (2). Analyzing the blood glucose status of centenarians can help identify the associations between blood glucose and longevity, thereby providing suggestions for clinical blood glucose control that will facilitate healthy aging. A study in Italian centenarians showed that fasting plasma glucose (FPG) levels were significantly lower in centenarians than in cohabitants (3). The results of the China Hainan centenary cohort study and the China Hainan senior cohort study also showed that blood glucose levels and the prevalence of diabetes were lower in centenarians than those in non-centenarians (4), while another study in Polish centenarians reported that their blood glucose levels were significantly lower than in elderly individuals (5). A previous study on glycemic control only focused on periodic glucose measures and average level of glycated hemoglobin (HbA1c). However, the relative importance of glycemic variation over time has attracted increasing attention, although only a few relevant studies have been carried out in centenarians. There is evidence that compared with hyperglycemia, an increase in the variability of glucose levels may damage vascular endothelial cells and that such changes are associated with a higher risk of cardio-cerebrovascular and microvascular complications (6, 7). Continuous glucose monitoring (CGM) may therefore be more applicable to accurately evaluating variability in glucose levels. Research has shown that HbA1c levels are reduced to a greater extent after CGM compared with that achieved by glucometer testing (8). It has also been shown that CGM can markedly reduce the incidence of hypoglycemia in elderly people (9). However, the use of CGM in centenarians has not been reported extensively in the current literature. To address this situation, we recruited centenarians from Rugao City, an area famous for the longevity of its population (10). The first-generation of the centenarians' offspring and non-longevity individuals were recruited as control groups. A comprehensive examination of the relationship between glycemic variation and longevity was performed in order to provide a clinical basis to facilitate healthy aging in the population.

MATERIALS AND METHODS

Study Participants

We recruited individuals from Rugao City, Jiangsu Province China from April 2020 to May 2021. The cohort included 60 centenarians, 60 first-generation offspring, and 20 non-cohabitant controls aged 60–80 years from the same region who were selected randomly during the same period. Each individual was asked to complete a household survey. Exclusion criteria included acute diabetic complications, being long-term bedridden, secondary DM, malignancies, cognitive impairment, severe infection, recent trauma, surgery or other emergencies, severe systemic diseases, individuals currently using glucocorticoids, and those who did not have sufficient time to carry out continuous glucose monitoring (CGM). The final cohort included 53 centenarians, 53 first-generation offspring cohabitants, and 20 controls. The demographic characteristics of these three groups are summarized in **Table 1**. The study followed the Declaration of Helsinki thoroughly and was approved by the medical research ethics committee of the Second Affiliated Hospital of Nantong University.

Data Acquisition

Basic Data Collection

Demographic data including gender, age, educational level, marital and fertility history, smoking and drinking status were collected using professional medical questionnaires.

Glucose Monitoring

Continuous blood glucose was measured by a flash glucose monitoring (FGM) and CGM system (Abbott, FreeStyle Libre H, hospital version) (11) using standard protocols. The measurements were recorded over at least 12 consecutive 24-h periods, with the first and last 24-h periods excluded from the analysis.

CGM Index

Mean blood glucose (MBG), standard deviation of blood glucose (SDBG), coefficient of variation (CV), estimated glycated hemoglobin (eHbA1c), glucose management indicator (GMI), mean-fasting plasma glucose (M-FPG), mean-postprandial plasma glucose (M-PPG), M-PPG-1h, M-PPG-2h, M-PPG-3h, J index, M value, low blood glucose index (LBGI), high blood glucose index (HBGI), average daily risk range (ADRR), continuous overlapping net glycemic action (CONGA), mean absolute glucose (MAG), lability index (Li), postprandial plasma glycemic excursion (PPGE), PPGE-1, PPGE-2, PPGE-3, mean amplitude of glycemic excursion (MAGE), largest amplitude of

TABLE 1 | Clinical characteristics of the participants, according to group assignment.

Parameter	Centenarian group ($n = 53$)	First-generation offspring group ($n = 53$)	Control group ($n = 20$)	*P*
Age (yr)	100.0 (100.0, 101.0)	69.0 (63.0, 75.0)[a1]	69.0 (65.0, 77.0)[b1]	<0.001
SBP (mmHg)	155.0 (134.3, 178.8)	142.0 (124.5, 156.0)[a2]	130.3 (121.0, 152.4)[b2]	0.001
DBP (mmHg)	73.5 (65.3, 84.0)	78.5 (71.8, 87.8)	73.0 (66.6, 85.9)	0.053
Gender				
Male	12 (22.6)	39 (73.6)[a1]	7 (35.0)[c2]	<0.001
Female	41 (77.4)	14 (26.4)	13 (65.0)	<0.001
Marital status *n* (%)				
Married	1 (1.9)	47 (88.7)[a1]	19 (95.0)[b1]	<0.001
Single	52 (98.1)	6 (11.3)	1 (5.0)	<0.001
Educational level *n* (%)				
Illiterate	40 (75.5)	3 (5.7)[a1]	8 (40.0)[b2c1]	<0.001
Primary	11 (20.7)	21 (39.6)	8 (40.0)	<0.001
Junior	1 (1.9)	21 (39.6)	3 (15.0)	
Senior and above	1 (1.9)	8 (15.1)	1 (5.0)	
Hypertension *n* (%)				
Yes	17 (32.7)	20 (37.7)	6 (30.0)	0.794
No	35 (67.3)	33 (62.3)	14 (70.0)	0.794
DM *n* (%)				
Yes	2 (3.8)	5 (9.4)	1 (5.0)	0.63
No	51 (96.2)	48 (90.6)	19 (95.0)	0.63
Smoking *n* (%)				
Never	43 (81.1)	26 (50.0)[a1]	10 (50.0)[b2]	
Quit	7 (13.2)	12 (23.1)	4 (20.0)	0.003
Yes	3 (5.7)	14 (26.9)	6 (30.0)	
Drinking *n* (%)				
Never	37 (71.1)	21 (40.4)[a1]	8 (40.0)[b2]	
Quit	8 (15.4)	5 (9.6)	4 (20.0)	0.001
Yes	7 (13.5)	26 (50.0)	8 (40.0)	

a1: centenarian group vs. first-generation offspring group, $p < 0.001$; a2: centenarian group vs. first-generation offspring group, $p < 0.05$; b1: centenarian group vs. control group, $p < 0.001$; b2: centenarian group vs. control group, $p < 0.05$; c1: first-generation offspring group vs. control group, $p < 0.001$; c2: first-generation offspring group vs. control group, $p < 0.05$.

glycemic excursion (LAGE), mean of daily differences (MODD), time in the target glucose range (TIR), time below the target glucose range (TBR), and time above the target glucose range (TAR). The definition and interpretation of these indices are shown in **Supplementary Table 1**.

Diagnostic Criteria

The diagnostic criteria for DM (12, 13) used in the study were as follows: (1) polydipsia, polyuria, polyphagia, loss of body weight, and a random blood glucose level ≥11.1 mmol/L, (2) fasting plasma glucose (FPG) ≥7.0 mmol/L, (3) oral glucose tolerance test (OGTT) 2-h postprandial blood glucose ≥11.1 mmol/L. Hypertension: elevation of systemic arterial pressure in the resting state and a doctor's office blood pressure ≥140/90 mmHg (14).

Statistical Analysis

Normally distributed continuous numerical variables were expressed as mean ± standard deviation (SD). For each group, the differences were analyzed using *t*-tests. Non-normally distributed data were first logarithmically transformed and if still skewed, the Mann-Whitney U or Kruskal-Wallis tests were used to test the differences, expressed as median (interquartile range). Categorical variables were expressed as *n* (%) and analyzed using Chi-square tests. The association between longevity and blood glucose indices were analyzed by logistic regression analyses. Because the genetic relationship between centenarians and their offspring would affect the results of the correlation analysis in the study, the logistic regression analyses were only conducted between centenarian and control groups, who did not have a genetic relationship. The *P*-values were two-tailed, with $P < 0.05$ considered statistically significant. IBM SPSS for Windows, version 26.0 software was used for the statistical analyses.

RESULTS

Study Cohort

Age and systolic blood pressure were significantly higher in the centenarian group than in the first-generation offspring and control groups ($P < 0.05$). The centenarian and control group

TABLE 2 | Continuous blood glucose parameters according to group assignment.

Parameter	Centenarian group ($n = 53$)	First-generation offspring group ($n = 53$)	Control group ($n = 20$)	*P*
MBG (mmol/L)	5.31 (4.8, 5.6)	5.6 (5.3, 5.9)[a1]	5.7 (5.1, 6.2)[b1]	0.001
SDBG (mmol/L)	1.4 ± 0.4	1.4 ± 0.5	1.6 ± 0.6	0.398
CV	0.2 ± 0.06	0.2 ± 0.06[a2]	0.3 ± 0.06	0.023
eHbA1c (%)	5.9 (5.1, 6.3)	6.4 (5.8, 6.9) [a1]	6.5 (5.5, 7.2) [b2]	0.001
GMI (mmol/mol)	37.6 (35.4, 38.9)	39.3 (37.6, 40.8) [a1]	39.7 (36.8, 41.9) [b2]	0.001
M-FPG (mmol/L)	4.1 (3.9, 4.6)	4.9 (4.5, 5.3)[a1]	4.8 (4.4, 5.1)[b1]	<0.001
M-PPG1h (mmol/L)	4.4 (4.0, 5.3)	5.2 (4.7, 5.8) [a1]	4.9 (4.6, 6.9)[b2]	<0.001
M-PPG2h (mmol/L)	5.7 ± 1.6	6.4 ± 1.8	6.6 ± 1.9	0.064
M-PPG3h (mmol/L)	6.2 ± 1.7	6.8 ± 2.2	6.4 ± 1.7	0.238
J index (mmol/L)	15.1 ± 5.2	18.5 ± 12.0	20.3 ± 12.7	0.080
M Value	29.8 (16.8, 46.2)	21.5 (15.5, 39.1)	33.2 (16.1, 64.2)	0.318
LBGI	3.7 ± 2.1	2.0 ± 1.7[a1]	2.1 ± 1.3 [b2]	<0.001
HBGI	0.2 (0.1, 0.6)	0.1 (0.1, 0.6)	0.6 (0.1, 1.2)	0.246
ADRR	33.2 (32.9, 33.8)	32.7 (32.4, 33.2)[a1]	32.7 (32.2, 33.4)[b2]	<0.001
CONGA1 (mmol/L)	5.5 (5.0, 5.8)	5.8 (5.4, 6.1)[a2]	5.9 (5.2, 6.5)[b2]	0.006
MAG (mmol/L)	2.94 ± 0.92	3.00 ± 1.16	3.21 ± 1.07	0.256
Li	1.3 (1.1, 1.6)	1.5 (1.0, 2.0)	1.6 (1.2, 2.)	0.158
PPGE (mmol/L)	2.7 ± 0.9	2.7 ± 0.9	2.9 ± 1.1	0.656
PPGE-1 (mmol/L)	3.3 ± 1.5	3.2 ± 1.7	2.8 ± 1.5	0.400
PPGE-2 (mmol/L)	2.1 ± 1.1	2.3 ± 1.3	3.2 ± 1.3[b2c1]	0.002
PPGE-3 (mmol/L)	2.7 ± 1.2	2.6 ± 1.1	2.8 ± 1.2	0.811
MAGE (mmol/L)	1.7 ± 0.4	1.7 ± 0.5	1.8 ± 0.5	0.707
LAGE (mmol/L)	5.3 ± 1.4	5.3 ± 1.9	5.7 ± 1.8	0.615
MODD (mmol/L)	0.9 ± 0.3	0.9 ± 0.4	1.1 ± 0.4	0.057
TIR	0.9 (0.8, 0.9)	0.9 (0.8, 0.9)	0.8 (0.8, 0.9)	0.094
TBR	0.06 ± 0.11	0.07 ± 0.09	0.10 ± 0.13	0.379
TAR	0.03 ± 0.09	0.02 ± 0.06	0.07 ± 0.20	0.676

a1: centenarian group vs. first-generation offspring group, p < 0.001; a2: centenarian group vs. first-generation offspring group, p < 0.05; b1: centenarian group vs. control group, p < 0.001; b2: centenarian group vs. control group, p < 0.05; c1: first-generation offspring group vs. control group, p < 0.05.

had lower proportions of males and married individuals than the first-generation offspring group ($P < 0.001$), while the illiteracy rate was the highest in the centenarian group ($P < 0.001$). The centenarian group was also more likely to include non-smokers or drinkers ($P < 0.05$). There were no differences in diastolic blood pressure, income, hypertension, and DM status between the three groups ($P > 0.05$) (**Table 1**).

Comparison of Continuous Blood Glucose Indexes Among the Three Groups

In the centenarian group, MBG, CONGA1, eHbA1c, GMI, M-FPG, and M-PPG1h were lower ($P < 0.05$) and LBGI and ADRR higher ($P < 0.001$) than those measured in the first-generation offspring and control groups. The control group had higher PPGE-2 than the centenarian and first-generation offspring groups ($P = 0.002$). There was no significant difference in TIR ($P > 0.05$) between the three groups (**Table 2**).

Association Between Blood Glucose Fluctuation and Anti-longevity

The risk of not achieving longevity increased with each unit increase in the blood glucose indices MBG by 126% [OR (95%CI): 2.26 (1.05–4.91)], eHbA1c by 67% [OR (95%CI): 1.67 (1.03–2.72)], GMI by 568% [OR (95%CI): 6.68 (1.11–40.30)], M-FPG by 365% [OR (95%CI): 4.65 (1.57–13.75)], and M-PPG1h by 98% [OR (95%CI): 1.98 (1.18–3.31)]. The risk of not achieving longevity also increased with each unit increase in CONGA1 by 102% [OR (95%CI): 2.02 (1.01–4.06)], Li by 200% [OR (95%CI): 3.00 (1.04–8.61)], and PPGE-2 by 150% [OR (95%CI): 2.50 (1.39–4.50)]. However, the risk of not achieving longevity decreased with each unit increase of LBGI by 53% [OR (95%CI): 0.47 (0.28–0.80)], ADRR by 60% [OR (95%CI): 0.40 (0.18–0.86)], and TBR by 11% [OR (95%CI): 0.89 (0.80–0.98)]. Among these indicators of blood glucose variability, GMI had the largest OR value and therefore showed the strongest correlation with longevity (**Table 3**). However, associations between not achieving longevity and SDBG, M-PPG2h, M-PPG3h, J index, M Value, HBGI, MAG,

TABLE 3 | Logistic regression analysis of not achieving longevity and CGM indices.

Parameter	OR (95 % CI)[a]	*P*	OR (95 % CI)[b]	*P*
MBG (mmol/L)	1.93 (1.11–3.36)	0.020	2.26 (1.05–4.91)	0.038
SDBG (mmol/L)	1.86 (0.66–5.26)	0.241	1.27 (0.34–4.69)	0.724
CV (%)	0.97 (0.89–1.06)	0.460	0.90 (0.80–1.01)	0.086
eHbA1c (%)	1.51 (1.07–2.14)	0.020	1.67 (1.03–2.72)	0.038
GMI (mmol/mol)	4.61 (1.28–16.69)	0.020	6.68 (1.11–40.30)	0.038
M-FPG (mmol/L)	3.33 (1.46–7.62)	0.004	4.65 (1.57–13.75)	0.005
M-PPG1h (mmol/L)	1.75 (1.15–2.67)	0.010	1.98 (1.18–3.31)	0.010
M-PPG2h (mmol/L)	1.32 (0.99–1.76)	0.060	1.22 (0.88–1.69)	0.238
M-PPG3h (mmol/L)	1.06 (0.78–1.44)	0.710	0.98(0.65–1.48)	0.936
J index (mmol/L)	1.08 (1.01–1.16)	0.038	1.09 (0.98–1.20)	0.100
M Value	1.01 (1.00–1.02)	0.098	1.01 (1.00–1.02)	0.262
LBGI	0.58 (0.40–0.85)	0.005	0.47 (0.28–0.80)	0.005
HBGI	1.51 (0.98–2.35)	0.064	1.55 (0.84–2.88)	0.161
ADRR	0.49 (0.28–0.85)	0.012	0.40 (0.18–0.86)	0.020
CONGA1 (mmol/L)	1.81 (1.09–3.01)	0.023	2.02 (1.01–4.06)	0.050
MAG (mmol/L)	1.33 (0.78–2.26)	0.292	1.22 (0.63–2.35)	0.553
Li	2.69 (1.12–6.45)	0.027	3.00 (1.04–8.61)	0.041
PPGE (mmol/L)	1.27 (0.74–2.19)	0.387	1.19 (0.61–2.34)	0.603
PPGE-1 (mmol/L)	0.76 (0.52–1.12)	0.164	0.54 (0.31–0.97)	0.040
PPGE-2 (mmol/L)	2.14 (1.34–3.42)	0.002	2.50 (1.39–4.50)	0.002
PPGE-3 (mmol/L)	1.08 (0.68–1.74)	0.736	1.03 (0.60–1.78)	0.910
MAGE (mmol/L)	1.41 (0.42–4.68)	0.580	1.05 (0.25–4.45)	0.943
LAGE (mmol/L)	1.22 (0.85–1.73)	0.279	1.12 (0.73–1.72)	0.612
MODD (mmol/L)	8.03 (1.52–42.44)	0.014	6.38 (0.82–49.60)	0.077
TIR (%)	1.02 (0.98–1.07)	0.287	1.05 (0.99–1.11)	0.103
TBR (%)	0.91 (0.84–0.99)	0.020	0.89 (0.80–0.98)	0.018
TAR (%)	1.09 (0.99–1.19)	0.054	1.10 (0.97–1.24)	0.130

[a]: Unadjusted; [b]: Adjusted for systolic blood pressure, gender, marital status and educational level, smoking and drinking status.

Li, PPGE, PPGE-1, PPGE-3, MAGE, LAGE, MODD, TIR, TBR, TAR were not found in this study.

DISCUSSION

The results of this study showed that the CGM indices (MBG, eHbA1c, GMI, M-FPG, M-PPG1h, and CONGA1) were significantly lower in centenarians than in first-generation offspring and control groups. The centenarian group also had significantly higher LBGI and ADRR than the first-generation offspring and control groups. The risk of not achieving longevity increased with each one unit increase of MBG (126%), eHbA1c (67%), GMI (568%), M-FPG (365%), M-PPG1h (98%), CONGA1 (102%), Li (200%), and PPGE-2 (150%), although decreased with each one unit increase of LBGI (53%), ADRR (60%), and TBR (11%).

By providing information on glucose trends and measuring fluctuations, CGM provides a better tool for glucose management, treatment guidance, and patient motivation (15). As shown in previous studies, marked blood glucose fluctuations cause greater harm than persistent hyperglycemia (16). Such fluctuations by generating reactive oxygen species (ROS), vascular endothelial cell damage, and inflammation (17–19) can result in kidney damage, diabetic retinopathy, vascular damage, and pancreatic β-cell dysfunction (20–24), thereby limiting individual lifespan. Mean blood glucose levels and fluctuations are associated significantly with all-cause mortality, with high levels resulting in reduced life expectancy (25, 26). However, recent studies on longevity have focused mainly on the effect of point blood glucose measurements, and have shown that FPG levels and DM-associated morbidity are lower in centenarians than in non-centenarians (4). It has also been reported that there is an association between HbA1c and risk of all-cause mortality (27). In recent years, many CGM indices have been used to describe blood glucose variability with evidence showing that MBG and SDBG reflect blood glucose variability, while MBG affects HbA1c level (28). TIR, MOOD, CV, CONGA, and Li have also been shown to reflect daily blood glucose variability (29–31). LBGI and ADRR and TBR are better markers of hypoglycemia and are known to be associated with a greater risk of developing this condition (5, 32). However, there are fewer comprehensive indices for CGM and many gaps in research remain in the use of CGM in centenarians.

Using dynamic blood glucose monitoring, we found that: (1) blood glucose parameters including MGB, eHbA1c, GMI, and M-FPG were lowest in the centenarian group; (2) there was no difference in blood glucose fluctuation parameters such as MAGE, MODD, and MAG among the three groups; (3) CONGA1 was lowest in the centenarian group, PPGE-2 was higher in the control group than in the centenarian and sub-generation groups, and TIR which reflects blood glucose compliance time was the same in the three groups. We also found that MBG, eHbA1c, GMI, M-FPG, M-PPG1h, CONGA1, Li, and PPGE-2 were associated with not achieving longevity, indicating that increased blood glucose levels and fluctuations are not conducive to longevity.

LBGI and ADRR were highest in the centenarian group suggesting a higher risk of hypoglycemia than in the first-generation offspring and control groups. Increases in LBGI, ADRR, and TBR were associated with an increased likelihood of longevity. To further examine these associations, we divided the centenarian TBR into 2 groups according to their hypoglycemia status (<3.0 mmol/L and 3.1–3.9 mmol/L) and found that in the range of 3.1–3.9 mmol/L the rate of TBR was only 6.94%. TBR in the <3.0 mmol/L group was close to zero, indicating an absence of symptomatic hypoglycemia. Our results also showed that centenarians had the lowest blood glucose levels of the three groups, suggesting that these lower levels were conducive to longevity. The results are shown in **Supplementary Table 2**.

The cross-sectional nature of our study limits our ability to definitively identify the causal links between the measured variables. However, given the potential importance of these findings, we consider that follow-up cohort studies should be carried out to further test these relationships. In addition, as this study was conducted during the COVID-19 pandemic, the sample size of the control group was smaller than would be normally possible. We are therefore currently addressing

this situation with additional recruitment which will allow for independent validation of the outcomes.

Finally, on the basis of these data, we strongly encourage clinicians to monitor blood glucose dynamics in addition to obtaining "point" blood glucose measurements by utilizing CGM. In addition, therapy should be oriented toward reducing the magnitude of glycemic excursions, which may help patients to achieve better health and result in them living longer.

Glucose metabolism disorders can lead to a series of complications, such as microvascular pathologies of the nervous, renal, and vision systems and an increased risk of adverse macrovascular cardiovascular outcomes,that then lead to an increase in mortality and a reduction in life expectancy (33). Rozing's study show that blood glucose homeostasis of centenarian offspring was better than that of a control group (non-centenarian offspring) (34). Therefore, maintaining the steady state of glucose metabolism is conducive to realization of healthy aging. The use of continuous blood glucose monitoring can effectively reduce the average blood glucose and occurrence of adverse events (such as hypoglycemia), thereby improving the stability of blood glucose levels (35).

The mechanism of mild blood glucose fluctuations in centenarians is unclear. Paolisso's study showed that the level of insulin resistance in centenarians was low, and the insulin resistance was related closely to the occurrence of type 2 diabetes and fluctuations in blood glucose levels (36). Although insulin sensitivity will decrease with an increase in age due to a decrease in muscle content and associated weakness (37) some studies have reported that insulin sensitivity in centenarians was higher (38, 39). This may also be a reason why the blood glucose status of centenarians is relatively stable. However, the current study was a household survey, and blood sampling involved household blood collection. Due to the limited site and laboratory conditions, an oral glucose tolerance test (OGTT) and euglycemic glucose clamp could not be conducted. However, TyG, as a new index to measure insulin resistance, also has certain credibility (40). The results in **Supplementary Table 4** show that for each unit increase in TyG, the risk of not achieving longevity increased by 73% [OR (95% CI):1.73 (1.14,2.64)]. This result also indicates that high insulin resistance is not conducive to longevity.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Medical Research Ethics Committee of Affiliated Hospital of Nantong University (2019-K045). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

J-lG and Y-jG contributed to the design of the study and revised the manuscript. S-hJ and CD conducted the study, collected the data, explain the data, and completed the proof. RC and C-cS collected the data. JX analyzed the data. All authors have read and approved the final manuscript.

FUNDING

The study was supported by the Science and Technology Support Program of Nantong (MS22019005 and MS12020015).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.955101/full#supplementary-material

REFERENCES

1. Collaboration NCDRF. 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) 387:1377–96. doi: 10.1016/S0140-6736(16)30054-X
2. Herman WH, Zimmet P. Type 2 diabetes: an epidemic requiring global attention and urgent action. *Diabetes Care*. (2012) 35:943–4. doi: 10.2337/dc12-0298
3. Daniels LB, Antonini P, Marino R, Rizzo M, Navarin S, Lucibello SG, et al. Cardiovascular health of nonagenarians in southern Italy: a cross-sectional, home-based pilot study of longevity. *J Cardiovasc Med*. (2020) 21:89–98. doi: 10.2459/JCM.0000000000000910
4. Liu M, Yang S, Wang S, Zhao Y, Zhu Q, Ning C, et al. Distribution of blood glucose and prevalence of diabetes among centenarians and oldest-old in China: based on the China Hainan Centenarian Cohort Study and China Hainan Oldest-old Cohort Study. *Endocrine*. (2020) 70:314–22. doi: 10.1007/s12020-020-02403-4
5. Patton SR, Clements MA. Average daily risk range as a measure for clinical research and routine care. *J Diabetes Sci Technol*. (2013) 7:1370–75. doi: 10.1177/193229681300700529
6. Beck RW, Bergenstal RM, Riddlesworth TD, Kollman C, Li Z, Brown AS, et al. Validation of time in range as an outcome measure for diabetes clinical trials. *Diabetes Care*. (2019) 42:400–5. doi: 10.2337/dc18-1444
7. Lu J, Ma X, Zhou J, Zhang L, Mo Y, Ying L, et al. Association of time in range, as assessed by continuous glucose monitoring, with diabetic retinopathy in type 2 diabetes. *Diabetes Care*. (2018) 41:2370–6. doi: 10.2337/dc18-1131
8. Martens T, Beck RW, Bailey R, Ruedy KJ, Calhoun P, Peters AL, et al. Effect of continuous glucose monitoring on glycemic control in patients with type 2 diabetes treated with basal insulin: a randomized clinical trial. *JAMA*. (2021) 325:2262–72. doi: 10.1001/jama.2021.13478
9. Pratley RE, Kanapka LG, Rickels MR, Ahmann A, Aleppo G, Beck R, et al. Effect of continuous glucose monitoring on hypoglycemia in older adults with type 1 diabetes: a randomized clinical trial. *JAMA*. (2020) 323:2397–406. doi: 10.1001/jama.2020.6928
10. Liu Z, Wang Y, Zhang Y, Chu X, Wang Z, Qian D, et al. Cohort profile: the rugao longevity and ageing study (RuLAS). *Int J Epidemiol*. (2016) 45:1064–73. doi: 10.1093/ije/dyv101
11. Ajjan RA, Jackson N, Thomson SA. Reduction in HbA1c using professional flash glucose monitoring in insulin-treated type 2 diabetes patients managed in primary and secondary care settings: a pilot,

multicentre, randomised controlled trial. *Diab Vasc Dis Res*. (2019) 16:385–95. doi: 10.1177/1479164119827456
12. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. (1998) 15:539–53.
13. Gabir MM, Hanson RL, Dabelea D, Imperatore G, Roumain J, Bennett PH, et al. Plasma glucose and prediction of microvascular disease and mortality: evaluation of 1997 American Diabetes Association and 1999 World Health Organization criteria for diagnosis of diabetes. *Diabetes Care*. (2000). 23:1113–18.
14. Xiao J, Ren WL, Liang YY, Shen H, Gao YX, Chu MJ, et al. Effectiveness of lifestyle and drug intervention on hypertensive patients: a randomized community intervention trial in rural China. *J Gen Intern Med*. (2020) 35:3449–57. doi: 10.1007/s11606-019-05601-7
15. Park C, Le QA. The effectiveness of continuous glucose monitoring in patients with type 2 diabetes: a systematic review of literature and meta-analysis. *Diabetes Technol Ther*. (2018) 20:613–21. doi: 10.1089/dia.2018.0177
16. Okada K, Hibi K, Gohbara M, Kataoka S, Takano K, Akiyama E, et al. Association between blood glucose variability and coronary plaque instability in patients with acute coronary syndromes. *Cardiovasc Diabetol*. (2015) 14:111. doi: 10.1186/s12933-015-0275-3
17. Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes*. (2008) 57:1349–54. doi: 10.2337/db08-0063
18. Ge QM, Dong Y, Zhang HM, Su Q. Effects of intermittent high glucose on oxidative stress in endothelial cells. *Acta Diabetol*. (2010) 47 (Suppl. 1):97–103. doi: 10.1007/s00592-009-0140-5
19. Ying C, Liu T, Ling H, Cheng M, Zhou X, Wang S, et al. Glucose variability aggravates cardiac fibrosis by altering AKT signalling path. *Diab Vasc Dis Res*. (2017) 14:327–35. doi: 10.1177/1479164117698917
20. Nyiraty S, Pesei F, Orosz A, Coluzzi S, Vagi OE, Lengyel C, et al. Cardiovascular autonomic neuropathy and glucose variability in patients with type 1 diabetes: is there an association? *Front Endocrinol*. (2018) 9:174. doi: 10.3389/fendo.2018.00174
21. Yang YF, Li TC, Li CI, Liu CS, Lin WY, Yang SY, et al. Visit-to-visit glucose variability predicts the development of end-stage renal disease in type 2 diabetes: 10-year follow-up of taiwan diabetes study. *Medicine*. (2015) 94:e1804. doi: 10.1097/MD.0000000000001804
22. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*. (2012) 35:556–64. doi: 10.2337/dc11-1909
23. Ying C, Zhou X, Chang Z, Ling H, Cheng X, Li W. Blood glucose fluctuation accelerates renal injury involved to inhibit the AKT signaling pathway in diabetic rats. *Endocrine*. (2016) 53:81–96. doi: 10.1007/s12020-016-0867-z
24. Torimoto K, Okada Y, Mori H, Tanaka Y. Relationship between fluctuations in glucose levels measured by continuous glucose monitoring and vascular endothelial dysfunction in type 2 diabetes mellitus. *Cardiovasc Diabetol*. (2013). 12:1.
25. Lu J, Wang C, Shen Y, Chen L, Zhang L, Cai J, et al. Time in range in relation to all-cause and cardiovascular mortality in patients with type 2 diabetes: a prospective cohort study. *Diabetes Care*. (2021) 44:549–55. doi: 10.2337/dc20-1862
26. Russo MP, Fosser SNM, Elizondo CM, Giunta DH, Fuentes NA, Grande-Ratti MF. In-hospital mortality and glycemic control in patients with hospital hyperglycemia. *Rev Diabet Stud*. (2021) 17:50–6. doi: 10.1900/RDS.2021.17.50
27. Cavero-Redondo I, Peleteiro B, Alvarez-Bueno C, Rodriguez-Artalejo F, Martinez-Vizcaino V. Glycated haemoglobin A1c as a risk factor of cardiovascular outcomes and all-cause mortality in diabetic and non-diabetic populations: a systematic review and meta-analysis. *BMJ Open*. (2017) 7:e015949. doi: 10.1136/bmjopen-2017-015949
28. McCarter RJ, Hempe JM, Chalew SA. Mean blood glucose and biological variation have greater influence on HbA1c levels than glucose instability: an analysis of data from the diabetes control and complications trial. *Diabetes Care*. (2006) 29:352–65. doi: 10.2337/diacare.29.02.06.dc05-1594
29. McDonnell CM, Donath SM, Vidmar SI, Werther GA, Cameron FJ. A novel approach to continuous glucose analysis utilizing glycemic variation. *Diabetes Technol Ther*. (2005) 7:253-263. doi: 10.1089/dia.2005.7.253
30. Puhr S, Calhoun P, Welsh JB, Walker TC. The effect of reduced self-monitored blood glucose testing after adoption of continuous glucose monitoring on hemoglobin A1c and time in range. *Diabetes Technol Ther*. (2018) 20:557–60. doi: 10.1089/dia.2018.0134
31. Dong M, Liu W, Luo Y, Li J, Huang B, Zou Y, et al. Glycemic variability is independently associated with poor prognosis in five pediatric ICU centers in Southwest China. *Front Nutr*. (2022) 9:757982. doi: 10.3389/fnut.2022.757982
32. Kovatchev B, Meng Z, Cali AMG, Perfetti R, Breton MD. Low blood glucose index and hypoglycaemia risk: insulin glargine 300 U/mL versus insulin glargine 100 U/mL in type 2 diabetes. *Diabetes Ther*. (2020) 11:1293–302. doi: 10.1007/s13300-020-00808-y
33. Xiang L, Mittwede P, Clemmer J. Glucose homeostasis and cardiovascular alterations in diabetes. *Compr Physiol*. (2015) 5:1815–39. doi: 10.1002/cphy.c150001
34. Rozing M, Mooijaart S, Beekman M, Wijsman C, Maier A, Bartke A, et al. C-reactive protein and glucose regulation in familial longevity. *Age*. (2011) 33:623–30. doi: 10.1007/s11357-011-9206-8
35. Kropff J, Choudhary P, Neupane S, Barnard K, Bain S, Kapitza C, et al. Accuracy and longevity of an implantable continuous glucose sensor in the PRECISE study: a 180-day, prospective, multicenter, pivotal trial. *Diabetes Care*. (2017) 40:63–8. doi: 10.2337/dc16-1525
36. Paolisso G, Barbieri M, Rizzo M, Carella C, Rotondi M, Bonafè M, et al. Low insulin resistance and preserved beta-cell function contribute to human longevity but are not associated with TH-INS genes. *Exp Gerontol*. (2001) 37:149–56. doi: 10.1016/S0531-5565(01)00148-6
37. Kamwa V, Welch C, Hassan-Smith Z. The endocrinology of sarcopenia and frailty. *Minerva Endocrinol*. (2021) 46:453–68. doi: 10.23736/S2724-6507.20.03198-3
38. Krentz A, Viljoen A, Sinclair A. Insulin resistance: a risk marker for disease and disability in the older person. *Diabet Med*. (2013) 30:535–48. doi: 10.1111/dme.12063
39. Wijsman C, Rozing M, Streefland T, le Cessie S, Mooijaart S, Slagboom P, et al. Familial longevity is marked by enhanced insulin sensitivity. *Aging cell*. (2011) 10:114–21. doi: 10.1111/j.1474-9726.2010.00650.x
40. Yoon J, Hong A, Choi W, Park J, Kim H, Kang H. Association of triglyceride-glucose index with bone mineral density in non-diabetic Koreans: KNHANES 2008-2011. *Calcif Tissue Int*. (2021) 108:176–87. doi: 10.1007/s00223-020-00761-9

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Nutrition

TYPE Systematic Review
PUBLISHED 15 August 2022
DOI 10.3389/fnut.2022.963471

OPEN ACCESS

EDITED BY
Devin Wahl,
Colorado State University,
United States

REVIEWED BY
Marta Gonzalez-Freire,
Balearic Islands Health Research Institute (IdISBa), Spain
Gloria Solano-Aguilar,
Agricultural Research Service (USDA), United States

*CORRESPONDENCE
Le Ma
male@mail.xjtu.edu.cn
Jing Lin
linjing0127@xjtu.edu.cn
Mao Ma
mamao2007@163.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

RECEIVED 15 June 2022
ACCEPTED 26 July 2022
PUBLISHED 15 August 2022

CITATION
Li Z, Lei H, Jiang H, Fan Y, Shi J, Li C, Chen F, Mi B, Ma M, Lin J and Ma L (2022) Saturated fatty acid biomarkers and risk of cardiometabolic diseases: A meta-analysis of prospective studies. *Front. Nutr.* 9:963471. doi: 10.3389/fnut.2022.963471

Saturated fatty acid biomarkers and risk of cardiometabolic diseases: A meta-analysis of prospective studies

Zhaoqing Li[1†], Haoyuan Lei[1†], Hong Jiang[1†], Yahui Fan[1], Jia Shi[1], Chao Li[1], Fangyao Chen[1], Baibing Mi[1], Mao Ma[2*], Jing Lin[1*] and Le Ma[1,3*]

[1]School of Public Health, Xi'an Jiaotong University Health Science Center, Xi'an, China, [2]The First Affiliated Hospital, Xi'an Jiaotong University Health Science Center, Xi'an, China, [3]Key Laboratory of Environment and Genes Related to Diseases (Xi'an Jiaotong University), Ministry of Education of China, Xi'an, China

Background and aims: Evidence regarding associations of circulating saturated fatty acids (SFAs) with chronic diseases is mixed. The objective of this study was to determine the associations between total or individual SFA biomarkers and the risk of cardiometabolic diseases.

Methods: Four electronic databases were searched from inception to March 2022. Three investigators independently assessed for inclusion and extracted data. Random-effects or fixed-effects models was used to estimate the pooled relative risks (RRs) and corresponding 95% confidence intervals (CIs) for the association of total or individual SFA biomarkers, including even-chain SFAs (e.g., 14:0, myristic acid; 16:0, palmitic acid; 18:0, stearic acid), odd-chain SFAs (e.g., 15:0, pentadecanoic acid; 17:0, margaric acid) and very-long-chain SFAs (VLCSFAs; e.g., 20:0, arachidic acid; 22:0, behenic acid; 24:0, lignoceric acid), with risk of incident type 2 diabetes (T2D), cardiovascular disease [CVD; coronary heart disease (CHD) inclusive of stroke], CHD and stroke.

Results: A total of 49 prospective studies reported in 45 articles were included. Higher concentration of circulating total SFAs was associated with an increasing risk of cardiometabolic diseases, the risk increased significantly by 50% for CVD (95%CI:1.31–1.71), 63% for CHD (95%CI:1.38–1.94), 38% for stroke (95%CI:1.05–1.82), respectively. Similarly, levels of even-chain SFAs were positively associated with higher risk of chronic diseases, with RRs ranging from 1.15 to 1.43. In contrast, the risk of cardiometabolic diseases was reduced with increasing odd-chain SFA levels, with RRs ranging from 0.62 to 0.91. A higher level of VLCSFAs corresponded to 19% reduction in CVD. Further dose-response analysis indicated that each 50% increment in percentage of total SFAs in circulating was associated with an 8% higher risk of T2D (RR: 1.08, 95%CI: 1.02–1.14) and trends toward higher risk of CVD (RR: 1.15, 95%CI: 0.98–1.34). Inverse linear relationships were observed between 17:0 biomarker and T2D or CVD risk.

Conclusion: Our findings support the current recommendations of reducing intake of saturated fat as part of healthy dietary patterns. Further studies are needed to confirm our findings on these SFAs in relation to cardiometabolic outcomes and to elucidate underlying mechanisms.

Systematic review registration: [https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42022329182], identifier [CRD42022329182].

KEYWORDS

saturated fatty acid biomarker, cardiometabolic disease, type 2 diabetes, coronary heart disease, stroke, cardiovascular disease, meta-analysis

Introduction

Limiting intake of saturated fatty acids (SFAs) has been considered as a key component to reduce the risk of chronic disorders for decades (1). The 2020 Dietary Guidelines for Americans recommended that SFAs comprise below 10% of total daily energy intake starting at age 2 years (2). The American Heart Association/American College of Cardiology guidelines have also set a target to decrease SFA intake to 5–6% of calories (3). *In vitro* and animal studies have demonstrated that SFAs can accelerate the atherosclerotic process, induce cellular inflammation and influence the insulin sensitivity (4, 5). Results from a meta-analysis of randomized controlled trials conducted by the American Heart Association demonstrated that replacing SFAs with unsaturated fats, especially polyunsaturated fats, will lower the incidence of cardiovascular disease (CVD) by approximately 30% (6). Therefore, it is important to accurately evaluate the effects of SFAs on health for the control of cardiometabolic diseases.

Associations of dietary SFAs with the incidence of major cardiometabolic diseases have been reported by some large prospective cohort studies, and findings of these studies were inconsistent (7–9). Interpretation has been complicated by the imprecision of dietary questionnaires, measurement errors, different study designs, variation in population characteristics, as well as bioavailability of these fatty acids (10–12). To reduce these limitations, biomarkers which are free of misclassification, reporting bias and other measurement errors can be used to elucidate associations between the intake of SFAs and disease risk (13, 14). An increasing number of prospective studies have been performed to investigate the associations between circulating total SFAs and risk of metabolic and cardiovascular event; however, the results were conflicting (15–17). In addition, previous studies mostly focused on the coronary heart disease (CHD), evidence regarding associations of circulating SFA with other cardiometabolic disease [such as type 2 diabetes (T2D) and stroke] is still needed to be explored. Furthermore, increasing evidence suggested that individual SFA tend to have different biological functions (18). Previous meta-analyses in 2014 reported that even-chain SFAs were positively associated with coronary risk (19); however, null or inverse association was observed for other subtypes of SFAs such as odd-chain SFAs and very-long-chain SFAs (VLCSFAs) in recent studies (20, 21). Therefore, the impact of individual SFA on cardiometabolic disease needs to be further studied and established.

To fill this knowledge gap, a dose-response meta-analysis of data from prospective studies was conducted to evaluate the associations between the circulating even-chain SFAs, odd-chain SFAs, VLCSFAs as well as the total SFAs and the incident of T2D, CVD, CHD, and stroke.

Materials and methods

This study was registered in PROSPERO (CRD42022329182) and reported in accordance with the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines.

Search strategy

A comprehensive literature search of Web of Science, PubMed, EMBASE, and Cochrane Library were conducted to identify relevant published articles from inception to March 2022. The following terms were used: "fatty acids" or "saturated fatty acid" or "saturated fatty acids" or "lauric acid" or "myristic acid" or "pentadecanoic acid" or "palmitic acid" or "margaric acid" or "stearic acid" or "arachidic acid" or "heneicosylic acid" or "behenic acid" or "tricosylic acid" or "lignoceric acid," AND "type 2 diabetes" or "diabetes mellitus" or "impaired glucose" or "impaired fasting insulin" or "cardiovascular disease" or "coronary heart disease" or "heart disease" or "ischemic heart disease" or "coronary artery disease" or "myocardial infarction"

or "stroke" or "ischemic stroke" or "haemorrhagic stroke," AND "blood" or "marker" or "biomarker" or "serum" or "plasma" or "whole blood" or "adipose tissue" or "circulating" or "erythrocytes" or "red blood cell" or "cholesterol esters," AND "prospective" or "nested case-control" or "cohort" or "case-cohort" or "follow-up" or "longitudinal." The search was restricted to human studies without any language restriction. Additionally, reference lists of retrieved articles, review articles, and meta-analyses were manually scanned to identify any other relevant studies. Corresponding authors of eligible articles were contacted for any further relevant work, published or unpublished, to reduce risk of publication bias.

Selection criteria

Studies were included in the meta-analysis if they satisfied the following criteria: (1) the study design was prospective (cohort, nested case-control, and case-cohort) with a follow-up more than 1 year; (2) the exposures of interest were total or individual SFA biomarkers [even-chain SFAs (e.g., 14:0, myristic acid; 16:0, palmitic acid; 18:0, stearic acid), odd-chain SFAs (e.g., 15:0, pentadecanoic acid; 17:0, margaric acid) and VLCSFAs (e.g., 20:0, arachidic acid; 22:0, behenic acid; 24:0, lignoceric acid)] measured in any type of tissue [circulating blood (whole blood, serum, plasma, and erythrocyte fraction) or adipose tissue]; (3) the endpoints of interest included incident T2D, CVD, CHD, and stroke; (4) the adjusted relative risks (RRs), odds ratios, or hazard ratios with corresponding 95% confidence intervals (CIs) or standard errors were presented or could be calculated. Whenever reports referred to identical outcomes from the same population, only those with the highest number of cases or the longest follow-up times were retained to avoid data duplication. Potentially eligible studies were assessed independently by three investigators (ZQL, HYL, and HJ), with discrepancies resolved by discussion until consensus was reached.

Data extraction and quality assessment

Data extraction were conducted independently by 3 investigators (ZQL, HYL, and HJ). A standardized data collection form was applied to extract the following baseline characteristics from each study: first author name, publication year, study location, study name, study design, follow-up duration, participant characteristics (age, proportion of men, and number of participants), SFA subtype, biological sample type (plasma, serum, erythrocyte membrane, and adipose tissue), assessment method [gas chromatography (GC), gas-liquid chromatography (GLC), and nuclear magnetic resonance-based profiling (NMR)], outcomes of interest (type and number of cases), covariates adjusted for in the multivariable model, and the risk estimate with 95%CIs for all categories of each biomarkers. When studies reported multiple results based on different numbers of covariates included in statistical adjustments, the results that adjusted for the most number of variables were extracted.

Study quality was performed by the same authors according to the validated Newcastle-Ottawa Scale (NOS) (22), which awards 0–9 points and incorporates information on selection (range 0–4 points), comparability (range 0–2 points), and outcome assessment (range 0–3 points). We considered NOS scores of 0–3, 4–6, and 7–9 as low, medium, and high quality, respectively. Any discrepancy in data extraction or quality assessments between investigators were discussed or resolved by a senior author (LM) until a joint consensus was reached.

Data synthesis and statistical analysis

In this meta-analysis, the RRs were used as the common measures of associations across studies, and odds ratios and hazard ratios were considered approximations of RRs. As different studies might report different exposure categories, methods previously described were used to derive estimates of associations corresponding to the comparison between the top and bottom third of SFAs distributions for the meta-analysis (19). The strategy was to harmonize different comparison groups used in individual studies, such as quartiles, quintiles, or other categorizations, or per standard deviation (SD) change. In brief, for studies that provided RRs per SD change of SFAs, we applied a factor of 2.18 to the log RR to derive the RRs comparing extreme thirds, assuming a normal distribution. Similarly, the factor of 2.54 or 2.80 was applied to convert estimates for comparing extreme quartiles or quintiles, respectively. The standard error of the transformed log RRs was calculated after applying the same factors (23). When studies used multiple measures as biomarker, the overall risk estimate was based on different duration of intake reflection according to the following list: adipose tissue, erythrocyte phospholipids, plasma phospholipids, total plasma or serum, and cholesterol esters. Studies that separately reported results by sex without presenting overall estimates were pooled to derive a single effect size for the study. Assessment of heterogeneity between the studies was based on Q test and I^2 statistic (24). A Cochran's Q $P < 0.10$ and I^2 value $> 50\%$ was considered to indicate significant heterogeneity and the random-effects model was used. Otherwise, fixed-effects model was performed. Meta-regression was conducted to examine sources of heterogeneity and the influence of potential residual confounding factors, such as study design [prospective cohort study (PC), prospective case-cohort study (PCC), or nested case-control study (NCC)], geographical location (the United States, Europe, or Asia), assessment method (GC, GLC, or NMR), biomarker type

(plasma, serum, erythrocyte membrane, or adipose tissue), number of cases ($<$ 300 or $\geq$ 300), as well as study quality (moderate or high). Dose-response analyses were conducted based on the method proposed by Greenland and Longnecker (25), studies that reported RRs with 95%CIs for at least three exposure categories were included. When studies reported only the total number of cases or total person-years and the exposure was defined in quantile, the distribution of cases or person-years was calculated by dividing the total number by the number of quantiles. For the studies that did not present the median or mean doses of SFAs, we chose the midpoint of each category as the alternative. When the highest category did not have an upper bound, the length of the open-ended interval was assumed to be the same as that of the adjacent interval. When the lowest category was open-ended, the lower bound was set to zero. Sensitivity analyses were conducted by omitting one study in each turn to assess the impact of individual studies on the overall estimated risk. Potential publication bias was explored by using visual appreciation of funnel plots, Begg's rank correlation and Egger's weighted regression tests. The influence of a potential publication bias on findings was explored by using the Duval and Tweedie trim and fill procedure (26). All analyses were performed using Stata, version 12.0 (Stata Corporation, College Station, TX, United States). All *P*-values reported are two-sided and a *P*-value $<$ 0.05 was considered statistically significant, except where otherwise specified.

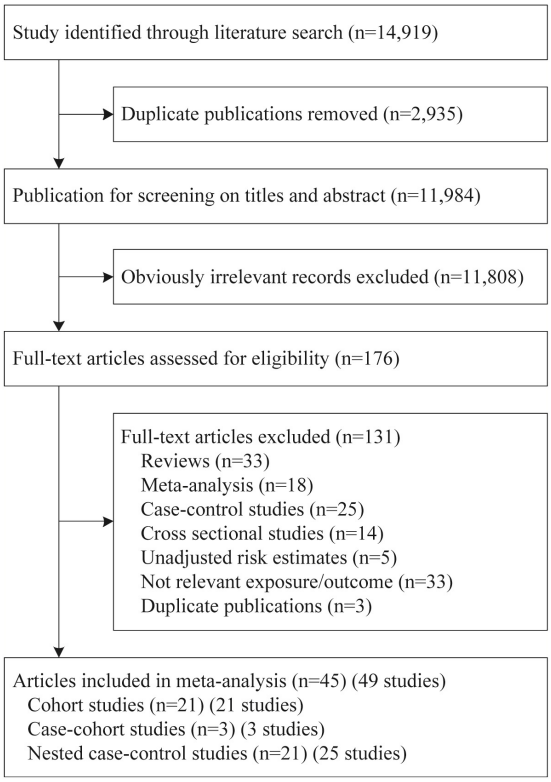


FIGURE 1
Flow chart of study selection.

Results

Studies retrieved and characteristics

A total of 14,919 potentially relevant citations were identified by search strategy, of which 2,935 duplicates were initially excluded. After screening titles and abstracts, 11,808 records were further removed, leaving 176 articles retrieved for full-text review. Finally, a total of 49 studies reported in 45 articles were included for the present meta-analysis (**Figure 1**) (15–17, 20, 21, 27–66).

The main characteristics of the included prospective studies are listed in **Supplementary Table 1**. The 49 identified studies comprised of 21 prospective cohort studies, three case-cohort studies, and 25 nested case-control studies. The number of participants in each included study varied from 188 to 95,854, with a total of 220,590 participants and 46,355 cases (21,252 T2D cases, 14,613 CVD cases, 7,314 CHD cases, and 3,176 stroke cases). 24 studies were conducted in the United States, 15 in Europe, and 10 in Asia. The duration of follow-up in prospective studies ranged from 1.5 to 30.7 years. The average age of participants ranged from 48.9 to 79.2 years. 32 studies comprised both men and women as participants, nine studies included men only and eight included women only. Concentrations of circulating SFAs were quantified by GC (n = 28), GLC (n = 18), NMR (n = 2), and GC-tandem mass spectrometry (n = 1). 48 studies measured SFA biomarkers in circulating blood (21 in plasma, 15 in erythrocyte membrane, and 12 in serum) and one in adipose tissue. Most studies were adjusted for age (n = 47) and smoking (n = 46), and alcohol drinking (n = 40), physical activity (n = 39) and body mass index (n = 38) were also controlled for in many studies. The results of the study quality assessment showed that 44 studies were graded as high quality, others were graded as moderate quality (**Supplementary Table 2**).

Association between saturated fatty acid biomarkers and type 2 diabetes

Twenty-two studies were assessed to estimate risk between the concentrations of SFA biomarkers and T2D, including 166,745 participants and 21,252 T2D events (17, 20, 27–44). The pooled estimate indicated that a higher level of total SFAs was associated with a non-significant higher risk of T2D (RR: 1.14, 95%CI: 0.83–1.56, P = 0.43; $P_{heterogeneity}$ $<$ 0.001; **Figure 2** and **Supplementary Figure 1**). For even-chain SFAs, compared with participants in the lowest tertile, those in the highest tertile of 16:0 level had a 28% significantly

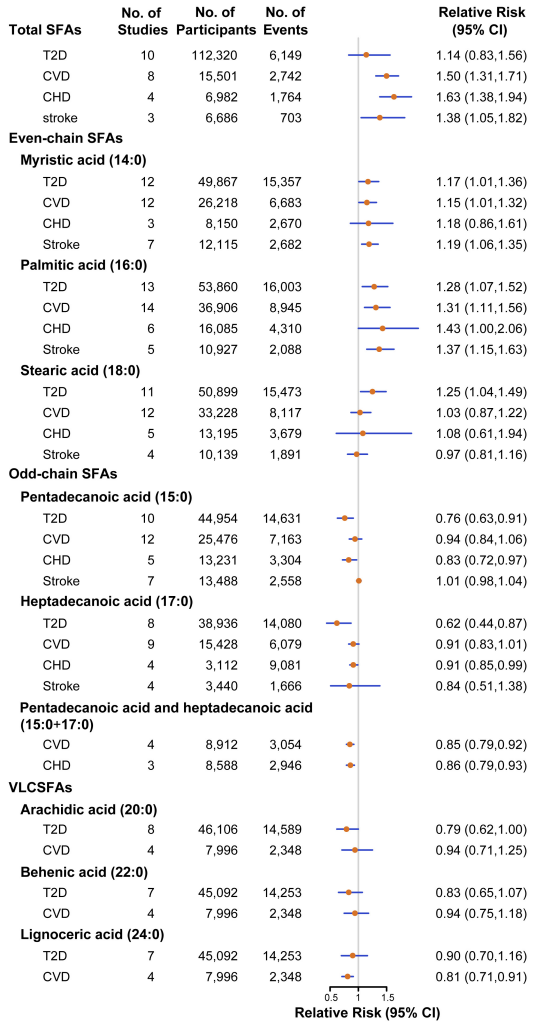


FIGURE 2
Pooled relative risks of T2D, CVD, CHD, stroke comparing the highest with the lowest tertile of SFA biomarkers. CHD, coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; SFAs, saturated fatty acids; T2D, type 2 diabetes; VLCSFAs, very-long-chain SFAs.

higher T2D risk (RR: 1.28, 95%CI: 1.07–1.52, P = 0.01; $P_{heterogeneity}$ < 0.001; Supplementary Figure 2). Similar association was also found between other two even-chain SFAs and CVD risk, with the RR of 1.17 (95%CI: 1.01–1.36, P = 0.03; $P_{heterogeneity}$ = 0.004; Supplementary Figure 3) for 14:0 and 1.25 (95%CI: 1.04–1.49, P = 0.01; $P_{heterogeneity}$ < 0.001; Supplementary Figure 4) for 18:0, respectively. By contrast, when comparing the extreme tertiles of odd-chain SFA level, the risk in T2D decreased significantly by 24% for 15:0 (RR: 0.76, 95%CI: 0.63–0.91, P = 0.003; $P_{heterogeneity}$ = 0.002; Supplementary Figure 5), 38% for 17:0 (RR: 0.62, 95%CI: 0.44–0.87, P = 0.01; $P_{heterogeneity}$ < 0.001; Supplementary Figure 6). Higher VLCSFAs status was associated with a non-significant lower risk of T2D [RR was 0.79 for 20:0 (95%CI: 0.62–1.00, P = 0.06; $P_{heterogeneity}$ < 0.001; Supplementary Figure 7), 0.83 for 22:0 (95%CI: 0.65–1.07, P = 0.16; $P_{heterogeneity}$ < 0.001; Supplementary Figure 8), 0.90 for 24:0 (95%CI: 0.70–1.16, P = 0.42; $P_{heterogeneity}$ = 0.001; Supplementary Figure 9)]. The association between circulating SFAs and T2D risk was not significantly modified by study type, sex, geographical location, number of cases, assessment method, biomarker type and study quality (Supplementary Figures 10–12). The result of dose-response analysis found that each 50% increment in percentage of total SFAs in circulating corresponded to an 8% (RR: 1.08, 95%CI: 1.02–1.14, P = 0.01; $P_{linearity}$ = 0.84) higher risk of T2D. For every 50% increase in percentage of odd-chain SFAs concentration in total SFAs, the risk of T2D was reduced by 8% for 15:0 (RR: 0.92, 95%CI: 0.83–1.02, P = 0.11; $P_{linearity}$ = 0.25), 16% for 17:0 (RR: 0.84, 95%CI: 0.76–0.92, P < 0.001; $P_{linearity}$ = 0.12), respectively.

Association between saturated fatty acid biomarkers and cardiovascular disease

Association between circulating SFAs and risk of CVD were investigated in 27 studies, with 53,845 participants and 14,613 CVD events (15, 16, 21, 45–66). Participants in the highest category of circulating total SFAs had a 50% higher risk with CVD compared with those in the lowest category (RR: 1.50, 95%CI: 1.31–1.71, P < 0.001; $P_{heterogeneity}$ = 0.56; Supplementary Figure 13). For even-chain SFAs, the pooled results indicated that higher 16:0 status was associated with a 31% increased risk of CVD (RR: 1.31, 95%CI: 1.11–1.56, P = 0.002; $P_{heterogeneity}$ < 0.001; Supplementary Figure 14). Similarly, higher levels of 14:0 was also associated with a 15% higher risk of CVD (RR: 1.15, 95%CI: 1.01–1.32, P = 0.03; $P_{heterogeneity}$ < 0.001; Supplementary Figure 15). For odd-chain SFAs, higher levels of the sum of 15:0 and 17:0 had a 15% lower risk of CVD (RR: 0.85, 95%CI: 0.79–0.92, P < 0.001; $P_{heterogeneity}$ = 0.15; Supplementary Figure 16). For VLCSFAs, in comparison with the lowest category, the highest category of 24:0 was associated with a 19% (RR: 0.81, 95%CI: 0.71–0.91, P = 0.001; $P_{heterogeneity}$ = 0.45; Supplementary Figure 17) reduced risk of CVD. 15:0, 17:0, 18:0, 20:0, and 22:0 biomarkers were not significantly associated with incidence of CVD (Supplementary Figures 18–22). In stratified analyses, the association between 16:0 status and CVD risk was significantly stronger among NCC studies (RR: 1.86, 95%CI: 1.32–2.62) compared with PC studies (Supplementary Figures 10–12). In the dose-response analysis, a non-significant linear association was noted between total SFAs biomarker and CVD risk, with the RR of 1.15 (95%CI: 0.98–1.34, P = 0.09; $P_{linearity}$ = 0.96) for each 50% increment in percentage of total SFAs in circulating. For every 50% increment in percentage of 17:0 and 24:0 concentrations of total SFAs, the RR of CVD

decreased by 18% for 17:0 (RR:0.82, 95%CI: 0.70–0.97, $P = 0.02$; $P_{linearity} = 0.84$), 10% for 24:0 (RR:0.90, 95%CI: 0.82–0.98, $P = 0.02$; $P_{linearity} = 0.14$), respectively.

Association between saturated fatty acid biomarkers and coronary heart disease

A total of 14 studies with 7,314 cases in 26,772 participants were included to investigate the relationship between circulating SFAs and CHD risk (15, 16, 49–59). The overall effect estimates of CHD comparing the top to bottom categories were 1.63 for total SFAs (95%CI: 1.38–1.94, $P < 0.001$; $P_{heterogeneity} = 0.45$; **Supplementary Figure 23**). For even-chain SFAs, the pooled result indicated that a higher level of 16:0 was associated with a higher risk of CHD (RR: 1.43, 95%CI: 1.00–2.06, $P = 0.05$; $P_{heterogeneity} = 0.003$; **Supplementary Figure 24**). The levels of odd-chain SFAs was associated with a significantly lower CHD risk by 17% for 15:0 (RR: 0.83, 95%CI: 0.72–0.97, $P = 0.02$; $P_{heterogeneity} = 0.21$; **Supplementary Figure 25**), 9% for 17:0 (RR: 0.91, 95%CI: 0.85–0.99, $P = 0.03$; $P_{heterogeneity} = 0.22$; **Supplementary Figure 26**) and 14% for the sum of 15:0 and 17:0 (RR: 0.86, 95%CI: 0.79–0.93, $P < 0.001$; $P_{heterogeneity} = 0.23$; **Supplementary Figure 27**), respectively. No significant associations were observed between 14:0, 18:0 concentrations and incidence of CHD (**Supplementary Figures 28, 29**). In stratified analyses, no evidence of heterogeneity was detected between subgroups. Due to the limited number of included studies, evaluation for dose-response analysis between SFA biomarkers and risk of CHD was not performed.

Association between saturated fatty acid biomarkers and stroke

The association between circulating SFAs with stroke risk were investigated in nine studies, comprising 16,589 participants and 3,176 stroke events (49, 60–66). The estimated RR of stroke for the comparison of extreme categories was 1.38 for total SFAs (95%CI: 1.05–1.82, $P = 0.02$; $P_{heterogeneity} = 0.76$; **Supplementary Figure 30**). For even-chain SFAs, both 16:0 and 14:0 biomarkers showed positive associations with stroke risk, with the pooled effect estimates of 1.37 (95%CI: 1.15–1.63, $P < 0.001$; $P_{heterogeneity} = 0.11$; **Supplementary Figure 31**) and 1.19 (95%CI: 1.06–1.35, $P = 0.004$; $P_{heterogeneity} = 0.10$; **Supplementary Figure 32**), respectively. No significant associations were detected for circulating 15:0, 17:0 or 18:0 and stroke risk (**Supplementary Figures 33–35**). No evidence of heterogeneity was detected within any of the subgroups. Dose-response analysis between circulating SFAs and stroke risk was limited by a lack of studies.

Sensitivity analysis and publication bias

Sensitivity analysis showed that no single study substantially changed the statistical significance or direction of the combined RR for any of the outcomes. Visual inspection of the funnel plot did not reveal substantial asymmetry (**Supplementary Figures 36–67**). Similarly, the results of the Egger's tests and Begg's tests indicated that no evidence of publication bias was found for associations between circulating SFAs and incidence of these cardiometabolic diseases ($P > 0.05$ for both tests).

Discussion

In the present meta-analysis, higher concentration of circulating total SFAs was associated with an increasing risk of CVD, CHD, and stroke, rather than T2D. Compared with the adverse effect of even-chain SFAs, odd-chain SFAs and VLCSFAs showed a significant inverse association with these cardiometabolic diseases. Our study suggested that individual circulating SFAs may not be equally associated with the risk of chronic diseases, which raises the possibility of generating public health recommendations and nutritional guidelines of SFAs in these cardiometabolic diseases prevention.

Previous studies have found that higher dietary intake of SFAs was associated with an increasing risk of chronic health condition (67). Data from prospective cohort studies also showed that dietary replacement of SFAs with polyunsaturated fat or whole grain carbohydrates might significantly reduce the incidence of cardiometabolic disease (8). Nevertheless, investigations into the effects of dietary SFA consumption on chronic disease in humans have been limited by challenges in accurately assessing saturated fat intake from dietary questionnaires, due to errors in recall and wide variations in SFA content of otherwise similar foods. In this setting, measurements of circulating fatty acids provide a more valid and objective biomarker of dietary fat. Up to date, the associations between circulating saturated fats and the risk of vascular disease still remain controversial. A meta-analysis conducted in 2013 have found essentially null associations between circulating SFAs and coronary risk (19), while a recent matched case-control study among 2,428 postmenopausal women in the Women's Health Initiative Observational Study showed that 1 mol% increase in plasma phospholipid SFAs was associated with a 20% higher CHD risk (59). Similarly, among 7,354 participants from the European Prospective Investigation into Cancer study who were free of CHD at baseline, the risk of developing CHD almost doubled in participants with highest level of circulating SFAs, when compared with those with lowest level (68). Consistent with these recent findings, the present studies suggested that circulating total SFAs was associated with an increased risk of cardiovascular events. Meanwhile, this result was also supported by subgroups analysis of even-chain SFAs which increment

in circulating myristic acid, palmitic acid and stearic acid corresponded to the increased risk of these diseases. As the major components of SFAs, 14:0, 16:0, and 18:0 contribute more than 90% of the fatty acid composition of plasma (69). The strength of the positive associations with cardiometabolic risk were similar for this fatty acid subtype and total SFAs, indicating that the detrimental effects of SFAs may mainly be attributed to even-chain SFAs. The results of the present study also indicated that the positive association with cardiometabolic diseases was more pronounced for high palmitic acid level, as compared with other even-chain SFAs (such as: myristic acid, and stearic acid). In a study of 6,379 postmenopausal women followed up for 10 years, participants in the highest category of circulating palmitic acid level experienced a 24% higher T2D risk compared with those in the lowest category, whereas a weak or no association was found for other even-chain SFAs (39). Similarly, the results from the Cardiovascular Health Study also showed an increased risk of cardiometabolic diseases with increasing circulating concertation of palmitic acid, rather than stearic acid (38). Therefore, our observations, together with evidence from previous studies, indicated that decreasing even-chain SFA level, especially palmitic acid, confers benefits for the prevention of cardiometabolic diseases.

The potential mechanisms through which circulating SFAs especially even-chain SFAs increase the risk of chronic disorders have been proposed, including producing inflammation, inducing lipoprotein disorders, and activating endoplasmic reticulum (ER) stress (70–73). Systemic inflammation is recognized as an important contributor to insulin resistance and atherosclerosis, all of which drives the development of metabolic diseases and CVD (72, 74). In previous *in vitro* experiments, Pal et al. found that even-chain SFAs could induce proinflammatory cytokine expression in adipocytes through the FetA and TLR4 pathway, resulting in insulin resistance (75). Animal experiments have also suggested that SFAs like 16:0 could activate the NLRP3 inflammasome in macrophages by an AMPK-autophagy-ROS signaling pathway (76). Along with the activating of proinflammatory responses, SFAs was thought to impair the LDL-receptor activity and consequently increase the concentration of LDL-cholesterol (LDL-C), an apolipoprotein B-containing lipoprotein that can become trapped in the artery wall and ultimately implicated in the generation of atherosclerosis (77, 78). The adverse effect of palmitic acid on lipoprotein metabolism might explain part of the stronger association between palmitic acid and risk of cardiometabolic diseases. A previous animal study suggested that increasing the absolute amount of dietary 16:0 could increase LDL-cholesterol (LDL-C) more than other even-chain SFAs, through specific modulation of the expression of the LDL receptor and apolipoprotein B genes (79). Clinical trials also showed that serum total cholesterol, apo A-I concentrations and plasma cholesteryl ester transfer protein activity significantly increased in the palmitic acid diet as compared with the stearic acid diet (80, 81). Meanwhile, emerging evidence has suggested that palmitic acid but not myristic or stearic acid exerts adverse effects on ER function by stimulating stress signaling XBP1 and ATF6 (82), which may play a pivotal role in arresting the cell cycle progression in islet (83) and initiating a positive feedback loop in production of even-chain SFAs *via de novo* lipogenesis (84), ultimately contributes to the occurrence of metabolic diseases.

Compared with the deleterious effects of even-chain SFAs, the present results suggested that a higher level of odd-chain SFAs was associated with a lower risk of chronic conditions. Data from Northern Sweden Health and Disease Study suggested that each percent increase of the proportion of this fatty acid species was significantly associated with a risk reduction of a first myocardial infarction (54). The Västerbotten Intervention Programme has also reported that for participants who were free from diabetes at baseline, per SD increase in circulating 15:0 and 17:0 confer relative reductions in T2D incidence of 29 and 46%, respectively (30). The protection against dysregulated lipid metabolism and low-grade inflammation have been proposed to underlie the beneficial role of odd-chain SFAs because the disorder of fat homeostasis and the expression of inflammatory proteins trigger atherosclerosis and other metabolic syndrome (85). In the European Prospective Investigation into Cancer and Nutrition-InterAct study, inverse associations have been observed between plasma phospholipid 15:0 and 17:0 and lipid markers, such as total cholesterol, triglycerides, apolipoprotein A-1, apolipoprotein B. This study also suggested that increasing odd-chain SFAs was accompanied by a decreased C-reactive protein, per 1-*SD* increment of the sum of 15:0 and 17:0 was associated with a 10% decrement of this inflammatory marker (86). Additionally, emerging evidence has indicated that this subclass of fatty acids may provide protection against incident T2D by ameliorating the degree of insulin resistance and β-cell dysfunction. In a multicenter study analyzing different race groups in America without diabetes at baseline, Santaren et al. found that serum concentrations of pentadecanoic acid was positively associated with insulin sensitivity and β cell function (Disposition Index), suggesting that this fatty acid species may play a role in T2D prevention (87). Moreover, as the primary source of odd-chain SFAs, similar association was also found in dairy products (53, 88–93). Two recent meta-analyses of randomized controlled trials reported significant reduction in cardiovascular events with dairy foods intake (94, 95). Despite the possible interactions among these two odd-numbered FAs and multiple bioactive substances in dairy products are largely unknown, the previous studies for dairy consumption still lend support to accumulating evidences of an inverse association between odd-chain SFAs and chronic disease.

For VLCSFAs, the present study of an inverse association between circulating VLCSFAs and CVD risk was in line with the results from previous epidemiological studies (20, 96). While diet is unlikely the main source of these SFAs in the human body (97), the VLCSFAs can be synthesized endogenously (98, 99) and serve as essential components of sphingolipids that is the key constituents of membranes in the human body (100, 101). Previous studies have demonstrated a potential association between sphingolipid metabolism and early markers of the latter chronic disease condition such as adiponectin. In a rat model treated with dietary sphingolipids, a 45-day VLCSFAs supplementation significantly improved adiponectin signaling and consequently increased insulin sensitivity (102). Moreover, as the elongation products of long chain SFAs, this consistent association has also been observed between these fatty acids and plasmalogen (103), an ether lipid which may function as cellular antioxidants and scavenge a variety of reactive oxygen species (104).

Some limitations of this study should be mentioned. Firstly, individual blood SFAs were quantitatively measured once and changes of these SFAs over time might lead to potential random measurement error caused by within-person variation. However, among the Nurses' Health Study and Nurses' Health Study II participants, reasonable validity and reproducibility of concentration of SFAs such as 15:0, 16:0, and 24:0 have been demonstrated with intraclass correlation coefficients, indicating a single measurement of fatty acid biomarker levels can reliably represent long-term levels over time (105). Secondly, participants with high SFAs consumption tended to have more unhealthy dietary habits and engage in less physical activity (8) which associated with higher cardiometabolic risk (106). Despite all included studies have adjusted for potential confounding factors, we cannot fully exclude the impact of residual or unmeasured confounding on the observed associations. Because of the prospective design of the current study, misclassification caused by potential confounders such as lifestyle was independent of the outcome ascertainment and was therefore more likely to be non-differential, which would tend to attenuate true associations toward the null. However, the positive association between 16:0 status and CVD risk appeared to be more evident among NCC studies compared with PC studies. This may be attributed to their inherent limitations as the NCC studies are more prone to be influenced by survival bias due to all the cases and controls are enrolled at the same time, which may lead to an overestimation of the magnitude of the association (107). Thirdly, different subtypes of SFAs may have possible additive or synergistic effects that results in the development of cardiometabolic diseases. Further additional studies are warranted to assess the potential interaction between levels of different SFA types and cardiovascular disease. Finally, publication bias should be ascertained. Although no significant publication bias was detected, the potential bias could not be completely ruled out.

Conclusion

Our meta-analysis of existing prospective studies indicated that a higher concentration of total SFAs and even-chain SFAs was associated with an increasing risk of cardiometabolic diseases, which supported the current recommendations of reducing intake of saturated fat as part of healthy dietary patterns. In addition, our finding of protective effects of odd-chain SFAs has potentially important clinical implications for preventing cardiometabolic diseases. Further studies are apparently needed to confirm our findings on these SFAs in relation to cardiometabolic outcomes and to elucidate underlying mechanisms.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author/s.

Author contributions

LM, JL, and MM generated the idea for the study, formulated an analytical plan, and supervised the study. ZL, HL, and HJ searched for relevant studies, conducted quality assessment, and extracted the data. ZL and HJ performed data analysis. ZL and HL wrote the manuscript and all other authors revised the manuscript. All authors approved the final manuscript for submission and acquired, analyzed, or interpreted the data.

Funding

This research was funded by the National Natural Science Foundation of China (NSFC-81473059, NSFC-81973025, NSFC-81202198, and NSFC-81803263), the Natural Science Foundation of Shaanxi Province of China (2017JM8041), the Fundamental Research Funds for the Central Universities (qngz2016004 and xzy032019008), and the Nutrition Research Foundation Fund of the Chinese Nutrition Society-DSM Special Research Foundation (CNSDSM 2016-041). The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.963471/full#supplementary-material

References

1. Hooper L, Martin N, Jimoh OF, Kirk C, Foster E, Abdelhamid AS. Reduction in saturated fat intake for cardiovascular disease. *Cochrane Database Syst Rev.* (2020) 5:CD011737. doi: 10.1002/14651858.CD011737.pub2

2. U.S. Department of Agriculture and U.S. Department of Health and Human Services. *Dietary guidelines for Americans, 2020-2025*. 9th ed. (2020). Available online at: https://health.gov/our-work/food-nutrition/current-dietary-guidelines (accessed December 1, 2020).

3. Eckel RH, Jakicic JM, Ard JD, de Jesus JM, Miller HN, Hubbard VS, et al. 2013 AHA/ACC guideline on lifestyle management to reduce cardiovascular risk: a report of the American College of Cardiology/American Heart Association task force on practice guidelines. *Circulation.* (2015) 131:E326. doi: 10.1161/01.cir.0000437740.48606.d1

4. Siri-Tarino PW, Chiu S, Bergeron N, Krauss RM. Saturated fats versus polyunsaturated fats versus carbohydrates for cardiovascular disease prevention and treatment. *Annu Rev Nutr.* (2015) 35:517–43. doi: 10.1161/01.cir.0000437740.48606.d1

5. Hunnicutt JW, Hardy RW, Williford J, McDonald JM. Saturated fatty acid-induced insulin resistance in rat adipocytes. *Diabetes.* (1994) 43:540–5. doi: 10.1146/annurev-nutr-071714-034449

6. Sacks FM, Lichtenstein AH, Wu JHY, Appel LJ, Creager MA, Kris-Etherton PM, et al. Dietary fats and cardiovascular disease: a presidential advisory from the American Heart Association. *Circulation.* (2017) 136:e1–23. doi: 10.1161/CIR.0000000000000510

7. de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, et al. Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *BMJ.* (2015) 351:h3978. doi: 10.1136/bmj.h3978

8. Zong G, Li Y, Wanders AJ, Alssema M, Zock PL, Willett WC, et al. Intake of individual saturated fatty acids and risk of coronary heart disease in US men and women: two prospective longitudinal cohort studies. *BMJ.* (2016) 355:i5796. doi: 10.1136/bmj.i5796

9. Jiao J, Liu G, Shin HJ, Hu FB, Rimm EB, Rexrode KM, et al. Dietary fats and mortality among patients with type 2 diabetes: analysis in two population based cohort studies. *BMJ.* (2019) 366:l4009. doi: 10.1136/bmj.l4009

10. Shim J-S, Oh K, Kim HC. Dietary assessment methods in epidemiologic studies. *Epidemiol Health.* (2014) 36:e2014009. doi: 10.4178/epih/e2014009

11. Hebert JR, Clemow L, Pbert L, Ockene IS, Ockene JK. Social desirability bias in dietary self-report may compromise the validity of dietary intake measures. *Int J Epidemiol.* (1995) 24:389–98. doi: 10.1093/ije/24.2.389

12. Hebert JR, Ma YS, Clemow L, Ockene IS, Saperia G, Stanek EJ, et al. Gender differences in social desirability and social approval bias in dietary self-report. *Am J Epidemiol.* (1997) 146:1046–55. doi: 10.1093/oxfordjournals.aje.a009233

13. Arab L. Biomarkers of fat and fatty acid intake. *J Nutr.* (2003) 133:925S–32S. doi: 10.1093/jn/133.3.925S

14. Cantwell MM. Assessment of individual fatty acid intake. *Proc Nutr Soc.* (2000) 59:187–91. doi: 10.1017/s0029665100000203

15. Clarke R, Shipley M, Armitage J, Collins R, Harris W. Plasma phospholipid fatty acids and CHD in older men: Whitehall study of London civil servants. *Br J Nutr.* (2009) 102:279–84. doi: 10.1017/S0007114508143562

16. Chei C-L, Yamagishi K, Kitamura A, Kiyama M, Sankai T, Okada T, et al. Serum fatty acid and risk of coronary artery disease-Circulatory Risk in Communities Study (CIRCS). *Circ J.* (2018) 82:3013–20. doi: 10.1253/circj.CJ-18-0240

17. Bragg F, Kartsonaki C, Guo Y, Holmes M, Du H, Yu C, et al. Circulating metabolites and the development of type 2 diabetes in Chinese adults. *Diabetes Care.* (2022) 45:477–80. doi: 10.2337/dc21-1415

18. Flock MR, Kris-Etherton PM. Diverse physiological effects of long-chain saturated fatty acids: implications for cardiovascular disease. *Curr Opin Clin Nutr Metab Care.* (2013) 16:133–40. doi: 10.1097/MCO.0b013e328359e6ac

19. Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Ann Intern Med.* (2014) 160:398–406. doi: 10.7326/m13-1788

20. Ardisson Korat AV, Malik VS, Furtado JD, Sacks F, Rosner B, Rexrode KM, et al. Circulating very-long-chain SFA concentrations are inversely associated with incident type 2 diabetes in US men and women. *J Nutr.* (2020) 150:340–9. doi: 10.1093/jn/nxz240

21. de Oliveira OMC, Lemaitre RN, Song X, King IB, Siscovick DS, Mozaffarian D. Serial measures of circulating biomarkers of dairy fat and total and cause-specific mortality in older adults: the Cardiovascular Health Study. *Am J Clin Nutr.* (2018) 108:476–84. doi: 10.1093/ajcn/nqy117

22. Wells GA, Shea B, O'Connell D, Robertson J, Peterson J, Welch V, et al. *The Newcastle-Ottawa Scale (NOS) for Assessing the Quality of Nonrandomised Studies in Meta-analyses*. (2020). Available online at: http://www.ohri.caprograms/clinical_epidemiology/oxford.asp (accessed April 21, 2020).

23. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease – meta-analyses of prospective studies. *JAMA.* (1998) 279:1477–82. doi: 10.1001/jama.279.18.1477

24. Higgins JPT, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ.* (2003) 327:557–60. doi: 10.1136/bmj.327.7414.557

25. Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol.* (1992) 135:1301–9. doi: 10.1093/oxfordjournals.aje.a116237

26. Duval S, Tweedie RA. non parametric "trim and fill" method of accounting for publication bias in meta-analysis. *J Am Stat Assoc.* (2000) 95:89–98. doi: 10.1080/01621459.2000.10473905

27. Laaksonen DE, Lakka TA, Lakka HM, Nyyssonen K, Rissanen T, Niskanen LK, et al. Serum fatty acid composition predicts development of impaired fasting glycaemia and diabetes in middle-aged men. *Diabet Med.* (2002) 19:456–64. doi: 10.1046/j.1464-5491.2002.00707.x

28. Wang L, Folsom AR, Zheng ZJ, Pankow JS, Eckfeldt JH. Plasma fatty acid composition and incidence of diabetes in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) study. *Am J Clin Nutr.* (2003) 78:91–8. doi: 10.1093/ajcn/78.1.91

29. Hodge AM, English DR, O'Dea K, Sinclair AJ, Makrides M, Gibson RA, et al. Plasma phospholipid and dietary fatty acids as predictors of type 2 diabetes:

interpreting the role of linoleic acid. *Am J Clin Nutr.* (2007) 86:189–97. doi: 10.1093/ajcn/86.1.189

30. Krachler B, Norberg M, Eriksson JW, Hallmans G, Johansson I, Vessby B, et al. Fatty acid profile of the erythrocyte membrane preceding development of type 2 diabetes mellitus. *Nutr Metab Cardiovasc Dis.* (2008) 18:503–10. doi: 10.1016/j.numecd.2007.04.005

31. Mozaffarian D, Cao H, King IB, Lemaitre RN, Song X, Siscovick DS, et al. Trans-palmitoleic acid, metabolic risk factors, and new-onset diabetes in U.S. adults: a cohort study. *Ann Intern Med.* (2010) 153:790–9. doi: 10.7326/0003-4819-153-12-201012210-00005

32. Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw K-T, Wareham NJ, et al. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort. *Am J Clin Nutr.* (2010) 92:1214–22. doi: 10.3945/ajcn.2010.29182

33. Kröger J, Zietemann V, Enzenbach C, Weikert C, Jansen EHJM, Döring F, et al. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam study. *Am J Clin Nutr.* (2011) 93:127–42. doi: 10.3945/ajcn.110.005447

34. Mozaffarian D, de Oliveira Otto MC, Lemaitre RN, Fretts AM, Hotamisligil G, Tsai MY, et al. trans-Palmitoleic acid, other dairy fat biomarkers, and incident diabetes: the Multi-Ethnic Study of Atherosclerosis (MESA). *Am J Clin Nutr.* (2013) 97:854–61. doi: 10.3945/ajcn.112.045468

35. Zong G, Zhu J, Sun L, Ye X, Lu L, Jin Q, et al. Associations of erythrocyte fatty acids in the de novo lipogenesis pathway with risk of metabolic syndrome in a cohort study of middle-aged and older Chinese. *Am J Clin Nutr.* (2013) 98:319–26. doi: 10.3945/ajcn.113.061218

36. Forouhi NG, Koulman A, Sharp SJ, Imamura F, Kroger J, Schulze MB, et al. Differences in the prospective association between individual plasma phospholipid saturated fatty acids and incident type 2 diabetes: the EPIC-InterAct case-cohort study. *Lancet Diabetes Endocrinol.* (2014) 2:810–8. doi: 10.1016/S2213-8587(14)70146-9

37. Lemaitre RN, Fretts AM, Sitlani CM, Biggs ML, Mukamal K, King IB, et al. Plasma phospholipid very-long-chain saturated fatty acids and incident diabetes in older adults: the cardiovascular health study. *Am J Clin Nutr.* (2015) 101:1047–54. doi: 10.3945/ajcn.114.101857

38. Ma W, Wu JHY, Wang Q, Lemaitre RN, Mukamal KJ, Djoussé L, et al. Prospective association of fatty acids in the de novo lipogenesis pathway with risk of type 2 diabetes: the cardiovascular health study. *Am J Clin Nutr.* (2015) 101:153–63. doi: 10.3945/ajcn.114.092601

39. Harris WS, Luo J, Pottala JV, Margolis KL, Espeland MA, Robinson JG. Red blood cell fatty acids and incident diabetes mellitus in the women's health initiative memory study. *PLoS One.* (2016) 11:e0147894. doi: 10.1371/journal.pone.0147894

40. Yakoob MY, Shi P, Willett WC, Rexrode KM, Campos H, Orav EJ, et al. Circulating biomarkers of dairy fat and risk of incident diabetes mellitus among men and women in the United States in two large prospective cohorts. *Circulation.* (2016) 133:1645–54. doi: 10.1161/CIRCULATIONAHA.115.018410

41. Akter S, Kurotani K, Sato M, Hayashi T, Kuwahara K, Matsushita Y, et al. High serum phospholipid dihomo-γ-linoleic acid concentration and low Δ5-desaturase activity are associated with increased risk of type 2 diabetes among Japanese adults in the Hitachi Health Study. *J Nutr.* (2017) 147:1558–66. doi: 10.3945/jn.117.248997

42. Lin J, Dong H, Chen G, Chen Z, Dong X, Zheng J, et al. Erythrocyte saturated fatty acids and incident type 2 diabetes in Chinese men and women: a prospective cohort study. *Nutrients.* (2018) 10:1393. doi: 10.3390/nu10101393

43. Lu Y, Wang Y, Zou L, Liang X, Ong CN, Tavintharan S, et al. Serum lipids in association with type 2 diabetes risk and prevalence in a Chinese population. *J Clin Endocrinol Metab.* (2018) 103:671–80. doi: 10.1210/jc.2017-02176

44. Zhuang P, Liu X, Li Y, Li H, Zhang L, Wan X, et al. Circulating fatty acids and genetic predisposition to type 2 diabetes: gene-nutrient interaction analysis. *Diabetes Care.* (2022) 45:564–75. doi: 10.2337/dc21-2048

45. Warensjo E, Sundstrom J, Vessby B, Cederholm T, Riserus U. Markers of dietary fat quality and fatty acid desaturation as predictors of total and cardiovascular mortality: a population-based prospective study. *Am J Clin Nutr.* (2008) 88:203–9. doi: 10.1093/ajcn/88.1.203

46. Woodward M, Tunstall-Pedoe H, Batty GD, Tavendale R, Hu FB, Czernichow S. The prognostic value of adipose tissue fatty acids for incident cardiovascular disease: results from 3944 subjects in the Scottish Heart Health Extended Cohort Study. *Eur Heart J.* (2011) 32:1416–23. doi: 10.1093/eurheartj/ehr036

47. Chien K-L, Lin H-J, Hsu H-C, Chen P-C, Su T-C, Chen M-F, et al. Comparison of predictive performance of various fatty acids for the risk of cardiovascular disease events and all-cause deaths in a community-based cohort. *Atherosclerosis.* (2013) 230:140–7. doi: 10.1016/j.atherosclerosis.2013.06.015

48. Fretts AM, Mozaffarian D, Siscovick DS, King IB, McKnight B, Psaty BM, et al. Associations of plasma phospholipid SFAs with total and cause-specific mortality in older adults differ according to SFA chain length. *J Nutr.* (2016) 146:298–305. doi: 10.3945/jn.115.222117

49. Trieu K, Bhat S, Dai Z, Leander K, Gigante B, Qian F, et al. Biomarkers of dairy fat intake, incident cardiovascular disease, and all-cause mortality: a cohort study, systematic review, and meta-analysis. *PLoS Med.* (2021) 18:e1003763. doi: 10.1371/journal.pmed.1003763

50. Simon JA, Hodgkins ML, Browner WS, Neuhaus JM, Bernert JT, Hulley SB. Serum fatty acids and the risk of coronary heart disease. *Am J Epidemiol.* (1995) 142:469–76. doi: 10.1093/oxfordjournals.aje.a117662

51. Wang L, Folsom AR, Eckfeldt JH, The ASI. Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) study. *Nutr Metab Cardiovasc Dis.* (2003) 13:256–66. doi: 10.1016/s0939-4753(03)80029-7

52. Warensjö E, Jansson J-H, Berglund L, Boman K, Ahrén B, Weinehall L, et al. Estimated intake of milk fat is negatively associated with cardiovascular risk factors and does not increase the risk of a first acute myocardial infarction. A prospective case-control study. *Br J Nutr.* (2004) 91:635–42. doi: 10.1079/BJN20041080

53. Sun Q, Ma J, Campos H, Hu FB. Plasma and erythrocyte biomarkers of dairy fat intake and risk of ischemic heart disease. *Am J Clin Nutr.* (2007) 86:929–37. doi: 10.1093/ajcn/86.4.929

54. Warensjö E, Jansson J-H, Cederholm T, Boman K, Eliasson M, Hallmans G, et al. Biomarkers of milk fat and the risk of myocardial infarction in men and women: a prospective, matched case-control study. *Am J Clin Nutr.* (2010) 92:194–202. doi: 10.3945/ajcn.2009.29054

55. Wu JHY, Lemaitre RN, Imamura F, King IB, Song X, Spiegelman D, et al. Fatty acids in the de novo lipogenesis pathway and risk of coronary heart disease: the cardiovascular health study. *Am J Clin Nutr.* (2011) 94:431–8. doi: 10.3945/ajcn.111.012054

56. Khaw KT, Friesen MD, Riboli E, Luben R, Wareham N. Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the EPIC-Norfolk prospective study. *PLoS Med.* (2012) 9:e1001255. doi: 10.1371/journal.pmed.1001255

57. Malik VS, Chiuve SE, Campos H, Rimm EB, Mozaffarian D, Hu FB, et al. Circulating very-long-chain saturated fatty acids and incident coronary heart disease in US men and women. *Circulation.* (2015) 132:260–8. doi: 10.1161/CIRCULATIONAHA.114.014911

58. Sun Y, Koh HWL, Choi H, Koh W-P, Yuan J-M, Newman JW, et al. Plasma fatty acids, oxylipins, and risk of myocardial infarction: the Singapore Chinese Health Study. *J Lipid Res.* (2016) 57:1300–7. doi: 10.1194/jlr.P066423

59. Liu Q, Matthan NR, Manson JE, Howard BV, Tinker LF, Neuhouser ML, et al. Plasma phospholipid fatty acids and coronary heart disease risk: a matched case-control study within the Women's Health Initiative Observational Study. *Nutrients.* (2019) 11:1672. doi: 10.3390/nu11071672

60. Iso H, Sato S, Umemura U, Kudo M, Koike K, Kitamura A, et al. Linoleic acid, other fatty acids, and the risk of stroke. *Stroke.* (2002) 33:2086–93. doi: 10.1161/01.str.0000023890.25066.50

61. Wiberg B, Sundström J, Aìrnlöv J, Tereìnt A, Vessby B, Zethelius B, et al. Metabolic risk factors for stroke and transient ischemic attacks in middle-aged men. *Stroke.* (2006) 37:2898–903. doi: 10.1161/01.STR.0000249056.24657.8b

62. Warensjo E, Smedman A, Stegmayr B, Hallmans G, Weinehall L, Vessby B, et al. Stroke and plasma markers of milk fat intake–a prospective nested case-control study. *Nutr J.* (2009) 8:21. doi: 10.1186/1475-2891-8-21

63. Yaemsiri S, Sen S, Tinker LF, Robinson WR, Evans RW, Rosamond W, et al. Serum fatty acids and incidence of ischemic stroke among postmenopausal women. *Stroke.* (2013) 44:2710–7. doi: 10.1161/STROKEAHA.111.000834

64. Yamagishi K, Folsom AR, Steffen LM, Aric Study Investigators. Plasma fatty acid composition and incident ischemic stroke in middle-aged adults: the Atherosclerosis Risk in Communities (ARIC) study. *Cerebrovasc Dis.* (2013) 36:38–46. doi: 10.1159/000351205

65. Yakoob MY, Shi P, Hu FB, Campos H, Rexrode KM, Orav EJ, et al. Circulating biomarkers of dairy fat and risk of incident stroke in U.S. men and women in 2 large prospective cohorts. *Am J Clin Nutr.* (2014) 100:1437–47. doi: 10.3945/ajcn.114.083097

66. Huang NK, Biggs ML, Matthan NR, Djousse L, Longstreth WT, Mukamal KJ, et al. Serum nonesterified fatty acids and incident stroke: the CHS. *J Am Heart Assoc.* (2021) 10:e022725. doi: 10.1161/JAHA.121.022725

67. Mann JI. Diet and risk of coronary heart disease and type 2 diabetes. *Lancet.* (2002) 360:783–9. doi: 10.1016/s0140-6736(02)09901-4

68. Katan MB, Khaw K-T, Friesen MD, Riboli E, Luben R, Wareham N. Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the EPIC-Norfolk prospective study. *PLoS Med.* (2012) 9:e1001255. doi: 10.1371/journal.pmed.1001255

69. Baylin A, Kim MK, Donovan-Palmer A, Siles X, Dougherty L, Tocco P, et al. Fasting whole blood as a biomarker of essential fatty acid intake in epidemiologic studies: comparison with adipose tissue and plasma. *Am J Epidemiol.* (2005) 162:373–81. doi: 10.1093/aje/kwi213

70. Dias JA, Wirfält E, Drake I, Gullberg B, Hedblad B, Persson M, et al. A high quality diet is associated with reduced systemic inflammation in middle-aged individuals. *Atherosclerosis.* (2015) 238:38–44. doi: 10.1016/j.atherosclerosis.2014.11.006

71. Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol.* (2012) 32:2045–51. doi: 10.1161/atvbaha.108.179705

72. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, et al. Low-grade systemic inflammation and the development of type 2 diabetes: the Atherosclerosis Risk in Communities study. *Diabetes.* (2003) 52:1799–805. doi: 10.2337/diabetes.52.7.1799

73. Vallim T, Salter AM. Regulation of hepatic gene expression by saturated fatty acids. *Prostaglandins Leukot Essent Fatty Acids.* (2010) 82:211–8. doi: 10.1016/j.plefa.2010.02.016

74. Libby P. Inflammation in atherosclerosis. *Nature.* (2002) 420:868–74. doi: 10.1038/nature01323

75. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nat Med.* (2012) 18:1279–85. doi: 10.1038/nm.2851

76. Wen H, Gris D, Lei Y, Jha S, Zhang L, Huang MT, et al. Fatty acid-induced NLRP3-ASC inflammasome activation interferes with insulin signaling. *Nat Immunol.* (2011) 12:408–15. doi: 10.1038/ni.2022

77. Ference BA, Kastelein JJP, Catapano AL. Update on lipids and lipoproteins-reply. *JAMA.* (2021) 325:400–1.

78. Jialal I, Devaraj S. Update on lipids and lipoproteins. *JAMA.* (2021) 325:400. doi: 10.1001/jama.2020.22621

79. Salter AM, Mangiapane EH, Bennett AJ, Bruce JS, Billett MA, Anderton KL, et al. The effect of different dietary fatty acids on lipoprotein metabolism: concentration-dependent effects of diets enriched in oleic, myristic, palmitic and stearic acids. *Br J Nutr.* (1998) 79:195–202. doi: 10.1079/bjn19980031

80. Snook JT, Park S, Williams G, Tsai Y-H, Lee N. Effect of synthetic triglycerides of myristic, palmitic, and stearic acid on serum lipoprotein metabolism. *Eur J Clin Nutr.* (1999) 53:597–605. doi: 10.1038/sj.ejcn.1600815

81. Schwab US, Maliranta HM, Sarkkinen ES, Savolainen MJ, Kesäniemi YA, Uusitupa MI. Different effects of palmitic and stearic acid-enriched diets on serum lipids and lipoproteins and plasma cholesteryl ester transfer protein activity in healthy young women. *Metabolism.* (1996) 45:143–9. doi: 10.1016/s0026-0495(96)90044-x

82. Cnop M, Abdulkarim B, Bottu G, Cunha DA, Igoillo-Esteve M, Masini M, et al. RNA sequencing identifies dysregulation of the human pancreatic islet transcriptome by the saturated fatty acid palmitate. *Diabetes.* (2014) 63:1978–93. doi: 10.2337/db13-1383

83. Babour A, Bicknell AA, Tourtellotte J, Niwa MA. surveillance pathway monitors the fitness of the endoplasmic reticulum to control its inheritance. *Cell.* (2010) 142:256–69. doi: 10.1016/j.cell.2010.06.006

84. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. *Physiol Rev.* (2018) 98:2133–223. doi: 10.1152/physrev.00063.2017

85. Tian XY, Ganeshan K, Hong C, Nguyen KD, Qiu Y, Kim J, et al. Thermoneutral housing accelerates metabolic inflammation to potentiate atherosclerosis but not insulin resistance. *Cell Metab.* (2016) 23:165–78. doi: 10.1016/j.cmet.2015.10.003

86. Zheng JS, Sharp SJ, Imamura F, Koulman A, Schulze MB, Ye Z, et al. Association between plasma phospholipid saturated fatty acids and metabolic markers of lipid, hepatic, inflammation and glycaemic pathways in eight European countries: a cross-sectional analysis in the EPIC-InterAct study. *BMC Med.* (2017) 15:203. doi: 10.1186/s12916-017-0968-4

87. Santaren ID, Watkins SM, Liese AD, Wagenknecht LE, Rewers MJ, Haffner SM, et al. Serum pentadecanoic acid (15:0), a short-term marker of dairy food intake, is inversely associated with incident type 2 diabetes and its underlying disorders. *Am J Clin Nutr.* (2014) 100:1532–40. doi: 10.3945/ajcn.114.092544

88. Lankinen M, Schwab U. Biomarkers of dairy fat. *Am J Clin Nutr.* (2015) 101:1101–2. doi: 10.3945/ajcn.114.104554

89. Mitri J, Tomah S, Furtado J, Tasabehji MW, Hamdy O. Plasma free fatty acids and metabolic effect in type 2 diabetes, an ancillary study from a randomized clinical trial. *Nutrients.* (2021) 13:1145. doi: 10.3390/nu13041145

90. Abdullah MM, Cyr A, Lepine MC, Labonte ME, Couture P, Jones PJ, et al. Recommended dairy product intake modulates circulating fatty acid profile in healthy adults: a multi-centre cross-over study. *Br J Nutr.* (2015) 113:435–44. doi: 10.1017/S0007114514003894

91. Pfeuffer M, Jaudszus A. Pentadecanoic and heptadecanoic acids: multifaceted odd-chain fatty acids. *Adv Nutr.* (2016) 7:730–4. doi: 10.3945/an.115.011387

92. Golley RK, Hendrie GA. Evaluation of the relative concentration of serum fatty acids C14:0, C15:0 and C17:0 as markers of children's dairy fat intake. *Ann Nutr Metab.* (2014) 65:310–6. doi: 10.1159/000368325

93. Brevik A, Veierød MB, Drevon CA, Andersen LF. Evaluation of the odd fatty acids 15:0 and 17:0 in serum and adipose tissue as markers of intake of milk and dairy fat. *Eur J Clin Nutr.* (2005) 59:1417–22. doi: 10.1038/sj.ejcn.1602256

94. Zhang K, Chen X, Zhang L, Deng Z. Fermented dairy foods intake and risk of cardiovascular diseases: a meta-analysis of cohort studies. *Crit Rev Food Sci Nutr.* (2020) 60:1189–94. doi: 10.1080/10408398.2018.1564019

95. Companys J, Pla-Pagà L, Calderón-Pérez L, Llauradó E, Solà R, Pedret A, et al. Fermented dairy products, probiotic supplementation, and cardiometabolic diseases: a systematic review and meta-analysis. *Adv Nutr.* (2020) 11:834–63. doi: 10.1093/advances/nmaa030

96. Gallo S, Egeland G, Meltzer S, Legault L, Kubow S. Plasma fatty acids and desaturase activity are associated with circulating adiponectin in healthy adolescent girls. *J Clin Endocrinol Metab.* (2010) 95:2410–7. doi: 10.1210/jc.2009-1975

97. Us Department of Agriculture, Agricultural Research Service. *Nutrient Data Laboratory. USDA National Nutrient Database for Standard Reference, Release 28 (Slightly revised).* (2016). Available online at: http://www.ars.usda.gov/nea/bhnrc/mafcl (accessed May 1, 2016)

98. Kihara A. Very long-chain fatty acids: elongation, physiology and related disorders. *J Biochem.* (2012) 152:387–95. doi: 10.1093/jb/mvs105

99. Jakobsson A, Westerberg R, Jacobsson A. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Prog Lipid Res.* (2006) 45:237–49. doi: 10.1016/j.plipres.2006.01.004

100. Maceyka M, Spiegel S. Sphingolipid metabolites in inflammatory disease. *Nature.* (2014) 510:58–67. doi: 10.1038/nature13475

101. Merrill AH Jr. De novo sphingolipid biosynthesis: a necessary, but dangerous, pathway. *J Biol Chem.* (2002) 277:25843–6. doi: 10.1074/jbc.R200009200

102. Yunoki K, Renaguli M, Kinoshita M, Matsuyama H, Mawatari S, Fujino T, et al. Dietary sphingolipids ameliorate disorders of lipid metabolism in Zucker fatty rats. *J Agric Food Chem.* (2010) 58:7030–5. doi: 10.1021/jf100722f

103. Yamazaki Y, Kondo K, Maeba R, Nishimukai M, Nezu T, Hara H. The Proportion of nervonic acid in serum lipids is associated with serum plasmalogen levels and metabolic syndrome. *J Oleo Sci.* (2014) 63:527–37. doi: 10.5650/jos.ess13226

104. Dean JM, Lodhi IJ. Structural and functional roles of ether lipids. *Protein Cell.* (2018) 9:196–206. doi: 10.1007/s13238-017-0423-5

105. Kotsopoulos J, Tworoger SS, Campos H, Chung F-L, Clevenger CV, Franke AA, et al. Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies. *Cancer Epidemiol Biomarkers Prev.* (2010) 19:938–46. doi: 10.1158/1055-9965.EPI-09-1318

106. Zhang YB, Chen C, Pan XF, Guo J, Li Y, Franco OH, et al. Associations of healthy lifestyle and socioeconomic status with mortality and incident cardiovascular disease: two prospective cohort studies. *BMJ.* (2021) 373:n604. doi: 10.1136/bmj.n604

107. Rothman KJ. *Modern Epidemiology*. Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins (2008).

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 09 September 2022
DOI 10.3389/fnut.2022.962834

OPEN ACCESS

EDITED BY
Zachary Clayton,
University of Colorado Boulder,
United States

REVIEWED BY
Mee Young Hong,
San Diego State University,
United States
Devin Wahl,
Colorado State University,
United States

*CORRESPONDENCE
Reza Tabrizi
kmsrc89@gmail.com
Reza Homayounfar
r_homayounfar@yahoo.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

RECEIVED 06 June 2022
ACCEPTED 22 August 2022
PUBLISHED 09 September 2022

CITATION
Keshavarz Z, Rahimlou M, Farjam M, Homayounfar R, Khodadost M, Abdollahi A and Tabrizi R (2022) Non-alcoholic fatty liver disease and dairy products consumption: Results from FASA Persian cohort study. *Front. Nutr.* 9:962834. doi: 10.3389/fnut.2022.962834

Non-alcoholic fatty liver disease and dairy products consumption: Results from FASA Persian cohort study

Zahra Keshavarz[1†], Mehran Rahimlou[2], Mojtaba Farjam[1†], Reza Homayounfar[3*], Mahmoud Khodadost[4], Ashkan Abdollahi[5] and Reza Tabrizi[1,6*]

[1]Noncommunicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran, [2]Department of Nutrition, School of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran, [3]National Nutrition and Food Technology Research Institute, Faculty of Nutrition Sciences and Food Technology, Shahid Beheshti University of Medical Sciences, Tehran, Iran, [4]Department of Public Health, School of Health, Larestan University of Medical Sciences, Larestan, Iran, [5]Student Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran, [6]Health Policy Research Center, Institute of Health, Shiraz University of Medical Sciences, Shiraz, Iran

Background/objectives: There are limited data on the association between dairy products consumption and nonalcoholic fatty liver disease (NAFLD). This study was conducted to evaluate the association between total intake of different dairy products and fatty liver index (FLI), a marker of subclinical fatty liver.

Methods: A total of 7,540 adults were included in this population-based cohort study. Dairy products consumption was evaluated by a validated interview questionnaire for food intake frequency. The FLI was calculated using the standard formula. Liver enzyme levels, lipid profiles, glycemic profiles and demographic characteristics were recorded for all participants. Univariate and multiple logistic regression models were used to respectively assess the mean percentage difference of mean FLI and odds ratios (ORs) for subclinical NAFLD across quantiles of dairy consumption.

Results: The mean age of all participants was 48.81 ± 9.631 years. FLI measurements for men and women were 26.71 ± 23.39 and 39.99 ± 26.64 respectively, which was significantly higher in women ($P < 0.05$). Multiple logistic regression analysis demonstrated that the amount of milk consumption was an independent preventive predictor of FLI (OR = 0.96; 95% CI: 0.94–0.99), conversely, it did not predict higher levels of liver enzymes. In term of cheese intake, participants in the third tertile of cheese intake had significantly lower FLI than lower tertiles ($P = 0.01$). However, there wasn't any significant association between cheese intake and the odds of FLI in the multivariate model ($P > 0.05$). We didn't find any significant association between yogurt consumption and NAFLD indicators ($P > 0.05$).

Conclusion: Higher milk consumption was inversely associated with FLI. However, there wasn't any significant association between other types of dairy products and NAFLD indicators.

KEYWORDS

nonalcoholic fatty liver diseases, dairy, yogurt, cheese, cohort

Introduction

Nonalcoholic Fatty Liver Disease (NAFLD) is a clinical histopathological condition in which triglyceride accumulates in the liver cells of patients with little or no history of alcohol consumption (1). This disease usually happens when fat accumulation reaches 5–10% of the liver's weight. NAFLD has a wide histological spectrum ranging from simple steatosis to non-alcoholic steatohepatitis (NASH) and more severe complications like cirrhosis or liver fibrosis (2, 3).

The total Prevalence of NAFLD based on a Meta-analytic study from 22 countries, has been reported as 25.24 % (4). In Asia, the number of patients with NAFLD is gradually increasing (5) due to lifestyle changes (high-fat diet, low physical activity, central obesity, and type 2 diabetes mellitus), which is comparable to Western countries (6).

NAFLD can lead to cirrhosis, cardiovascular disease, type 2 diabetes mellitus, and chronic kidney disease if left untreated (7).

Today, various studies have shown that improving the microbial profile of the intestine plays an important role in the prevention and treatment of NAFLD by reducing oxidative stress and inflammation, as well as decreasing triglycerides (TG) accumulation in the liver (8–12). As a result, the chance of hepatic steatosis will decrease. Dairy products are widely included in daily diet due to their high nutrition contents of protein, fat, minerals, and vitamins (13). Currently, it is recommended to limit the consumption of dairy products due to high levels of saturated fat and cholesterol, in order for reducing the risk of cardiovascular disease. However, a cross-sectional analysis of the Oslo Health Study has shown that increasing the frequency of consumption of some dairy products, such as cheese, is positively associated with improved serum concentrations of high-density lipoprotein (HDL) and is inversely related to serum triglyceride levels (14). Moreover, the consumption of some dairy products, especially those fortified with probiotics, appears to reduce the level of low-density lipoprotein (LDL) cholesterol in human intervention studies (15).

This study aims to focus on the relevance of dairy products consumption with NAFLD and fatty liver index in a large-population cohort in Fasa, Iran.

Abbreviations: NAFLD, Nonalcoholic fatty liver disease; FLI, Fatty liver index; OR, Odds ratio; FFQ, Food frequency questionnaire; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; SBP, systolic blood pressure; DBP, diastolic blood pressure; ANOVA, One-way Analysis of Variance.

Patients and methods

Study population

In a population-based cohort, at least 10,000 people within the age range of 35–70 years old from Sheshdeh, the suburb of Fasa city and its 24 satellite villages were recruited. A detailed demographic, socioeconomic, anthropometric, nutrition, and medical history was obtained for each individual besides limited physical examinations and determination of physical activity and sleep patterns supplemented by body composition and electrocardiographic records (16). Participants older than 35 years with one of the following conditions were included in the study: alanine aminotransferase level >30 U/L, Gamma-glutamyltransferase >35 U/L.

Individuals with evidence of alcohol consumption of more than 20 grams per day, hepatitis, drug-induced liver disease, Wilson's disease, hereditary hemochromatosis, encephalopathy, or variceal bleeding were excluded from the study. Routine laboratory assessments were done, and a comprehensive biobank was compiled for future biological investigations. All data were stored online using a dedicated software.

Measurement of clinical parameters and biochemical analysis

Fatty liver index (FLI) is a standard predictor of hepatic steatosis severity. The following formula was designed and validated by Bogoni et al. for calculating FLI using laboratory and anthropometric data, including gamma glutamyl transferase concentration, triglyceride, Body mass index and waist circumference (17).

$$FLI = (e\ 0.953 \times \log e\ (TG) + 0.139 \times BMI + 0.718 \times \log e\ (GGT) + 0.053 \times WC - 15.745) / (1 + e\ 0.953 \times \log e\ (TG) +$$

0.139 × BMI + 0.718 × loge (GGT) + 0.053 × WC − 15.745) × 100

To calculate the amount of dairy intake, a validated food frequency questionnaire (FFQ) was used. Different dairy products consumption was recorded separately according to the amount of daily, weekly, monthly and annual intake units. The questionnaire included 100 items with a specified serving size, 7 response frequency categories from "almost never eat" to "twice or more per day" for foods, and 8 response frequency categories from "almost never drink" to "Four or more times a day" for beverages. Total daily energy and nutrient intake are calculated based on the latest available table of Iranian food composition. Frequency of consumption of dairy products (milk, yogurt, cheese, doogh, curd and cream and any other dairy products) in the last month was assessed using 7 categories of answers as follows: Almost never, less than once a week, once a week, 2–3 times a week, 4–6 times a week, once a day and twice a day. Detailed information about the design, foods included and the validity of this questionnaire has been reported elsewhere (18). Previous validation studies of this FFQ designed specifically for Iranian adults revealed good correlation between dietary intakes assessed by a similar FFQ and multiple days of 24-h dietary recalls (18, 19).

Statistical analysis

The characteristics of the participants across this study were showed in quartiles of FLI. Continuous variables were presented as mean ± SD and categorical variables as frequency and percentages (%). Data were analyzed using SPSS (version 18; SPSS Inc., Chicago, IL, USA) by running the student's *t*-test and one way ANOVA, and chi-squared tests. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were estimated from univariate and multiple logistic regression for being classified with an undesirable level of FLI, ALT, AST and GGT and increasing quartile groups (Q1–Q4) for intake of dairy products. Multiple logistic regression models using backward elimination method were applied, adjusted for age, energy, physical activity, BMI, vitamin D levels, selenium levels, smoking status, hypertension, hypercholesterolemia, and diabetes mellitus, and for each relevant food items. Statistical tests were two-sided and $P < 0.05$ was considered statistically significant.

Results

Characteristics of study participants by sex and age

Characteristics of the study participants are presented in the Table 1. Data from 7,540 participants (59% female) were included in the final analysis. The mean age of all participants in this study was 48.81 ± 9.631 years. BMI was significantly higher in females as compared with males (26.63 ± 4.79 kg/m^2 vs. 23.27 ± 4.17 kg/m^2; $P < 0.001$). FLI was higher in females than males (39.991 ± 26.64 vs. 26.708 ± 23.39; $P < 0.05$). Also, serum levels of HDL, TC, LDL, FBS, SBP and DBP were significantly higher in females than males ($P < 0.001$). Dietary intakes of study participants across quartile of FLI score are presented in Table 2. A greater FLI score was significantly associated with the higher intake of carbohydrates, fruits, vegetables, meats, beans, dairy, vitamin D and selenium ($P < 0.05$).

Association of fatty liver index with dairy products

Crude and multiple-adjusted models for mean intake of dairy intake across quartiles of FLI are shown in Table 3. After controlling for potential confounders, participants in the highest quartile of FLI didn't have higher odds for dairy products intake [(OR = 1.00; 95% CI: 0.99, 1.00; $P = 0.084$) for males and (OR = 1.00; 95% CI: 0.99, 1.01; $P = 0.306$) for females]. Also, both females and males in the highest quartile of FLI didn't have higher odds for consumption of milk, yogurt, cheese, doogh, curd and cream ($P > 0.05$). We also examined the association between ALT and AST quartiles and odds of dairy consumption. The results showed that individuals in the highest ALT quartile did not have odds of more dairy products consumption. Participants in the highest quartile of AST had higher odds only for cream intake (OR= 0.97; 95% CI: 0.95, 0.99; $P_{trend} = 0.011$). There wasn't any significant association between other dairy products and ALT and AST quartiles ($P > 0.05$).

The results of univariate and multiple analyses for determining independent association between fatty liver index and dairy consumption are shown in the Table 4. The mean FLI in the tertile 3 milk intake was 34.08 (95% CI: 32.99, 35.17) that wasn't significantly different from tertile 1 ($P = 0.171$). Mean liver enzymes did not differ significantly in different milk consumption tertiles ($P = 0.08$ for AST, $P = 0.174$ for ALT, and $P = 0.491$ for GGT).

The association between the amount of dairy consumption and severity of fatty liver disease is shown in Table 4. When controlled for confounding factors, multiple logistic regression analysis demonstrated that the amount of milk consumption was the independent preventive predictor of FLI (OR = 0.96; 95% CI: 0.94, 0.99; $P = 0.025$), but was not the predictors of higher levels of AST, ALT and GGT ($P > 0.05$). For the cheese intake, participants in the tertile 3 had significantly lower FLI than the lowest tertile 35.04 (95% CI: 33.98, 36.10; $P = 0.01$). Individuals in the highest tertile of cheese intake had higher levels of ALT than lowest tertile 18.24 mg/dl (95% CI:

TABLE 1 Baseline characteristics of study participants.

Variables	Total ($n = 7,540$)	Men ($n = 3,048$)	Women ($n = 4,492$)	P-value
Age	48.81 ± 9.631	49.48 ± 9.666	48.36 ± 9.581	<0.001
BMI (kg/m2)	25.27 ± 4.84	23.27 ± 4.17	26.63 ± 4.79	<0.001
Waist circumference (cm)	92.274 ± 11.8734	87.483 ± 10.7930	95.526 ± 11.4660	<0.001
Current smoker (%)	1,985 (26.3 %)	1,770 (58.1 %)	215 (4.8 %)	<0.001
Physical activities (Met-h/day)	41.379 ± 11.078	45.622 ± 14.346	38.499 ± 6.772	<0.001
Total cholesterol (mg/dl)	182.70 ± 37.808	174.03 ± 34.775	188.58 ± 38.645	<0.001
Triglycerides (mg/dl)	122.89 ± 71.818	121.63 ± 72.313	123.74 ± 71.476	0.210
HDL-cholesterol (mg/dl)	51.66 ± 16.026	47.64 ± 14.525	54.39 ± 16.419	<0.001
LDL-cholesterol (mg/dl)	106.41 ± 31.882	101.99 ± 29.820	109.41 ± 32.874	<0.001
Fasting glucose (mg/dl)	90.90 ± 25.815	88.66 ± 21.220	92.42 ± 28.417	<0.001
AST (U/l)	20.20 ± 4.584	21.07 ± 4.633	19.60 ± 4.454	<0.001
ALT (U/l)	18.02 ± 5.385	18.65 ± 5.677	17.59 ± 5.133	<0.001
GGT (U/l)	17.08 ± 7.882	18.58 ± 8.311	16.07 ± 7.408	<0.001
ALP (U/I)	203.27 ± 62.184	205.58 ± 58.175	201.71 ± 64.723	0.008
SBP (mmHg)	110.69 ± 18.655	109.19 ± 17.692	111.70 ± 19.216	<0.001
DBP (mmHg)	74.08 ± 11.916	73.15 ± 11.654	74.72 ± 12.050	<0.001
FLI	34.621 ± 26.202	26.708 ± 23.398	39.991 ± 26.641	<0.001

BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; SBP, systolic blood pressure; DBP, diastolic blood pressure; FLI; fatty liver index.
Data are mean ± standard deviation or as n (%), The data analyzed using Student's t-test or chi-square test.

18.02, 18.45; $P = 0.001$). However, there wasn't any significant association between cheese intake and odds of fatty liver index ($P > 0.05$).

Participants in the highest tertile of yogurt intake had significantly higher FLI scores 36.70 (95% CI: 35.56, 37.85; $P < 0.001$) and AST concentration 20.03 mg/dl (95% CI: 19.84, 20.22; $P = 0.026$). We did not find a significant association between yogurt consumption and fatty liver index or biomarkers ($P > 0.05$).

Discussion

The results of this study clearly indicate that participants with higher FLI score consume more dairy, cheese and yogurt compared to the subjects with lower FLI. Moreover, there is no strong correlation between dairy consumption and fatty liver indicators and only milk consumption is a preventive indicator of FLI. Also, in our study, the consumption of dairy products in women had more protective effects against NAFLD than in men.

The impacts of dairy products on the amounts of liver-derived lipoproteins have been widely studied and discussed previously (20, 21). Several studies have looked at dietary factors, including dairy intake as the key interventional variable on NAFLD-related parameters in NAFLD patients. In some observational studies, researchers have reported an inverse association between low-fat dairy intake and the severity of NAFLD. Ferolla et al. in a cross-sectional study of 96 patients with NAFLD observed an inverse association between low fat dairy intake and NAFLD severity (22). Similar findings were found in another study (23). Conversely, it has been shown that some dietary regiments which contain adequate amounts of dairy products such as DASH (Dietary Approaches to Stop Hypertension) can have a positive effect on reducing the symptoms of NAFLD. Razavi Zade et al. conducted a clinical trial with 3 daily servings with low dairy products to evaluate the effects of DASH diet on the NAFLD severity and showed adherence to DASH eating pattern leads to a significant reduction in serum levels of ALT, AST, and insulin resistance (24). However, part of the positive effects of DASH diet on fatty liver can be attributed to high amounts of fibers and phytochemicals in the DASH diet (25).

In the present study, except for milk and FLI correlation, we did not observe any correlations between dairy products and fatty liver biomarkers. Contrary to our findings, in a 6-week randomized controlled trial, Dugan et al. found a significant reduction in ALT, AST, hepatic steatosis index, and mRNA expression of IL-6 and IL-1β in peripheral blood mononuclear cells (PBMCs), following the consumption of 3 daily servings of low-fat dairy (296 mL 1% milk, 170 g non-fat yogurt, 56.7 g 2% cheese) as compared with isocaloric carbohydrate-based control foods (26). A recent meta-analysis was done on the

TABLE 2 Participant characteristic and dietary intake according to FLI quartile.

	Total FLI, quartiles				
	Quartile 1 ($n = 1,885$)	Quartile 2 ($n = 1,884$)	Quartile 3 ($n = 1886$)	Quartile 4 ($n = 1885$)	*P*-value
FLI	6.395 ± 2.852	19.195 ± 4.799	40.314 ± 7.263	72.569 ± 12.454	<0.001
BMI, kg/m^2	19.999 ± 2.211	23.630 ± 2.135	26.435 ± 2.225	31.042 ± 3.783	<0.001
Age	47.49 ± 9.469	48.94 ± 9.948	49.16 ± 9.812	49.65 ± 9.147	<0.001
Smoking status, %	779 (41.4%)	490 (26.0%)	407 (21.6%)	309 (16.4%)	<0.001
Diabetes, %	81 (4.3%)	173 (9.2%)	237 (12.6%)	339 (18.0%)	<0.001
Hypertension, %	147 (7.8%)	301 (16.0%)	410 (21.7%)	651 (34.5%)	<0.001
Hypercholesterolemia, %	153 (8.1%)	249 (13.2%)	327 (17.3%)	455 (24.1%)	<0.001
physical activity (Met-h/day)	44.523 ± 12.922	42.266 ± 11.873	40.320 ± 9.875	38.407 ± 8.059	<0.001
Energy, kcal	2,949.230 ± 1,159.784	2,976.097 ± 1,114.890	2,886.361 ± 1,121.060	2,909.666 ± 1,145.891	0.071
Protein, g	94.558 ± 40.842	92.953 ± 37.827	92.357 ± 38.295	92.013 ± 38.633	0.191
Carbohydrate, g	501.27 ± 204.83	490.19 ± 197.97	491.97 ± 206.10	470.85 ± 188.48	<0.001
Fruit, g/d	353.096 ± 316.174	379.641 ± 302.302	399.193 ± 318.931	423.674 ± 335.956	<0.001
Vegetables, g/d	597.414 ± 338.962	636.174 ± 331.090	664.631 ± 350.200	717.518 ± 403.156	<0.001
Grains, g/d	372.769 ± 204.430	381.838 ± 212.351	379.374 ± 211.520	382.936 ± 211.981	0.447
Meats and beans g/d	150.558 ± 91.025	145.492 ± 83.309	140.905 ± 80.312	140.087 ± 83.842	<0.001
Dairy, g/d	201.501 ± 181.891	200.343 ± 162.967	209.012 ± 177.231	223.612 ± 184.515	<0.001
Milk, g/d	41.894 ± 64.497	41.918 ± 63.989	42.078 ± 79.580	41.478 ± 69.028	0.994
Cheese, g/d	18.448 ± 19.933	16.865 ± 16.244	17.243 ± 17.721	19.509 ± 21.458	<0.001
Yogurt, g/d	88.754 ± 92.848	89.789 ± 87.997	95.806 ± 94.337	103.108 ± 100.118	<0.001
Calcium, mg	1,417.222 ± 712.958	1,393.980 ± 657.089	1,400.386 ± 671.894	1,398.778 ± 663.375	0.737
Riboflavin, mg	2.453 ± 1.075	2.399 ± 1.002	2.378 ± 0.992	2.393 ± 1.005	0.120
Vitamin D, IUs	1.435 ± 1.178	1.412 ± 1.030	1.348 ± 1.016	1.345 ± 0.975	0.014
Vitamin E, mg	8.388 ± 4.124	8.533 ± 4.096	8.299 ± 3.781	8.484 ± 4.019	0.282
Selenium	152.895 ± 77.523	147.591 ± 73.298	144.941 ± 73.542	142.119 ± 72.438	<0.001

FLI; fatty liver index; BMI, body mass index.
Data are mean ± standard deviation or as n (%), The data analyzed using Student's t-test or chi-square test.

association between food groups and the risk of NAFLD which included three cross sectional studies and one case control study. The cross-sectional studies didn't find a significant correlation between dairy consumption and the likelihood of NAFLD. However, the case–control study showed a positive association between dairy product consumption and the possibility of NAFLD (27).

The diversity in the type of dairy products and their fat percentages may partly account for the difference observed in the results. Most previous studies that have reported significant changes on fatty liver related indicators following dairy consumption have examined the effect of low-fat dairy products, while in our study participants consumed both high-fat and low-fat dairy products. In fact, the diet of Iranian people especially those in rural areas is mostly based on high consumption of high-fat dairy products (28). Esmaillzadeh et al. studied 486 healthy Iranian women aged 40–60 years and found an inverse association between low fat dairy intake and C-reactive protein, IL-6 and soluble vascular cell adhesion molecule (29). Moreover, they reported an increase in serum amyloid A and soluble vascular cell adhesion molecule-1 among women with high fat dairy consumption (30). High-fat dairy products contain high levels of saturated fatty acids which play a key role in dyslipidemia, insulin resistance and inflammation, that altogether are major risk factors for NAFLD (31).

In contrast with our findings, Zhang et al. in a cross-sectional study of 24,389 adults found that participants with yogurt consumption more than 4 times per weeks had lower likelihood for NAFLD (32). Other similar studies have shown a preventative role for yogurt consumption in the development of other chronic diseases such as cardiovascular disease and type 2 diabetes (29, 33). Most studies that have shown the positive effect of yogurt consumption on liver enzymes have used a variety of probiotic yogurts (9, 34).

In our study, participants in the higher FLI quartile consumed significantly more yogurt than subjects in the lower quartile of FLI. The present study was a cross-sectional study and due to the nature of cross-sectional studies it is not possible to identify the causal relationship between two variables. However, it seems that this difference might be attributed to the fact

TABLE 3 Odds ratio (95% CI) from multiple logistic regression by dairy food type.

FLI	Sex	Crude model					Adjusted model[a]				
		Q1 ($M = 1{,}112$, $W = 773$)	Q2 ($M = 818$, $W = 1{,}066$)	Q3 ($M = 649$, $W = 1{,}237$)	Q4 ($M = 469$, $W = 1{,}416$)	*P*-trend	Q1 ($M = 1{,}112$, $W = 773$)	Q2 ($M = 818$, $W = 1{,}066$)	Q3 ($M = 649$, $W = 1{,}237$)	Q4 ($M = 469$, $W = 1{,}416$)	*P*-trend
Dairy products	M	1 (Ref)	1.00 (0.99–1.00) $P = 0.611$	1.00 (1.00–1.00) $P = 0.501$	1.00 (1.00–1.00) $P = 0.084$	0.201	1 (Ref)	1.00 (0.99–1.00) $P = 0.350$	1.00 (0.99–1.00) $P = 0.686$	1.00 (0.99–1.00) $P = 0.701$	0.811
	W	1 (Ref)	1.00 (1.00–1.00) $P = 0.036$	1.00 (1.00–1.00) $P = 0.000$	1.00 (1.00–1.00) $P = 0.000$	<0.001	1 (Ref)	1.00 (1.00–1.00) $P = 0.291$	1.00 (0.99–1.00) $P = 0.447$	1.00 (0.99–1.01) $P = 0.306$	0.671
Milk	M	1 (Ref)	1.00 (0.99–1.00) $P = 0.855$	1.00 (0.99–1.00) $P = 0.896$	0.99 (0.99–1.00) $P = 0.316$	0.777	1 (Ref)	1.00 (0.99–1.00) $P = 0.953$	1.00 (0.99–1.00) $P = 0.813$	0.99 (0.99–1.00) $P = 0.188$	0.404
	W	1 (Ref)	1.00 (0.99–1.00) $P = 0.313$	1.00 (1.00–1.00) $P = 0.192$	1.00 (1.00–1.00) $P = 0.113$	0.421	1 (Ref)	1.00 (1.00–1.01) $P = 0.119$	1.00 (0.99–1.00) $P = 0.263$	1.00 (0.99–1.01) $P = 0.346$	0.452
Cheese	M	1 (Ref)	0.99 (0.99–0.99) $P = 0.030$	0.99 (0.98–0.99) $P = 0.028$	1.00 (0.99–1.01) $P = 0.318$	0.006	1 (Ref)	0.99 (0.99–1.01) $P = 0.291$	0.99 (0.98–1.01) $P = 0.215$	1.01 (0.99–1.02) $P = 0.274$	0.224
	W	1 (Ref)	1.00 (0.99–1.01) $P = 0.991$	1.00 (0.99–1.01) $P = 0.166$	1.01 (1.00–1.02) $P = 0.000$	<0.001	1 (Ref)	0.99 (0.98–1.01) $P = 0.568$	1.00 (0.99–1.01) $P = 0.972$	1.01 (0.99–1.02) $P = 0.310$	0.126
Yogurt	M	1 (Ref)	1.00 (0.99–1.00) $P = 0.603$	1.00 (1.00–1.00) $P = 0.057$	1.00 (1.00–1.00) $P = 0.012$	0.047	1 (Ref)	1.00 (0.99–1.00) $P = 0.593$	1.00 (0.99–1.00) $P = 0.991$	1.00 (0.99–1.00) $P = 0.861$	0.890
	W	1 (Ref)	1.00 (1.00–1.00) $P = 0.182$	1.00 (1.00–1.00) $P = 0.001$	1.00 (1.00–1.01) $P = 0.000$	<0.001	1 (Ref)	0.99 (0.99–1.00) $P = 0.542$	0.99 (0.99–1.00) $P = 0.498$	1.00 (0.99–1.00) $P = 0.838$	0.798
Dough	M	1 (Ref)	1.00 (0.99–1.00) $P = 0.437$	1.00 (0.99–1.00) $P = 0.636$	1.00 (1.00–1.00) $P = 0.139$	0.229	1 (Ref)	0.99 (0.99–1.00) $P = 0.321$	1.00 (0.99–1.00) $P = 0.937$	1.00 (0.99–1.00) $P = 0.972$	0.667
	W	1 (Ref)	1.00 (1.00–1.00) $P = 0.182$	1.00 (1.00–1.00) $P = 0.001$	1.00 (1.00–1.01) $P = 0.000$	<0.001	1 (Ref)	1.00 (1.00–1.01) $P = 0.088$	1.00 (0.99–1.00) $P = 0.144$	1.00 (0.99–1.00) $P = 0.142$	0.384
Curd	M	1 (Ref)	0.98 (0.96–1.00) $P = 0.089$	0.98 (0.96–1.01) $P = 0.129$	1.01 (0.98–1.03) $P = 0.404$	0.058	1 (Ref)	0.97 (0.95–1.01) $P = 0.146$	0.98 (0.94–1.02) $P = 0.389$	0.99 (0.95–1.04) $P = 0.878$	0.391
	W	1 (Ref)	0.99 (0.97–1.03) $P = 0.447$	0.99 (0.97–1.01) $P = 0.461$	1.00 (0.98–1.02) $P = 0.985$	0.710	1 (Ref)	0.98 (0.95–1.01) $P = 0.295$	0.99 (0.95–1.03) $P = 0.492$	0.95 (0.89–1.01) $P = 0.101$	0.262
Cream	M	1 (Ref)	0.97 (0.95–0.99) $P = 0.042$	0.98 (0.96–1.01) $P = 0.125$	0.96 (0.93–0.99) $P = 0.022$	0.029	1 (Ref)	1.01 (0.96–1.06) $P = 0.693$	1.02 (0.97–1.07) $P = 0.484$	1.02 (0.97–1.08) $P = 0.423$	0.791
	W	1 (Ref)	1.00 (0.98–1.03) $P = 0.754$	1.01 (0.98–1.03) $P = 0.682$	0.99 (0.97–1.02) $P = 0.932$	0.925	1 (Ref)	0.99 (0.96–1.02) $P = 0.592$	0.98 (0.94–1.03) $P = 0.460$	0.99 (0.95–1.04) $P = 0.962$	0.622

(Continued)

10.3389/fnut.2022.962834

TABLE 3 (Continued)

ALT	Sex	Crude model					Adjusted model[a]				
		Q1 ($M = 699$, $W = 1,230$)	Q2 ($M = 689$, $W = 1,200$)	Q3 ($M = 737$, $W = 1,106$)	Q4 ($M = 923$, $W = 956$)	*P*-trend	Q1 ($M = 699$, $W = 1,230$)	Q2 ($M = 689$, $W = 1,200$)	Q3 ($M = 737$, $W = 1,106$)	Q4 ($M = 923$, $W = 956$)	*P*-trend
Dairy products	M	1 (Ref)	1.00 (1.00–1.00) $P = 0.171$	1.00 (1.00–1.00) $P = 0.632$	1.00 (1.00–1.00) $P = 0.002$	0.006	1 (Ref)	1.00 (0.99–1.00) $P = 0.873$	1.00 (0.99–1.00) $P = 0.153$	1.00 (1.00–1.00) $P = 0.335$	0.063
	W	1 (Ref)	1.00 (1.00–1.00) $P = 0.436$	1.00 (1.00–1.00) $P = 0.216$	1.00 (1.00–1.00) $P = 0.178$	0.514	1 (Ref)	1.00 (0.99–1.00) $P = 0.847$	1.00 (0.99–1.00) $P = 0.665$	1.00 (0.99–1.00) $P = 0.729$	0.975
Milk	M	1 (Ref)	1.00 (0.99–1.00) $P = 0.639$	0.99 (0.99–1.00) $P = 0.407$	1.00 (0.99–1.00) $P = 0.397$	0.318	1 (Ref)	0.99 (0.99–1.00) $P = 0.134$	0.99 (0.99–1.00) $P = 0.054$	1.00 (0.99–1.00) $P = 0.703$	0.107
	W	1 (Ref)	1.00 (0.99–1.00) $P = 0.784$	1.00 (0.99–1.00) $P = 0.934$	1.00 (0.99–1.00) $P = 0.293$	0.669	1 (Ref)	1.00 (0.99–1.00) $P = 0.971$	1.00 (0.99–1.00) $P = 0.551$	1.00 (0.99–1.00) $P = 0.488$	0.641
Cheese	M	1 (Ref)	1.00 (0.99–1.01) $P = 0.099$	0.99 (0.99–1.01) $P = 0.775$	1.01 (1.00–1.01) $P = 0.034$	0.030	1 (Ref)	1.00 (0.99–1.01) $P = 0.183$	0.99 (0.99–1.00) $P = 0.417$	1.00 (0.99–1.01) $P = 0.164$	0.053
	W	1 (Ref)	0.99 (0.99–1.00) $P = 0.576$	1.00 (0.99–1.00) $P = 0.697$	1.01 (1.00–1.01) $P = 0.035$	0.052	1 (Ref)	0.99 (0.99–1.00) $P = 0.554$	1.00 (0.99–1.01) $P = 0.948$	1.00 (0.99–1.01) $P = 0.086$	0.122
Yogurt	M	1 (Ref)	1.00 (0.99–1.00) $P = 0.591$	1.00 (0.99–1.00) $P = 0.747$	1.00 (1.00–1.00) $P = 0.068$	0.236	1 (Ref)	1.00 (0.99–1.01) $P = 0.518$	0.99 (0.99–1.00) $P = 0.234$	1.00 (0.99–1.00) $P = 0.982$	0.489
	W	1 (Ref)	1.00 (1.00–1.00) $P = 0.236$	1.00 (1.00–1.00) $P = 0.173$	1.00 (0.99–1.00) $P = 0.847$	0.438	1 (Ref)	1.00 (0.99–1.00) $P = 0.815$	1.00 (0.99–1.00) $P = 0.811$	0.99 (0.99–1.00) $P = 0.139$	0.324
Dough	M	1 (Ref)	1.00 (1.00–1.01) $P = 0.005$	1.00 (1.00–1.01) $P = 0.035$	1.00 (1.00–1.01) $P = 0.000$	0.001	1 (Ref)	1.00 (1.00–1.01) $P = 0.135$	1.00 (0.99–1.00) $P = 0.529$	1.00 (1.00–1.01) $P = 0.098$	0.077
	W	1 (Ref)	1.00 (0.99–1.00) $P = 0.789$	1.00 (1.00–1.00) $P = 0.240$	1.00 (1.00–1.00) $P = 0.241$	0.522	1 (Ref)	1.00 (0.99–1.00) $P = 0.657$	1.00 (0.99–1.00) $P = 0.981$	1.00 (0.99–1.00) $P = 0.916$	0.968
Curd	M	1 (Ref)	0.99 (0.97–1.01) $P = 0.694$	0.99 (0.97–1.02) $P = 0.538$	1.01 (0.98–1.02) $P = 0.475$	0.508	1 (Ref)	0.99 (0.97–1.02) $P = 0.480$	0.98 (0.96–1.01) $P = 0.352$	1.00 (0.98–1.02) $P = 0.769$	0.481
	W	1 (Ref)	0.99 (0.97–1.01) $P = 0.334$	0.99 (0.98–1.01) $P = 0.613$	1.00 (0.98–1.02) $P = 0.952$	0.723	1 (Ref)	0.98 (0.97–1.01) $P = 0.220$	0.99 (0.97–1.01) $P = 0.331$	0.99 (0.97–1.02) $P = 0.770$	0.579
Cream	M	1 (Ref)	0.98 (0.96–1.01) $P = 0.254$	0.98 (0.96–1.01) $P = 0.190$	0.99 (0.96–1.01) $P = 0.267$	0.522	1 (Ref)	0.98 (0.96–1.01) $P = 0.322$	0.98 (0.96–1.01) $P = 0.252$	0.99 (0.97–1.01) $P = 0.436$	0.657
	W	1 (Ref)	1.01 (0.98–1.02) $P = 0.570$	0.99 (0.97–1.02) $P = 0.945$	1.00 (0.98–1.02) $P = 0.906$	0.912	1 (Ref)	1.01 (0.98–1.03) $P = 0.464$	1.00 (0.97–1.02) $P = 0.872$	1.01 (0.98–1.03) $P = 0.580$	0.846

(Continued)

10.3389/fnut.2022.962834

TABLE 3 (Continued)

AST	Sex	Crude model					Adjusted model[a]				
		Q1 ($M = 551$, $W = 1,354$)	Q2 ($M = 737$, $W = 1,191$)	Q3 ($M = 820$, $W = 1,039$)	Q4 ($M = 940$, $W = 908$)	*P*-trend	Q1 ($M = 551$, $W = 1,354$)	Q2 ($M = 737$, $W = 1,191$)	Q3 ($M = 820$, $W = 1,039$)	Q4 ($M = 940$, $W = 908$)	*P*-trend
Dairy products	M	1 (Ref)	1.00 (0.99–1.00) $P = 0.439$	1.00 (0.99–1.00) $P = 0.633$	1.00 (1.00–1.00) $P = 0.318$	0.191	1 (Ref)	1.00 (0.99–1.00) $P = 0.273$	1.00 (0.99–1.00) $P = 0.163$	1.00 (0.99–1.00) $P = 0.985$	0.280
	W	1 (Ref)	1.00 (0.99–1.00) $P = 0.159$	1.00 (0.99–1.00) $P = 0.520$	1.00 (0.99–1.00) $P = 0.132$	0.376	1 (Ref)	1.00 (0.99–1.00) $P = 0.215$	1.00 (0.99–1.00) $P = 0.289$	1.00 (0.99–1.00) $P = 0.097$	0.366
Milk	M	1 (Ref)	0.99 (0.99–1.00) $P = 0.182$	1.00 (0.99–1.00) $P = 0.631$	1.00 (0.99–1.00) $P = 0.587$	0.199	1 (Ref)	0.99 (0.99–1.00) $P = 0.137$	0.99 (0.99–1.00) $P = 0.287$	1.00 (0.99–1.00) $P = 0.866$	0.323
	W	1 (Ref)	1.00 (0.99–1.00) $P = 0.658$	1.00 (0.99–1.00) $P = 0.558$	1.00 (0.99–1.00) $P = 0.937$	0.803	1 (Ref)	1.00 (0.99–1.00) $P = 0.708$	1.00 (0.99–1.00) $P = 0.712$	1.00 (0.99–1.00) $P = 0.831$	0.897
Cheese	M	1 (Ref)	1.00 (0.99–1.01) $P = 0.621$	0.99 (0.99–1.00) $P = 0.761$	1.00 (0.99–1.01) $P = 0.239$	0.362	1 (Ref)	1.00 (0.99–1.01) $P = 0.747$	0.99 (0.99–1.00) $P = 0.511$	1.00 (0.99–1.01) $P = 0.484$	0.450
	W	1 (Ref)	0.99 (0.99–1.00) $P = 0.099$	0.99 (0.99–1.00) $P = 0.687$	0.99 (0.99–1.00) $P = 0.104$	0.225	1 (Ref)	0.99 (0.99–1.00) $P = 0.215$	1.00 (0.99–1.00) $P = 0.877$	0.99 (0.99–1.01) $P = 0.476$	0.496
Yogurt	M	1 (Ref)	1.00 (0.99–1.00) $P = 0.886$	1.00 (0.99–1.00) $P = 0.417$	1.00 (0.99–1.00) $P = 0.551$	0.454	1 (Ref)	1.00 (0.99–1.00) $P = 0.964$	0.99 (0.99–1.00) $P = 0.141$	1.00 (0.99–1.00) $P = 0.917$	0.297
	W	1 (Ref)	1.00 (0.99–1.00) $P = 0.571$	1.00 (0.99–1.00) $P = 0.772$	0.99 (0.99–1.00) $P = 0.057$	0.180	1 (Ref)	1.00 (0.99–1.00) $P = 0.713$	1.00 (0.99–1.00) $P = 0.584$	0.99 (0.99–1.00) $P = 0.050$	0.199
Dough	M	1 (Ref)	0.99 (0.99–1.00) $P = 0.313$	1.00 (0.99–1.00) $P = 0.498$	1.00 (0.99–1.00) $P = 0.275$	0.109	1 (Ref)	0.99 (0.98–1.00) $P = 0.204$	1.00 (0.99–1.00) $P = 0.963$	1.00 (0.99–1.00) $P = 0.708$	0.296
	W	1 (Ref)	0.99 (0.99–1.00) $P = 0.118$	0.99 (0.99–1.00) $P = 0.128$	1.00 (0.99–1.00) $P = 0.655$	0.294	1 (Ref)	0.99 (0.99–1.00) $P = 0.123$	0.99 (0.99–1.00) $P = 0.055$	1.00 (0.99–1.00) $P = 0.378$	0.188
Curd	M	1 (Ref)	1.00 (0.97–1.02) $P = 0.988$	0.99 (0.96–1.01) $P = 0.422$	0.99 (0.97–1.01) $P = 0.556$	0.784	1 (Ref)	0.99 (0.97–1.02) $P = 0.876$	0.98 (0.96–1.01) $P = 0.277$	0.99 (0.96–1.01) $P = 0.375$	0.612
	W	1 (Ref)	0.99 (0.98–1.01) $P = 0.756$	0.98 (0.96–1.00) $P = 0.159$	1.00 (0.98–1.02) $P = 0.984$	0.480	1 (Ref)	0.99 (0.98–1.01) $P = 0.744$	0.98 (0.95–1.00) $P = 0.057$	0.99 (0.97–1.01) $P = 0.576$	0.236
Cream	M	1 (Ref)	0.98 (0.97–1.01) $P = 0.329$	0.96 (0.93–0.98) $P = 0.005$	0.97 (0.95–0.99) $P = 0.031$	0.017	1 (Ref)	0.98 (0.96–1.01) $P = 0.293$	0.96 (0.94–0.98) $P = 0.004$	0.97 (0.95–0.99) $P = 0.020$	0.011
	W	1 (Ref)	1.01 (0.98–1.03) $P = 0.372$	1.01 (0.98–1.02) $P = 0.960$	1.01 (0.98–1.03) $P = 0.602$	0.813	1 (Ref)	1.01 (0.98–1.04) $P = 0.312$	1.01 (0.98–1.03) $P = 0.551$	1.01 (0.98–1.03) $P = 0.472$	0.726

W, women; M, men.

Odds ratio (95% CI) are from multiple logistic regression for being classified with an undesirable level of FLI, ALT, AST and GGT and increasing quintile groups (Q1–Q4) for intake of dairy products. Q1, lowest intake, is the reference category. Statistically significant p-values are in supers.

[a]**Multivariable-adjusted model adjusted** for age (years), energy (kcals/day), physical activity (minutes/week), BMI (kg/m2), Vitamin D levels, Selenium levels, smoking status (yes, no), hypertension (yes, no), hypercholesterolemia (yes, no), and diabetes (yes, no), and for each relevant food items.

10.3389/fnut.2022.962834

TABLE 4 Univariate and multiple analyses to determine the independent association between fatty liver index and dairy consumption.

	Milk			
	Tertile 1 ($n = 2,952$)	**Tertile 2 ($n = 2,368$)**	**Tertile 3 ($n = 2,220$)**	
Mean (95% CI)				***P*–value ANOVA**
FLI	35.32 (34.35–36.29)	34.25 (33.23–35.27)	34.08 (32.99–35.17)	0.171
AST	20.07 (19.91–20.24)	20.20 (20.02–20.39)	20.35 (20.16–20.55)	0.088
ALT	17.96 (17.77–18.15)	17.92 (17.71–18.14)	18.20 (17.97–18.42)	0.174
GGT	17.21 (16.92–17.51)	16.97 (16.68–17.25)	17.03 (16.68–17.37)	0.491
OR (95% CI)[a]				
FLI	1 (Ref)	0.99 (0.99–1.00)$^{0.230}$	0.96 (0.94–0.99)$^{0.025}$	
AST	1 (Ref)	1.00 (0.99–1.02)$^{0.608}$	1.00 (0.99–1.02)$^{0.223}$	
ALT	1 (Ref)	0.99 (0.98–1.01)$^{0.529}$	1.00 (0.99–1.01)$^{0.826}$	
GGT	1 (Ref)	0.99 (0.98–1.00)$^{0.260}$	0.99 (0.99–1.00)$^{0.439}$	
	Cheese			
	Tertile 1 ($n = 3,075$)	**Tertile 2 ($n = 1,996$)**	**Tertile 3 ($n = 2,469$)**	
Mean (95% CI)				***P*–value ANOVA**
FLI	35.55 (32.65–34.46)	35.73 (34.57–36.89)	35.04 (33.98–36.10)	0.010
AST	20.14 (19.98–20.30)	20.22 (20.03–20.42)	20.24 (20.06–20.43)	0.678
ALT	17.75 (17.56–17.94)	18.16 (17.93–18.40)	18.24 (18.02–18.45)	0.001
GGT	16.84 (16.59–17.08)	17.32 (16.96–17.67)	17.19 (16.85–17.54)	0.071
OR (95% CI)[a]				
FLI	1 (Ref)	1.00 (0.99–1.01)$^{0.137}$	1.00 (0.99–1.01)$^{0.936}$	
AST	1 (Ref)	1.01 (0.99–1.02)$^{0.326}$	1.00 (0.98–1.01)$^{0.850}$	
ALT	1 (Ref)	1.01 (0.99–1.02)$^{0.119}$	1.00 (0.99–1.01)$^{0.373}$	
GGT	1 (Ref)	1.01 (0.99–1.01)$^{0.110}$	1.00 (0.99–1.01)$^{0.598}$	
	Yogurt			
	Tertile 1 ($n = 2,514$)	**Tertile 2 ($n = 2,938$)**	**Tertile 3 ($n = 2,088$)**	
Mean (95% CI)				***P*–value ANOVA**
FLI	32.73 (31.71–33.74)	34.75 (33.81–35.69)	36.70 (35.56–37.85)	0.000
AST	20.13 (19.96–20.31)	20.37 (20.20–20.54)	20.03 (19.84–20.22)	0.026
ALT	17.84 (17.63–18.06)	18.10 (17.90–18.30)	18.11 (17.88–18.34)	0.143
GGT	16.86 (16.58–17.13)	17.05 (16.75–17.35)	17.39 (17.03–17.74)	0.073
OR (95% CI)[a]				
FLI	1 (Ref)	1.00 (0.99–1.00)$^{0.577}$	1.00 (0.99–1.01)$^{0.879}$	
AST	1 (Ref)	1.01 (0.99–1.02)$^{0.201}$	0.98 (0.97–1.00)$^{0.073}$	
ALT	1 (Ref)	0.99 (0.98–1.01)$^{0.815}$	0.99 (0.97–1.00)$^{0.099}$	
GGT	1 (Ref)	0.96 (1.00–0.99)$^{0.964}$	1.00 (0.99–1.01)$^{0.523}$	

[a]**Multivariable-adjusted model adjusted** for sex, age (years), energy (kcals/day), physical activity (minutes/week), BMI (kg/m2), Vitamin D levels, Selenium levels, smoking status (yes, no), hypertension (yes, no), hypercholesterolemia (yes, no), and diabetes (yes, no), and for each relevant food items.

that people who are at risk of NAFLD increase their yogurt consumption to get more probiotics because of the positive role of bacteria in yogurt against NAFLD, especially probiotic yogurt. The positive effect of yogurt on fatty liver has been attributed to the presence of various probiotic compounds in yogurt. Animal studies have shown that some bacterial strains, such as Lactobacillus, have the ability to reduce the inflammation caused by high levels of lipopolysaccharide (LPS) and subsequent hepatic toll-like receptor 4 (TLR4) activation in NAFLD patients (35). Additionally, part of the beneficial effects of dairy products, especially low-fat dairy products, might be due to high amounts of nutrients, especially magnesium, potassium, protein and calcium in dairy products, which in turn can increase the whole-body fat oxidation (36, 37).

In the present study, we conducted sex-specific analyses which showed that the consumption of dairy products in women had stronger protective effects against NAFLD than in men. While the exact mechanisms for this difference are unclear, several studies have reported a higher risk of NALFD in men and post-menopausal women than in pre-menopausal women (38, 39). Interestingly, experimental results suggest a protective role for estradiol in hepatic injuries by suppressing lipid accumulation and liver fibrosis (40). The presence of estrone (E1) and 17β-estradiol (E2) in raw whole cow's milk has been demonstrated (41). Dairy products have been estimated to account for up to 60% of estrogens in a German diet (42). Some studies have shown that the estrogen in dairy products may increase the concentration of estrogen in the serum (43).

The results of some studies contradicted with the findings of our study. Lee et al. in a study of 5171 adults showed higher dairy protein intake was significantly and inversely associated with the risk of incident NAFLD in men and women aged ≥50 years (44). They suggested that replacing macronutrients equivalent to carbohydrate intake with dairy protein intake may have contributed to lowering the risk of developing NAFLD (32, 44). It has also been suggested that some of the beneficial effects of dairy products in preventing NAFLD are related to the whey protein found in dairy products. Several human and animal studies have shown that whey protein or dairy products reduce weight and fat mass (45–47). Other mechanisms have been suggested for the beneficial effects of dairy products against NAFLD, some of which are related to insulin resistance. The population-based prospective Coronary Artery Risk Development in Young Adults study found an inverse association between frequency of dairy intake and development of insulin resistance syndrome (48). Also, a meta-analysis study showed that the consumption of dairy products, especially low-fat dairy products had a beneficial effect on Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), waist circumference, and body weight (49).

This study has several important advantages including the large sample size, and extensive information on lifestyle and dietary factors, which allowed us to control for many potential confounders. In this study, we adjusted the results for several variables, especially BMI which is a crude measure of body fat and is associated with NAFLD and may negatively affect the accuracy of the results (32). On the other hand, the present study was not free of limitations. Due to the structure of the questionnaires used, we were not able to examine low-fat and high-fat dairy products separately. Also, because of the nature of questionnaires filled in interviews, there is always possibility of bias which can affect the accuracy of the results. Another limitation of the current study was the presence of some potential confounding factors such as diabetes, hyperlipidemia, and high blood pressure and their variability between FLI quartile. However, in the adjusted models, we adjusted the effects of these confounding factors. Finally, because of the difficulties in budget management and the high sample size, we were not able to assess the liver through imaging studies. Evaluation of hepatic steatosis and fibrosis could increase the accuracy of the results in future studies.

Conclusion

In summary, this population-based cohort study didn't show any strong association between dairy products consumption and NAFLD indicators. However, a modest inverse correlation was observed between milk consumption and FLI. Therefore, based on the results of this study, consumption of appropriate amounts of milk, especially low-fat types (at least one unit of milk more than 5–6 times a week) can play a positive role in preventing NAFLD. The results of the present study seem to be in line with the existing recommendations for consumption of a healthy dietary pattern to prevent NAFLD.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The study protocol was approved by the Research Council and the Ethics Committee of Fasa University of Medical Sciences (IR.FUMS.REC.1400.085). The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZK, MF, RH, and RT make substantial contributions to conception, design, acquisition of data, analysis and interpretation of data, and drafting of this study. ZK, MR, AA, and MK participate in drafting the article or revising it. MF and RH gives final approval of the version to be submitted and any revised version. MF and RT are the guarantors of this work. All authors have read and approved the manuscript.

Funding

This study was supported by the Deputy of Research and Technology of Fasa University of Medical Sciences, Fasa, Iran (No. 400067).

Acknowledgments

The authors thank all the study participants for their valuable contributions. This paper was derived from a thesis for fulfillment of obtaining a MD degree.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, Mccullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology.* (1999) 116:1413–9. doi: 10.1016/S0016-5085(99)70506-8

2. Organization WH. *Global Status Report on Noncommunicable Diseases 2014.* Geneva: World Health Organization (2014).

3. Rezende AC, Souza LG, Jardim TV, Perillo NB, Araújo YCL, De Souza SG, et al. Is waist-to-height ratio the best predictive indicator of hypertension incidence? A cohort study. *BMC Public Health.* (2018) 18:1–11. doi: 10.1186/s12889-018-5177-3

4. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology.* (2016) 64:73–84. doi: 10.1002/hep.28431

5. Wong S-W, Chan W-K. Epidemiology of non-alcoholic fatty liver disease in Asia. *Indian J Gastroenterol.* (2020) 39:1–8. doi: 10.1007/s12664-020-01018-x

6. Ashtari S, Pourhoseingholi MA, Zali MR. Non-alcohol fatty liver disease in Asia: Prevention and planning. *World J Hepatol.* (2015) 7:1788. doi: 10.4254/wjh.v7.i13.1788

7. Mantovani A, Scorletti E, Mosca A, Alisi A, Byrne CD, Targher G. Complications, morbidity and mortality of nonalcoholic fatty liver disease. *Metabolism.* (2020) 111:154170. doi: 10.1016/j.metabol.2020.154170

8. Kim D-H, Kim H, Jeong D, Kang I-B, Chon J-W, Kim H-S, et al. Kefir alleviates obesity and hepatic steatosis in high-fat diet-fed mice by modulation of gut microbiota and mycobiota: targeted and untargeted community analysis with correlation of biomarkers. *J Nutr Biochem.* (2017) 44:35–43. doi: 10.1016/j.jnutbio.2017.02.014

9. Bakhshimoghaddam F, Shateri K, Sina M, Hashemian M, Alizadeh M. Daily consumption of synbiotic yogurt decreases liver steatosis in patients with nonalcoholic fatty liver disease: a randomized controlled clinical trial. *J Nutr.* (2018) 148:1276–84. doi: 10.1093/jn/nxy088

10. Chen Y, Feng R, Yang X, Dai J, Huang M, Ji X, et al. Yogurt improves insulin resistance and liver fat in obese women with nonalcoholic fatty liver disease and metabolic syndrome: a randomized controlled trial. *Am J Clin Nutr.* (2019) 109:1611–9. doi: 10.1093/ajcn/nqy358

11. Kong CY, Li ZM, Han B, Zhang ZY, Chen HL, Zhang SL, et al. Diet consisting of balanced yogurt, fruit, and vegetables modifies the gut microbiota and protects mice against nonalcoholic fatty liver disease. *Mol Nutr Food Res.* (2019) 63:1900249. doi: 10.1002/mnfr.201900249

12. Egresi A, Drexler D, Hagymási K, Blázovics A, Jakab Z, Kocsis I, et al. The potential role of organic and conventional yoghurt consumption in the treatment of non-alcoholic fatty liver disease. *Orv Hetil.* (2020) 161:1466–74. doi: 10.1556/650.2020.31839

13. Colín-Ramírez E, Rivera-Mancía S, Infante-Vázquez O, Cartas-Rosado R, Vargas-Barrón J, Madero M, et al. Protocol for a prospective longitudinal study of risk factors for hypertension incidence in a Mexico City population: the Tlalpan 2020 cohort. *BMJ Open.* (2017) 7:e016773. doi: 10.1136/bmjopen-2017-016773

14. Høstmark AT, Tomten SE. The Oslo Health Study: cheese intake was negatively associated with the metabolic syndrome. *J Am Coll Nutr.* (2011) 30:182–90. doi: 10.1080/07315724.2011.10719959

15. Guo Z, Liu X, Zhang Q, Shen Z, Tian F, Zhang H, et al. Influence of consumption of probiotics on the plasma lipid profile: a meta-analysis of randomised controlled trials. *Nutr Metabol Cardiovasc Dis.* (2011) 21:844–50. doi: 10.1016/j.numecd.2011.04.008

16. Farjam M, Bahrami H, Bahramali E, Jamshidi J, Askari A, Zakeri H, et al. A cohort study protocol to analyze the predisposing factors to common chronic non-communicable diseases in rural areas: Fasa Cohort Study. *BMC Public Health.* (2016) 16:1–8. doi: 10.1186/s12889-016-3760-z

17. Krauss RM, Winston M, Fletcher BJ, Grundy SM. Obesity: impact on cardiovascular disease. *Circulation.* (1998) 98:1472–6. doi: 10.1161/01.CIR.98.14.1472

18. Keshteli AH, Esmaillzadeh A, Rajaie S, Askari G, Feinle-Bisset C, Adibi P. A dish-based semi-quantitative food frequency questionnaire for assessment of dietary intakes in epidemiologic studies in Iran: design and development. *Int J Prev Med.* (2014) 5:29. Available online at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3915470/

19. Malekshah A, Kimiagar M, Saadatian-Elahi M, Pourshams A, Nouraie M, Goglani G, et al. Validity and reliability of a new food frequency questionnaire compared to 24 h recalls and biochemical measurements: pilot phase of Golestan cohort study of esophageal cancer. *Eur J Clin Nutr.* (2006) 60:971–7. doi: 10.1038/sj.ejcn.1602407

20. Huth PJ, Park KM. Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. *Adv Nutr.* (2012) 3:266–85. doi: 10.3945/an.112.002030

21. Astrup A. Yogurt and dairy product consumption to prevent cardiometabolic diseases: epidemiologic and experimental studies. *Am J Clin Nutr 99 1235s-1242s.* (2014). doi: 10.3945/ajcn.113.073015

22. Ferolla SM, Ferrari TC, Lima ML, Reis TO, Tavares WCJr, Couto OF, et al. Dietary patterns in Brazilian patients with nonalcoholic fatty liver disease: a cross-sectional study. *Clinics.* (2013) 68:11–7. doi: 10.6061/clinics/2013(01)OA03

23. Lei S, Liu ZW, Yun L, Cai G, Zhang H, Song LJ, et al. The prevalence of nonalcoholic fatty liver disease and its association with lifestyle/dietary habits among university faculty and staff in Chengdu. *Biomed Environ Sci.* (2012) 25:383–91. doi: 10.3967/0895-3988.2012.04.002

24. Razavi Zade M, Telkabadi MH, Bahmani F, Salehi B, Farshbaf S, Asemi Z. The effects of DASH diet on weight loss and metabolic status in adults with non-alcoholic fatty liver disease: a randomized clinical trial. *Liver Int.* (2016) 36:563–71. doi: 10.1111/liv.12990

25. Dreher ML. Dietary patterns, foods, nutrients and phytochemicals in non-alcoholic fatty liver disease. In: *Dietary Patterns and Whole Plant Foods in Aging and Disease.* New York, NY: Springer (2018). p. 291–311.

26. Dugan CE, Aguilar D, Park YK, Lee JY, Fernandez ML. Dairy consumption lowers systemic inflammation and liver enzymes in typically low-dairy consumers with clinical characteristics of metabolic syndrome. *J Am Coll Nutr.* (2016) 35:255–61. doi: 10.1080/07315724.2015.1022637

27. He K, Li Y, Guo X, Zhong L, Tang S. Food groups and the likelihood of non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Br J Nutr.* (2020) 124:1–13. doi: 10.1017/S0007114520000914

28. Salehi-Sahlabadi A, Sadat S, Beigrezaei S, Pourmasomi M, Feizi A, Ghiasvand R, et al. Dietary patterns and risk of non-alcoholic fatty liver disease. *BMC Gastroenterol.* (2021) 21:1–12. doi: 10.1186/s12876-021-01612-z

29. Chen M, Sun Q, Giovannucci E, Mozaffarian D, Manson JE, Willett WC, et al. Dairy consumption and risk of type 2 diabetes: 3 cohorts of US adults and an updated meta-analysis. *BMC Med.* (2014) 12:215. doi: 10.1186/s12916-014-0215-1

30. Esmaillzadeh A, Azadbakht L. Dairy consumption and circulating levels of inflammatory markers among Iranian women. *Public Health Nutr.* (2010) 13:1395–402. doi: 10.1017/S1368980009992126

31. Fan JG, Cao HX. Role of diet and nutritional management in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol.* (2013) 28:81–7. doi: 10.1111/jgh.12244

32. Zhang S, Fu J, Zhang Q, Liu L, Lu M, Meng G, et al. Association between habitual yogurt consumption and newly diagnosed non-alcoholic fatty liver disease. *Eur J Clin Nutr.* (2020) 74:491–9. doi: 10.1038/s41430-019-0497-7

33. Buendia JR, Li Y, Hu FB, Cabral HJ, Bradlee ML, Quatromoni PA, et al. Regular yogurt intake and risk of cardiovascular disease among hypertensive adults. *Am J Hypertens.* (2018) 31:557–65. doi: 10.1093/ajh/hpx220

34. Nabavi S, Rafraf M, Somi MH, Homayouni-Rad A, Asghari-Jafarabadi M. Effects of probiotic yogurt consumption on metabolic factors in individuals with nonalcoholic fatty liver disease. *J Dairy Sci.* (2014) 97:7386–93. doi: 10.3168/jds.2014-8500

35. Xue L, He J, Gao N, Lu X, Li M, Wu X, et al. Probiotics may delay the progression of nonalcoholic fatty liver disease by restoring the gut microbiota structure and improving intestinal endotoxemia. *Sci Rep.* (2017) 7:1–13. doi: 10.1038/srep45176

36. Melanson E, Sharp T, Schneider J, Donahoo W, Grunwald G, Hill J. Relation between calcium intake and fat oxidation in adult humans. *Int J Obes.* (2003) 27:196–203. doi: 10.1038/sj.ijo.802202

37. Zemel MB. Proposed role of calcium and dairy food components in weight management and metabolic health. *Phys Sportsmed.* (2009) 37:29–39. doi: 10.3810/psm.2009.06.1707

38. Yang JD, Abdelmalek MF, Pang H, Guy CD, Smith AD, Diehl AM, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. *Hepatology.* (2014) 59:1406–14. doi: 10.1002/hep.26761

39. Lonardo A, Nascimbeni F, Ballestri S, Fairweather D, Win S, Than TA, et al. Sex differences in nonalcoholic fatty liver disease: state of the art and identification of research gaps. *Hepatology.* (2019) 70:1457–69. doi: 10.1002/hep.30626

40. Lee C, Kim J, Jung Y. Potential therapeutic application of estrogen in gender disparity of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Cells.* (2019) 8:1259. doi: 10.3390/cells8101259

41. Abshirini M, Siassi F, Koohdani F, Qorbani M, Golpour-Hamedani S, Khosravi S, et al. Association between dairy consumption and menopausal symptoms: A cross-sectional study among Iranian postmenopausal women. *Int Dairy J.* (2020) 105:104688. doi: 10.1016/j.idairyj.2020.104688

42. Hartmann S, Lacorn M, Steinhart H. Natural occurrence of steroid hormones in food. *Food Chem.* (1998) 62:7–20. doi: 10.1016/S0308-8146(97)00150-7

43. Pape-Zambito D, Roberts R, Kensinger R. Estrone and 17β-estradiol concentrations in pasteurized-homogenized milk and commercial dairy products. *J Dairy Sci.* (2010) 93:2533–40. doi: 10.3168/jds.2009-2947

44. Lee JH, Lee HS, Ahn SB, Kwon YJ. Dairy protein intake is inversely related to development of non-alcoholic fatty liver disease. *Clin Nutr.* (2021) 40:5252–60. doi: 10.1016/j.clnu.2021.08.012

45. Frestedt JL, Zenk JL, Kuskowski MA, Ward LS, Bastian ED. A whey-protein supplement increases fat loss and spares lean muscle in obese subjects: a randomized human clinical study. *Nutr Metab.* (2008) 5:1–7. doi: 10.1186/1743-7075-5-8

46. Eller LK, Reimer RA. Dairy protein attenuates weight gain in obese rats better than whey or casein alone. *Obesity.* (2010) 18:704–11. doi: 10.1038/oby.2009.300

47. Trottier SK, Macpherson RE, Knuth CM, Townsend LK, Peppler WT, Mikhaeil JS, et al. Dairy attenuates weight gain to a similar extent as exercise in rats fed a high-fat, high-sugar diet. *Obesity.* (2017) 25:1707–15. doi: 10.1002/oby.21941

48. Pereira MA, Jacobs Jr DR, Van Horn L, Slattery ML, Kartashov AI, Ludwig DS. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA study. *JAMA.* (2002) 287:2081–9. doi: 10.1001/jama.287.16.2081

49. Sochol KM, Johns TS, Buttar RS, Randhawa L, Sanchez E, Gal M, et al. The effects of dairy intake on insulin resistance: a systematic review and meta-analysis of randomized clinical trials. *Nutrients.* (2019) 11:2237. doi: 10.3390/nu11092237

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 03 November 2022
DOI 10.3389/fnut.2022.1051964

OPEN ACCESS

EDITED BY
Devin Wahl,
Colorado State University,
United States

REVIEWED BY
Elena Franco Robles,
University of Guanajuato, Mexico
Pamela Senesi,
University of Milan, Italy

*CORRESPONDENCE
Quanyang Li
liquanyang@gxu.edu.cn
Yang Zhou
zhouyangtj@sina.com

SPECIALTY SECTION
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

RECEIVED 23 September 2022
ACCEPTED 19 October 2022
PUBLISHED 03 November 2022

CITATION
Zheng W, Li R, Zhou Y, Shi F, Song Y, Liao Y, Zhou F, Zheng X, Lv J and Li Q (2022) Effect of dietary protein content shift on aging in elderly rats by comprehensive quantitative score and metabolomics analysis. *Front. Nutr.* 9:1051964. doi: 10.3389/fnut.2022.1051964

Effect of dietary protein content shift on aging in elderly rats by comprehensive quantitative score and metabolomics analysis

Wenxuan Zheng[1], Ruiding Li[1], Yang Zhou[2]*, Fengcui Shi[1], Yao Song[1], Yanting Liao[1], Fan Zhou[1], Xiaohua Zheng[1], Jingwen Lv[1] and Quanyang Li[1]*

[1]School of Light Industry and Food Engineering, Guangxi University, Nanning, China, [2]Wangdingdi Hospital, Tianjin, China

In the protein nutrition strategy of middle-aged and elderly people, some believe that low protein is good for health, while others believe high protein is good for health. Facing the contradictory situation, the following hypothesis is proposed. There is a process of change from lower to higher ratio of protein nutritional requirements that are good for health in the human body after about 50 years of age, and the age at which the switch occurs is around 65 years of age. Hence, in this study, 50, 25-month-old male rats were randomly divided into five groups: Control (basal diet), LP (low-protein diet with a 30% decrease in protein content compared to the basal diet), HP (high-protein diet with a 30% increase in protein content compared to the basal diet), Model 1 (switched from LP to HP feed at week 4), and Model 2 (switched from LP to HP feed at week 7). After a total of 10 weeks intervention, the liver and serum samples were examined for aging-related indicators, and a newly comprehensive quantitative score was generated using principal component analysis (PCA). The effects of the five protein nutritional modalities were quantified in descending order: Model 1 > HP > LP > Control > Model 2. Furthermore, the differential metabolites in serum and feces were determined by orthogonal partial least squares discriminant analysis, and 15 differential metabolites, significantly associated with protein intake, were identified by Spearman's correlation analysis ($p < 0.05$). Among the fecal metabolites, 10 were positively correlated and 3 were negatively correlated. In the serum, tyrosine and lactate levels were positively correlated, and acetate levels were negatively correlated. MetaboAnalyst analysis identified that the metabolic pathways influenced by protein intake were mainly related to amino acid and carbohydrate metabolism. The results of metabolomic analysis elucidate the mechanisms underlying the preceding effects to some degree. These efforts

not only contribute to a unified protein nutrition strategy but also positively impact the building of a wiser approach to protein nutrition, thereby helping middle-aged and older populations achieve healthy aging.

KEYWORDS

comprehensive quantitative score, metabolomics, aging, dietary patterns, nuclear magnetic resonance

Introduction

The global prevalence of obesity has increased markedly, almost tripling in the past few decades, and it has become increasingly common in the elderly (1). Unhealthy lifestyles, especially poor nutritional intake, are to blame. Nutritional imbalances, especially excess food intake, are associated with many severe aging-related diseases, such as diabetes, cardiovascular disease, Alzheimer's disease, and cancer (2–4). Although dietary restriction may improve obesity in older adults, prolonged dietary restriction is undoubtedly unrealistic because of low adherence. Recent studies have shown that dietary restriction delays aging because of protein restriction rather than total dietary calorie restriction (5–7). Malondialdehyde (MDA), total antioxidant capacity (T-AOC), and interleukin-6 (IL-6) are typical biomarkers that reflect the body's aging status (8). Several randomized clinical trials and animal studies have found that low-protein (LP) diets are well suited to improve the body's metabolic health by increasing T-AOC levels, decreasing MDA levels, and lowering IL-6 concentrations, thus demonstrating the benefits of LP diets in combating oxidative stress and improving inflammation levels (9–11). In addition, protein restriction can lower serum insulin growth factor (IGF-1) and enhance insulin sensitivity. Both animal and human studies have shown that normal glucose metabolism, lower insulin levels, and higher insulin sensitivity can constitute markers of healthy aging and longevity (12). Insulin resistance can promote oxidative stress and inflammation through multiple pathways (13), cause cognitive dysfunction (14), accelerate cardiovascular aging (12), and increase the risk of frailty in older populations (15). Protein restriction can alter epigenetic modifications, and such alterations may have long-term effects on gene expression and organ function (16). In terms of epigenetic mechanisms, protein restriction can improve health by affecting transcription and translation as well as gene expression profile (17). For example, protein restriction can inhibit tumor growth through epigenetic modification and inhibition of the IGF/Akt/mTOR pathway (18), and can mediate effects on stem cell function through epigenetic changes (19). Thus, protein restriction can also influence lifespan by affecting epigenetic programs. Various dietary patterns that favor longevity have distinct features of LP diets. In the Okinawan dietary pattern, the proportion of carbohydrate energy supply is as high as 85%, whereas protein accounts for only 9% (20). Low protein intake is also a characteristic of the dietary longevity pattern of Guangxi, China, which is being studied by our team for many years (21).

However, contradictory phenomena have also been observed. Robert et al. reported that protein malnutrition in the elderly causes sarcopenia (22). Arthur et al. found that protein deficiency in the elderly is associated with sarcopenic obesity and cardiovascular diseases (23). Green et al. found that protein restriction promotes metabolic health in mice, but the specific benefits depend on sex, strain, restriction level, and age (24). These results raise questions about whether the elderly should also be on an LP diet. A growing body of evidence suggests that starting or continuing an LP diet later in life may be flawed for some adults over 65 years of age. Morgan et al. studied the relationship between protein intake and mortality from related diseases through a questionnaire in the United States (25). They suggested that "low protein intake in middle-aged people and high protein intake in older people may be beneficial for health and longevity." In the face of conflicting research results, years of exploration by a combined team led to the following hypothesis: the nutritional protein requirements are not constant after the age of 50, but there is a smooth change from the need for a lower to higher content rates. The switch occurs around the age of 65 years, and the drastic change is detrimental to health.

Some studies have demonstrated that supplementation with a high-protein (HP) diet in protein-malnourished older adults can increase muscle mass (26, 27). However, a short-term switch from an LP to an HP diet may negatively affect older adults with relatively poor self-regulation. Long-term epidemiological studies have shown that HP intake is associated with an increased risk of cardiovascular diseases, diabetes, and mortality (28, 29). The changes in metabolic function, the risk of kidney disease (30), and the risk of age-related diseases such as cancer, associated with abrupt dietary shifts indicate that the dietary patterns of older adults need to be studied more intensively and carefully (31). It is essential to understand the reasons behind the "harmful" and "beneficial" results of recommended LP and

HP diets in different age groups. What are the corresponding changes in the body metabolism? Does the rapid transition from an LP to HP diet cause metabolic disturbances in older adults? Research on these questions has rarely been reported. Therefore, it is necessary to explore the physical changes in middle-aged and elderly populations when changing from an LP to HP diet.

Changes and trajectories in metabolic profiles can provide direct and indirect information to explore alterations in metabolism during dietary interventions (32). Therefore, it is essential to use metabolomic techniques to explore the metabolic impact of changes in protein intake. As a downstream application of systems biology, metabolomics can accurately capture changes in the state of the organism and identify possible signals or biomarkers and has become an essential tool for understanding the mechanisms of organismal health. A growing number of studies have shown that metabolites play crucial roles in physiological and pathological aging. Aging and dietary interventions cause changes in metabolites, and alterations in metabolites and metabolic pathways can help to elucidate the mechanisms of diseases or treatments (33). In addition, changes in multiple metabolites have been associated with oxidative stress and inflammation. Several biomarkers such as polyunsaturated fatty acids, glucose, ornithine, arginine, and lactate are associated with dietary habits, apoptosis, mitochondrial dysfunction, inflammation, lipid metabolism, autophagy, and oxidative stress resistance (34, 35), and thus can serve as biomarkers of aging to some extent to reflect the aging status of individuals. Therefore, analyzing metabolomic profiles of feces and serum can provide comprehensive access to overall metabolic information and a strong foundation to explore the effect of protein intake shifts on aging.

Based on this, we aimed to explain the action pattern of protein nutrition by mimicking the human aging process in rats. Based on the intake of isocaloric diets, the diet of rats was changed from LP to HP nutrition in different groups and at different time points. By characterizing aging-related indicators and analyzing non-targeted fecal and serum metabolomic profiles, we verified the accuracy of the above mentioned hypothesis, explored the physical changes in middle-aged and elderly populations when changing from an LP to HP diet, and provide new ideas and references for developing healthy dietary patterns in middle-aged and elderly populations.

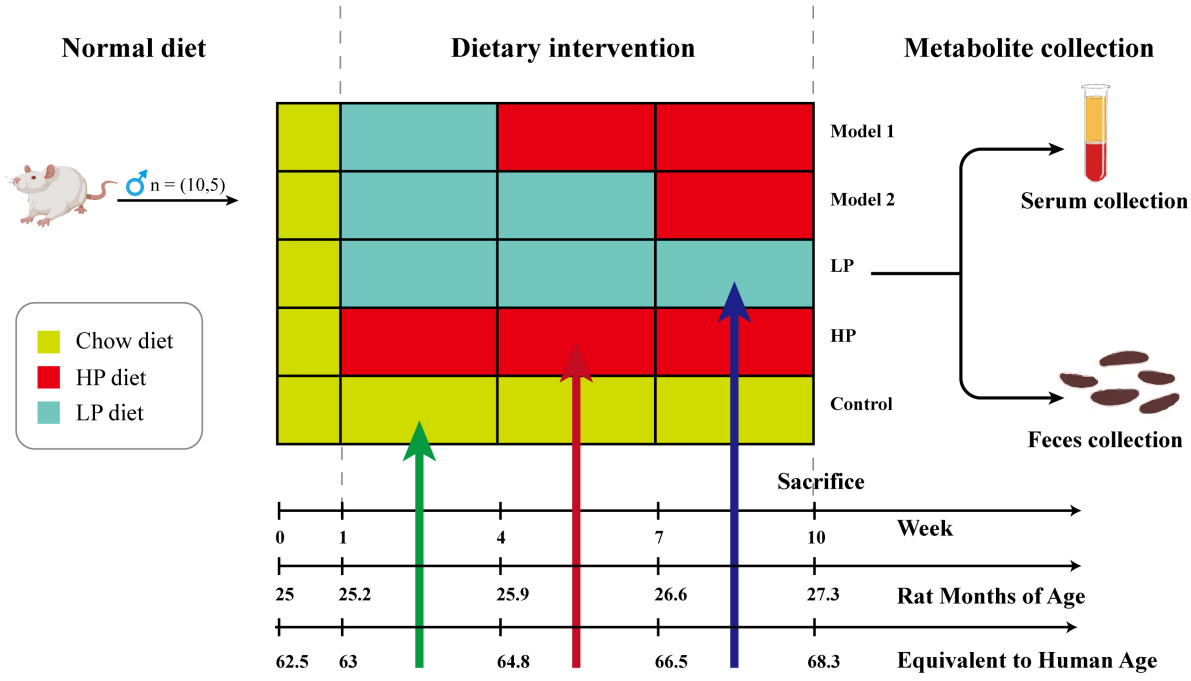
Materials and methods

Animals and experimental design

Fifty, 25-month-old, male SD rats (equivalent to human age 62.5 years) were purchased from Ziyuan Lab Animal Ltd. (Hangzhou, China) (36) (animal certification number SCXK-2019-0004). All animal experiments were conducted in accordance with the Animal Ethics Committee of Guangxi University (Approval No. GXU-2022-248). The rats were housed in a room with constant temperature (24 ± 2°C), maintained at a relative humidity of 60 ± 10%, controlled with a 12-h alternating day/night light/dark cycle, and fed ad libitum with the same food and water during the acclimation period. After 1 week of acclimatization, the rats were randomly divided into five groups and received a 10-week dietary intervention (10 rats per group): control group (basal diet), LP diet group (LP diet with a 30% decrease in protein content compared to the basal diet), HP diet group (HP diet with a 30% increase in protein content compared to the basal diet), Model 1 (switched from LP to HP feed at week 4), and Model 2 (switched from LP to HP feed at week 7). Rat feed was purchased from Keao Feed, Ltd. (Beijing, China). LP and HP diets were made from the standard chow as the base, with corn starch, maltodextrin, casein, L-cystine, sucrose, cellulose, lard, mixed vitamin V10037, mixed mineral S10022G, and hydrocholine bitartrate as the configuration ingredients, respectively. To ensure the same daily calorie intake for each group of rats, the weights of the LP and HP feeds were adjusted according to the ratio of total energy to obtain special feeds with total energy consistent with the standard feed. Body weight was measured weekly during the 10-week treatment by means of the ME10402 electronic balance (METTLER TOLEDO Instruments Co., Shanghai, China). After 10 weeks, rat feces were collected. After testing the open-field experiment, all rats were fasted for 12 h, and then were anesthetized with isoflurane and sacrificed by cervical dislocation, and serum and liver tissues were collected. The experimental design, animal grouping, and composition of the experimental diets are shown in **Figure 1**.

Open-field experiments

Open-field experiments were performed according to literature (37), and the experiments can assess rats' spontaneous exploration, locomotor, and anxiolytic abilities in new environments (38). The experimental setup consisted of a 100 × 100 × 40 cm blue medical ABS plastic box and SuperMaze animal behavior analysis software (XR-Xmaze, Shanghai Xinruan Information Technology Co., Shanghai, China) to monitor the behavioral trajectory of the rats. The open-field experiment was conducted after 10 weeks of dietary intervention and the rats were sacrificed on the day after the open-field experiment. The rats were brought into the experimental site 3 h in advance to adapt to the new environment while maintaining low light illumination and silence in the environment. Each rat was placed in the central area of the open field. The average movement speed, number of central area entries, and number of upright hind limbs of rats in the open field were recorded for 5 min. The data



	Chow diet	HP diet	LP diet
Nutrients	(g/kg diet)		
Protein	196.1	265.15	137.36
Fat	44.8	40.91	31.35
Carbohydrate	553.2	505.63	683.23
Energy (kcal/kg diet)	3400	3451.43	3565.06

FIGURE 1
Schematic diagram showing the experimental design, animal grouping, and composition of the experimental diet.

were analyzed using the SuperMaze software for statistical and trajectory processing.

Quantification of liver tissue malondialdehyde, serum total antioxidant capacity, and interleukin-6

The liver was immediately frozen in liquid nitrogen and stored at −80°C. On the day of the assay, the liver tissue was removed and weighed accurately for the assay. Add 4 times the volume of saline by weight and homogenize mechanically in an ice water bath to produce a liver tissue homogenate. MDA in liver tissue and T-AOC in serum were measured using the MDA assay kit (A003-1-1) and the T-AOC assay kit (A015-1-2), respectively (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China), and serum inflammatory factor IL-6 was measured using a rat interleukin-6 ELISA test kit (JL20896) (Shanghai Jianglai Biotechnology Co., Ltd., Shanghai, China).

Statistical analysis of comprehensive health indicators and quantitative evaluation of health indicators

To comprehensively characterize the effects of protein nutrition on aging of experimental animals, a new determination method termed as the comprehensive quantitative evaluation was established based on the multivariate statistical analysis theory. Principal component analysis (PCA) was used to quantitatively analyze the health status of the rats (39). This study selected five indicators: T-AOC, MDA, IL-6, mean movement speed, and number of upright hind limbs. To eliminate the effect of differences in the data scale of different indicators, the raw data of each indicator were standardized and transformed into dimensionless data. MDA and IL-6 levels, two negative indicators of health status, were normalized, and the control group data were used as a reference to investigate the change in health status. The health statuses of the rats were quantitatively expressed using

comprehensive health evaluation indices. The overall evaluation score (f_{sum}) was calculated using the following equation:

$$f_{sum} = \frac{v_1}{M}\left(\sum_{=1}^{4} a_i X_i\right) + \frac{v_2}{M}\left(\sum_{=1}^{4} b_i X_i\right) + \frac{v_3}{M}\left(\sum_{=1}^{4} c_i X_i\right) + \frac{v_4}{M}\left(\sum_{=1}^{4} b_i X_i\right) \quad (1)$$

where X_i denotes the standardized values of the indicators for each test group, a_i, b_i, c_i, and d_i are the first, second, third, and fourth principal component values for each group, respectively, v_i is the variance explained by the sample under each principal component, and M is the cumulative variance explained by the sample.

Fecal and serum untargeted metabolomics analysis based on ^{1}H-nuclear magnetic resonance

Fecal and serum sample preparation for metabolomic analysis

Feces were collected from each group of rats at week 10 by the massage abdomen method and stored in an ultra-low temperature refrigerator at −80°C. Fifty milligrams of feces were collected in a 2 ml centrifuge tube and 500 μl PBS/D_2O buffer (0.1 M, pH 7.4) containing 10% D_2O (v/v) and 0.005% sodium 3-(trimethylmethylsilyl)propionate (TSP) (w/v) was added. The samples were vortexed and mixed for 15 s, and then freeze-thawed three times repeatedly with liquid nitrogen. The mixture was homogenized for 2 min in a S10 handheld high-speed homogenizer (Ningbo Xinzhi Biotechnology Co., Ningbo, Zhejiang, China), followed by centrifugation (12,000 *g*, 15 min, 4°C). Supernatants were removed and the extraction procedure was repeated. The supernatant obtained after extraction was collected by centrifugation (12,000 *g* for 20 min at 4°C). The resulting supernatant (550 μl) was added to a 5 mm nuclear magnetic resonance (NMR) tube and mixed well for measurement.

The serum samples obtained were thawed on ice, and 200 μl of serum was aspirated into a 2 ml sterile eppendorf tube and 400 μl of PBS/D_2O buffer (0.1 M, pH 7.4) containing 15% D_2O (v/v) was added. The samples were mixed well and centrifuged (12,000 *g*, 20 min, 4°C). The supernatant (550 μl) was slowly aspirated into a 5 mm NMR tube and mixed well for measurement.

^{1}H-nuclear magnetic resonance parameters for metabolomics analysis

Prepared rat fecal and serum samples were placed on a Bruker Avance 500 MHz NMR spectrometer (Bruker, Karlsruhe, Germany) with a Prodigy liquid nitrogen cryogenic probe for NMR mapping. The water peaks were suppressed by pre-saturation with a NOESYPR1D pulse sequence (recycle delay − 90° − t_1 − 90° − t_m − 90° − acquisition) with the following parameters: number of scans, 64; temperature, 25°C; spectral width, 10,000 Hz; measurement frequency, 500.13 MHz; chirality delay, 2.0 s; sampling points, 65,536 points; sampling time, 3.277 s; fixed echo time, 2 ms; number of cycles, 16.

Nuclear magnetic resonance data processing and multivariate statistical analysis

After all tests, data from the fecal and serum samples were imported into MestreNova 14.2 (Mestrelab Research SL, Santiago de Compostela, Spain). An exponential window function with a broadening factor of 0.3 Hz was multiplied before performing the Fourier transform, and the phase was manually adjusted with respect to the baseline. Fecal spectra were calibrated using the methyl resonance at TSP (δ 0.0) as the reference, and serum spectra were calibrated using the methyl resonance at lactate (δ 1.33) as the reference. To eliminate the spurious effect of water suppression, the water peak integral at δ 4.70–5.15 ppm in the fecal spectrum and the water peak integral at δ 4.64–5.10 ppm in the serum spectrum were removed. The region of chemical shift interval δ 0.00–9.00 ppm was integrated into segments with δ 0.002, and the integration results were normalized.

Multivariate statistical analysis of the data was performed using MetaboAnalyst 5.0.[1] PCA was performed to determine the overall distribution of the samples. Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed to identify differences between the groups, and the stability of the model was ensured by cross-validating the parameters Q^2, R^2, and variance analysis of the cross-validated residuals (CV-ANOVA) ($p < 0.05$). Differential metabolites were screened according to variable projective importance (VIP >1) and independent sample *t*-tests ($p < 0.05$). Differential metabolites with Spearman correlation coefficient $|r| > 0.4$ and $p < 0.05$ were considered metabolites correlated with protein intake. The correlated differential metabolites were then subjected to metabolic pathway analysis based on the Kyoto Encyclopedia of Gene and Genomes (KEGG) database with an impact threshold value >0.08 for metabolic pathways. The results with interaction scores >0.4 were selected using Search Tool for Interactions of Chemicals (STITCH) to construct a metabolic pathway network.

Statistical analysis

SPSS26.0 software (SPSS Inc., Chicago, IL, USA) was used to statistically analyze the test data. The results are expressed as mean ± SD. Differences in continuous variables, such as body weight, were analyzed using repeated measures ANOVA followed by Tamhane T2 test after performing sphericity test and normality test to ensure the prerequisites for repeated measures

1 https://www.metaboanalyst.ca/

ANOVA. The Wilcoxon nonparametric test was used to assess differences in metabolites and biochemical indicators before and after the intervention. Spearman's correlation analysis was used to examine the relationship between changes in metabolite and protein intake.

Results

Effect of protein intake shift on behavioral and physiological parameters in rats

After dietary interventions, the physiological conditions of the rats were characterized by examining the following seven parameters: body weight, average movement speed during the open-field experiment, number of upright hind limbs, number of central region entries, liver MDA levels, serum T-AOC levels, and serum inflammatory factor IL-6 levels. The body weight results are shown in **Figure 2A**. The LP diet group lost 7.77% of their body weight over a 9-week period. The HP diet and control groups gained 4.86 and 6.67% of body weight, respectively. The rats in both Model 1 and Model 2 groups regained weight after switching the diet pattern. A 3.76% weight gain was observed in Model 1, and a 3.75% weight gain was observed in Model 2 within 6 weeks after switching the diet. Significant differences were found using repeated measures ANOVA between the LP group and Model 2 and that between HP and control groups ($p < 0.001$). The results indicated that the LP diet effectively reduced the body weight of the aged rats.

The results of the open-field experiment are shown in **Figures 2B–D**. Unlike the LP group, the HP group showed significant increases of 47.74 and 226.39% in the mean movement speed (**Figure 2B**) and the number of upright hind limbs (**Figure 2C**), respectively ($p < 0.05$). In contrast, although there was an increase in the Model 1 group, the difference was insignificant. In addition, the number of upright hind limbs and the number of passes through the center of the open field (**Figure 2D**) could also indicate the anxiety levels of the experimental animals. The higher the number of upright hind limbs and the higher the number of passes through the open field center, the lower were their anxiety. Although the number of times the rats passed through the center of the open field did not vary significantly among the groups ($p > 0.05$), it was evident that the members of the LP group crossed the center of the open field least, reflecting a higher level of anxiety. Overall, the LP diet increased the anxiety levels of the aging rats, while the HP diet reversed the high anxiety level of rats that were earlier on the LP diet. This also suggests that an LP diet that does not consider the demand pattern is detrimental to the health of the organism.

Malondialdehyde levels in the liver tissue (**Figure 2E**) were significantly decreased by 46.55% in the LP diet group compared to that in HP diet group ($p = 0.003$) and by 30.18 and 18.32% compared to those in the Model 1 and Model 2 groups, respectively, but the differences were not significant. Serum T-AOC levels (**Figure 2F**) were significantly higher by 186.78% ($p = 0.001$), and serum IL-6 levels (**Figure 2G**) were significantly lower by 35.77% ($p = 0.003$) in the LP group than in the HP diet group. Compared with HP diet group, T-AOC levels increased by 94.3 and 80.8%, and IL-6 levels decreased by 22.02 and 13.88% in Model 1 and Model 2, respectively, with no significant differences.

To comprehensively characterize the effects of protein nutrition on aging of experimental animals, the following five indices were selected: average movement speed, number of upright hind limbs, liver MDA levels, serum T-AOC levels, and serum inflammatory factor IL-6 levels. The above indicators were first scored comprehensively and quantitatively using PCA, and four principal components were extracted according to a cumulative variance contribution rate greater than 85%. The contribution of each variable and the factor-loading matrix are shown in **Figure 2H**, and the cumulative variance contribution rate reached 98.73%. The standardized indicators and factor loading matrix were substituted into Equation 1, and the violin plot of the obtained results is shown in **Figure 2I**. The results showed that Model 1 had the highest combined quantitative score, followed by the HP and LP groups, while Model 2 had the lowest combined quantitative score. Compared with total LP and HP diets, the switch from an LP to an HP diet produced two extreme divisions. Model 1 with the highest score, indicates that the switch from LP to HP nutrition can help healthy aging in test rats after a certain period of adaptation. Model 2 with the lowest score, indicates that a sudden switch from LP to HP nutrition can negatively impact on the health of the body in a short period (3 weeks).

Nuclear magnetic resonance metabolic profile of rat feces and serum

The fecal and serum metabolites of rats were identified based on the chemical shifts and signal diversity of each metabolite, combined with coupling information from the public database BMRB[2] and 2D NMR profiles from authentic literature (32, 40–42). Forty-three metabolites were detected in rat feces (**Figure 3A**), and forty were detected in rat serum (**Figure 3B**). The assignments of major metabolites from fecal and serum samples of rats are shown in **Supplementary Tables 1**, **2**. These metabolites include amino acids (e.g., tyrosine and valine), carbohydrates (e.g., glucose and xylose), intermediates of the TCA cycle (e.g., citrate, fumarate, and succinate),

2 http://www.bmrb.wisc.edu

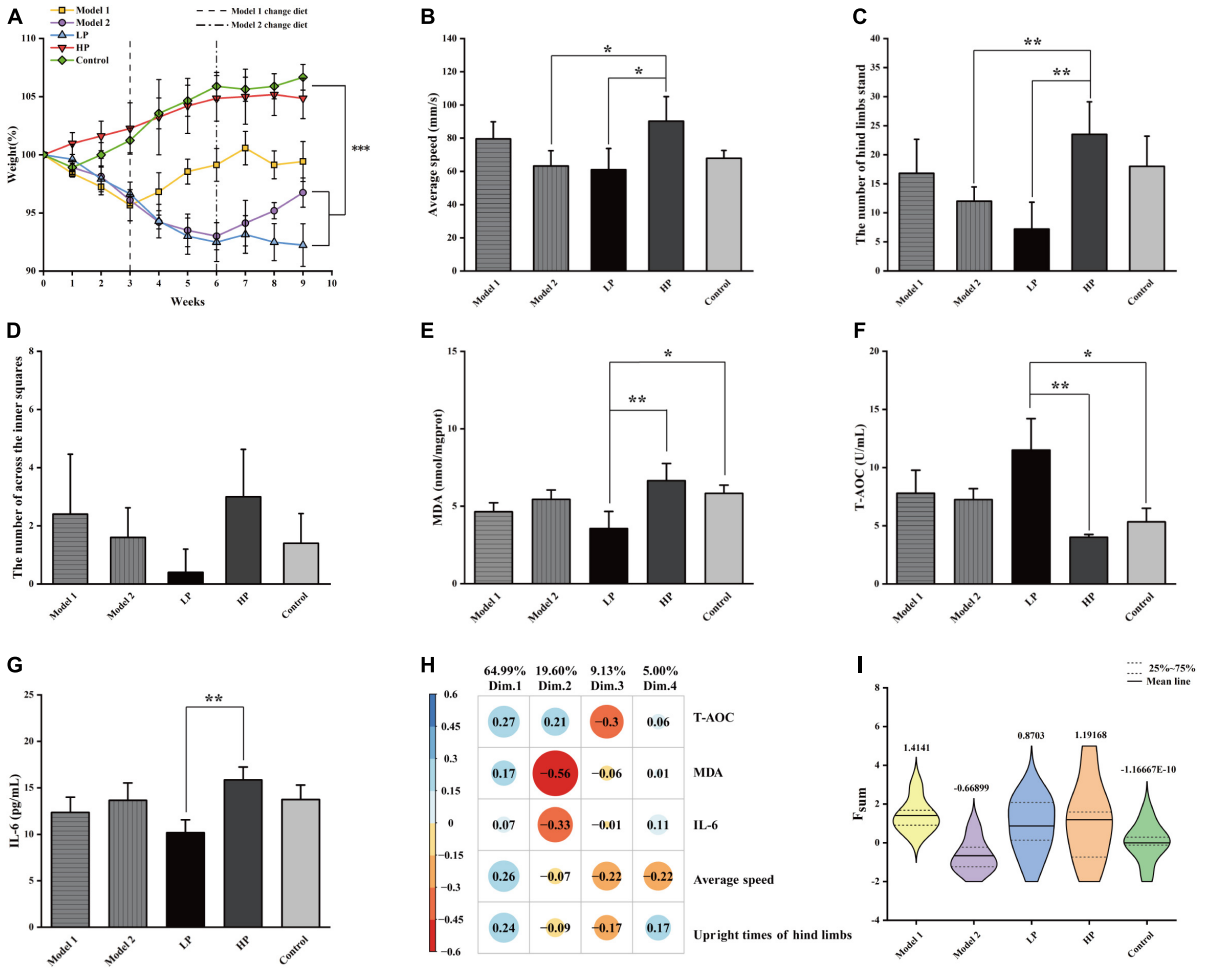


FIGURE 2
Basic physiological parameters of rats and the comprehensive assessment of aging status. **(A)** Body weight, **(B)** mean movement speed of rats in the open field experiment, **(C)** number of upright hind limbs of rats in the open field experiment, **(D)** number of central region entries of rats in the open field experiment, **(E)** liver MDA level, **(F)** serum T-AOC level, and **(G)** serum inflammatory factor IL-6 level. All values above are expressed as mean ± SD (n = 10), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. **(H)** Factor loading matrix of the principal components of the health index. **(I)** Violin plot of F_{sum} scores of rats in each group.

energy metabolites (e.g., pyruvate and lactate), short-chain fatty acids (SCFA) (e.g., acetate and butyrate), ketone bodies (e.g., acetone and 3-hydroxybutyrate), intestinal flora metabolites (e.g., dimethylamine and trimethylamine), glycoproteins (e.g., N-acetylglucosamine), and other substances.

Differential metabolites in rat fecal and serum samples

Fecal and serum ^{1}H-NMR data were analyzed to determine the variability of the metabolites between the groups. The unsupervised PCA scores of feces and serum are shown in Supplementary Figures 1A, 2A, respectively. The p-value of the PERMANOVA test for fecal and serum PCA was less than 0.001, which implied that the intervention of different protein nutritional modalities had a significant effect on the metabolic status of the rats. However, there were groups with non-significant differences in the Adonis paired test for PCA, suggesting that PCA did not completely distinguish between all groups of rats. To achieve maximum separation of fecal and serum metabolites in each group of rats, further analysis was performed using the OPLS-DA model. The OPLS-DA score plots and VIP score rankings for feces and serum are shown in Supplementary Figures 1B–E, 2B–E, respectively. The Q^2Y of all groups were close to one [$R^2 > 0.4$, p(CV) < 0.05]. The results of the model parameters indicated that the model fit was highly reliable and could effectively separate the fecal and serum metabolites of each group of rats.

Twenty and 13 differential metabolites were screened in feces and serum, respectively, compared to controls based on a combination of VIP values (VIP >1) and

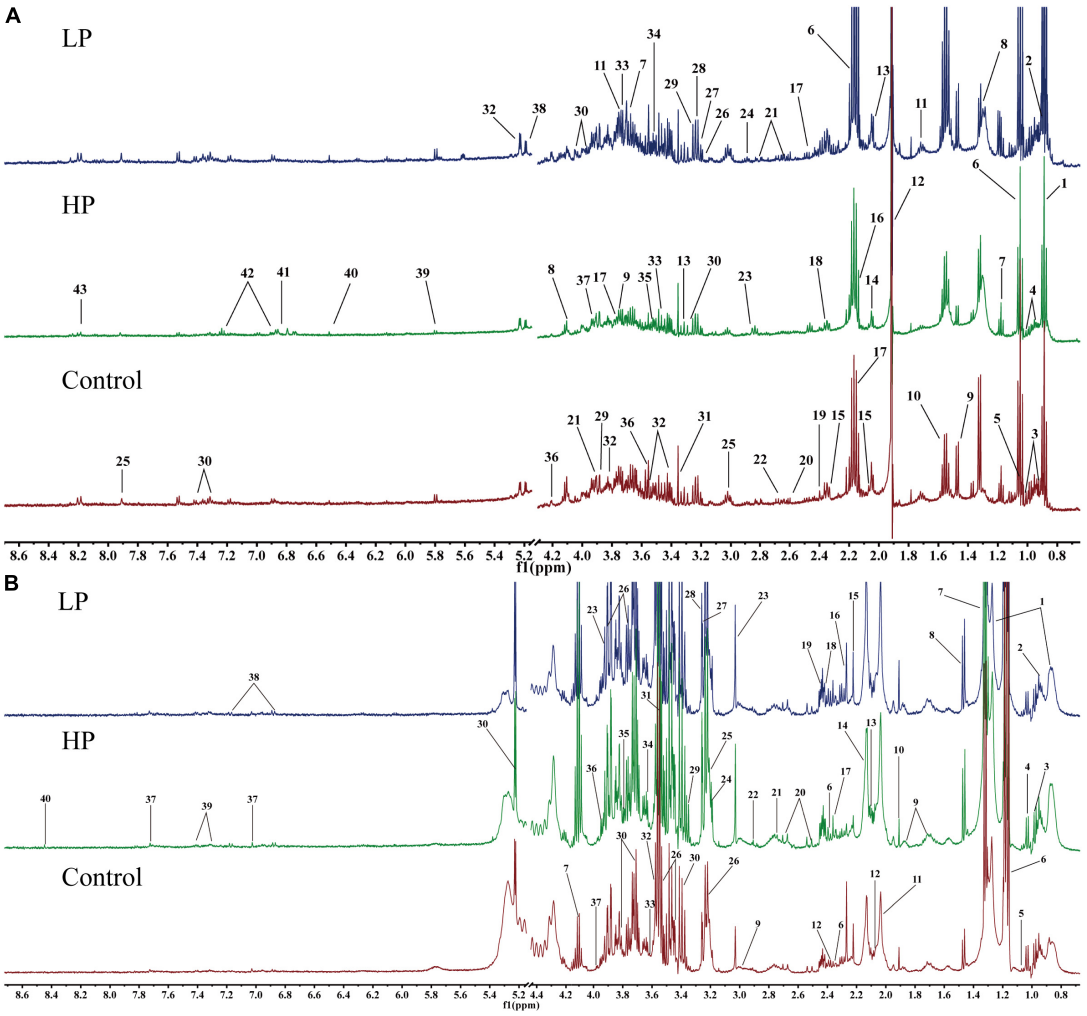


FIGURE 3
Representative ^{1}H-NMR metabolic profiles of rat feces and serum. **(A)** Representative ^{1}H-NMR metabolic profiles of rat feces. 1, lipid; 2, butyrate; 3, isoleucine; 4, leucine; 5, valine; 6, propionate; 7, ethanol; 8, lactate; 9, alanine; 10, citrulline; 11, arginine; 12, acetate; 13, proline; 14, N-acetylglucosamine; 15, glutamate; 16, methionine; 17, glutamine; 18, pyruvate; 19, succinate; 20 PUFA; 21, aspartate; 22, dimethylamine; 23, trimethylamine; 24, asparagine; 25, histidine; 26, choline; 27, β-xylose; 28, taurine; 29, betaine; 30, phenylalanine; 31, methanol; 32, α-glucose; 33, β-glucose; 34, inositol; 35, glycine; 36, threonine; 37, glycolate; 38, α-xylose; 39, uracil; 40, fumarate; 41, 3-hydroxyphenyl propionic acid; 42, tyrosine; 43, hypoxanthine. **(B)** Representative ^{1}H-NMR metabolic profiles of rat serum. 1, lipid; 2, isoleucine; 3, leucine; 4, valine; 5, isobutyrate; 6, 3-hydroxybutyrate; 7, lactate; 8, alanine; 9, lysine; 10, acetate; 11, N-acetylglucosamine; 12, glutamate; 13, glutamine; 14, O-acetyl glycoprotein; 15, acetone; 16, acetoacetate; 17, pyruvate; 18, succinate 19, carnitine; 20, citrate; 21, dimethylamine; 22, trimethylamine; 23, creatine; 24, choline; 25, phosphorylcholine; 26, β-glucose; 27, trimethylamine oxide; 28, betaine; 29, scyllo-inositol; 30, α-glucose; 31, glycine; 32, threonine; 33, propanetriol; 34, inositol; 35, arginine; 36, 1-methylhistidine; 37, histidine; 38, tyrosine; 39, phenylalanine; 40, formate.

the Wilcoxon nonparametric test results ($p < 0.05$). The differential metabolites identified in the feces and serum and their correlation coefficients are shown in Tables 1, 2, respectively. Among fecal metabolites, the levels of amino acids (arginine, histidine, glycine, leucine, tyrosine, glutamate, and threonine), hypoxanthine, and lipids were reduced while lactate, N-acetylglucosamine, and pyruvate levels were elevated in the LP diet group. In contrast, arginine, valine, and acetate levels were increased and the levels of succinate, polyunsaturated fatty acids, uracil, lipids, α-xylose, and N-acetylglucosamine were decreased in the HP diet group. Among the serum metabolites, the levels of alanine, lactate, and pyruvate increased and that of 3-hydroxybutyrate decreased in the LP diet group. Acetate, phenylalanine, tyrosine, and glutamate levels were increased and N-acetylglucosamine levels were decreased in the HP diet group.

To illustrate the changes in serum and fecal metabolites and the differences in metabolite levels between the groups of rats, we produced metabolite hierarchical clustering heat maps for feces and serum, as shown in Figures 4A,B, respectively. The graphs show that the variation of metabolites in response to diet was greater in feces than in serum. More differential metabolites were identified in feces, and the magnitude of

10.3389/fnut.2022.1051964

TABLE 1 Correlation coefficients of fecal differential metabolites between different paired comparison groups.

Metabolites	FC			
	Model 1 vs. control	Model 2 vs. control	LP vs. control	HP vs. control
α-Xylose		−0.63536		−1.2312
Acetate				+0.31746
Arginine			−0.85319	
Asparagine		−0.60505		
Glutamate		−0.62497	−0.81163	
Glycine	−0.59124	−0.57278	−0.75023	
Histidine	−0.52255	−0.9696	−1.5287	
Hypoxanthine		−0.4194	−0.55583	
Lactate	+1.2126	+1.2757	+0.96242	
Leucine	−0.91305	−1.324	−2.3603	
Lipid	−0.75249	−0.71621	−0.83976	−0.65864
N-acetylglucosamine			+0.71973	−0.75979
PUFA	−1.3631			−1.5501
Pyruvate			+1.0634	
Succinate	−1.4668			−1.8356
Threonine		−1.3134	−0.9945	
Trimethylamine				−0.35337
Tyrosine			−1.302	
Uracil	−0.83561	−0.75283		−1.1564
Valine				+0.66537

FC (fold change) indicates the fold change of metabolites between two groups as the logarithmic value of the mean ratio between two groups with a base of 2; + and − indicate the increase or decrease of metabolite concentration in the previous group, respectively.

TABLE 2 Correlation coefficients of serum differential metabolites between different paired comparison groups.

Metabolites	FC			
	Model 1 vs. control	Model 2 vs. control	LP vs. control	HP vs. control
3-Hydroxybutyrate	−0.32961	−0.52644	−0.27046	
Acetate	+0.3388			+0.6747
Alanine	+0.29435	+0.2173	+0.51014	
Choline		+0.44545		
Glutamate				+0.69206
Glycine	−0.33434	−0.48691		
Isobutyrate	+0.75203	+1.2462		
Lactate	+0.60182	+0.83173	+0.90002	+0.4537
Lysine	+0.53865			
N-acetylglucosamine				−0.58918
Phenylalanine		+1.3653		+0.97413
Pyruvate			+0.90236	
Tyrosine				+0.65458

FC (fold change) indicates the fold change of metabolites between two groups as the logarithmic value of the mean ratio between two groups with a base of 2; + and − indicate the increase or decrease of metabolite concentration in the previous group, respectively.

the metabolite changes was greater. The HP diet significantly increased the levels of amino acids and their derivatives and decreased the levels of carbohydrate metabolic intermediates in both feces and serum. The LP diet was found to have an effect opposite to that of the HP diet: the levels of amino acids and their derivatives were reduced, and the levels of carbohydrate metabolic intermediates were increased. Although the only difference between Model 1 and the high-protein group

was the 3-week LP diet intervention, the metabolite clustering heat map showed a significant difference between the metabolite concentrations of the two groups.

Correlation analysis between differential metabolites and protein intake

The differences in protein intake among the different groups of rats during the dietary intervention led to significant differences in the fecal and serum metabolites between these groups. Therefore, it was necessary to further investigate the correlation between protein intake and differential metabolites in rats using Spearman's correlation analysis. The selection criteria to identify correlated differential metabolites was set as $|r| > 0.4$ and $p < 0.05$. The normalized peak area box plots of all correlated differential metabolites are shown in **Figure 5**. The results showed that some essential amino acids (leucine and valine), conditionally essential amino acids (arginine and histidine), and some essential amino acids involved in physiological metabolic regulation (glutamate, tyrosine, alanine, and glycine) in feces were positively correlated with protein intake. In addition, asparagine and hypoxanthine levels were positively correlated with protein intake. In contrast, pyruvate, succinate, and N-acetylglucosamine levels were negatively correlated with protein intake. Serum tyrosine and acetate levels were positively correlated with protein intake, whereas lactate levels were negatively correlated with protein intake.

Metabolic pathway analysis

To clarify the effects of differential protein intake on biochemical pathways, we further mapped the newly identified differentially correlated metabolites to metabolic pathways using MetPA (Metabolomics Pathway Analysis). The results are shown in **Figure 6A**. Changes in protein intake mainly affected amino acid synthesis and metabolism, such as arginine biosynthesis; phenylalanine, tyrosine, and tryptophan biosynthesis; histidine metabolism; alanine, aspartate, and glutamate metabolism; arginine and proline metabolism; glycine, serine, and threonine metabolism; and glutathione metabolism. In addition, metabolic pathways related to carbohydrate metabolism, such as glycolysis/gluconeogenesis, glyoxylate, and dicarboxylate metabolism, pyruvate metabolism, and the TCA cycle, were also identified.

To explore the interaction between the correlated differential metabolites in these metabolic pathways, we constructed a metabolic network of correlated differential metabolites using STITCH. The results are shown in **Figure 6B**. The results indicate that all correlated differential metabolites interact with each other, directly or indirectly. The remaining differential metabolites were closely related, with the exception of hypoxanthine and N-acetylglucosamine. Strong interactions were observed between the amino acids alanine, glycine, arginine, histidine, glutamate, leucine, and asparagine and the metabolites lactate, pyruvate, succinate, and acetate (all interaction scores >0.9). This reflects the strong role of amino acid metabolism in the formation of essential or non-essential amino acids in organisms through amino-acid conversion and the interaction between amino acid and carbohydrate metabolisms. To better understand this situation, we generalized the metabolic pathways disturbed by protein intake. The results are shown in **Figure 6C**.

Discussion

Currently, there are different views on protein nutrition requirements in middle-aged and elderly people (43–45). To understand and reveal the underlying mechanisms, this study explored this issue using rat models to mimic the human aging process. The effects of different protein nutritional intakes on health status were examined comprehensively by characterizing aging-related indicators in rat models combined with non-targeted fecal and serum metabolomic changes. A comprehensive quantitative evaluation of behavioral factors, antioxidant capacity, and inflammatory factors in rats showed that a dietary pattern shift from low to high protein had better anti-aging effects after a certain period of adaptation compared to long-term LP or HP diets. The results of the metabolomic analysis also revealed the mechanisms underlying these effects.

Comprehensive quantitative assessment of aging status

This study found that an LP diet reduces body weight, oxidative stress, and inflammation. The HP diet compensated for the weight loss that occurred with the LP diet and maintained the exercise capacity of the rats. In a study by Mitchell et al., protein restriction was found to improve levels of oxidative stress and inflammation in mice (46). In the study by Mitsuishi et al., weight and exercise capacity were increased in older adults by a HP dietary intervention (47). These results are consistent with the findings in our study. A new quantitative method was established to characterize the health status of rats as scientific and comprehensive as possible. Through PCA, multiple analytical terms were converted into several key generalized indicators. A composite score was obtained using the component scores and variance interpretation rates to comprehensively assess aging status. Model 1 had a higher composite score than the long-term LP and HP diet groups, and Model 2 had the lowest composite score probably because of a 3-week difference in the duration of the HP diet. Walrand

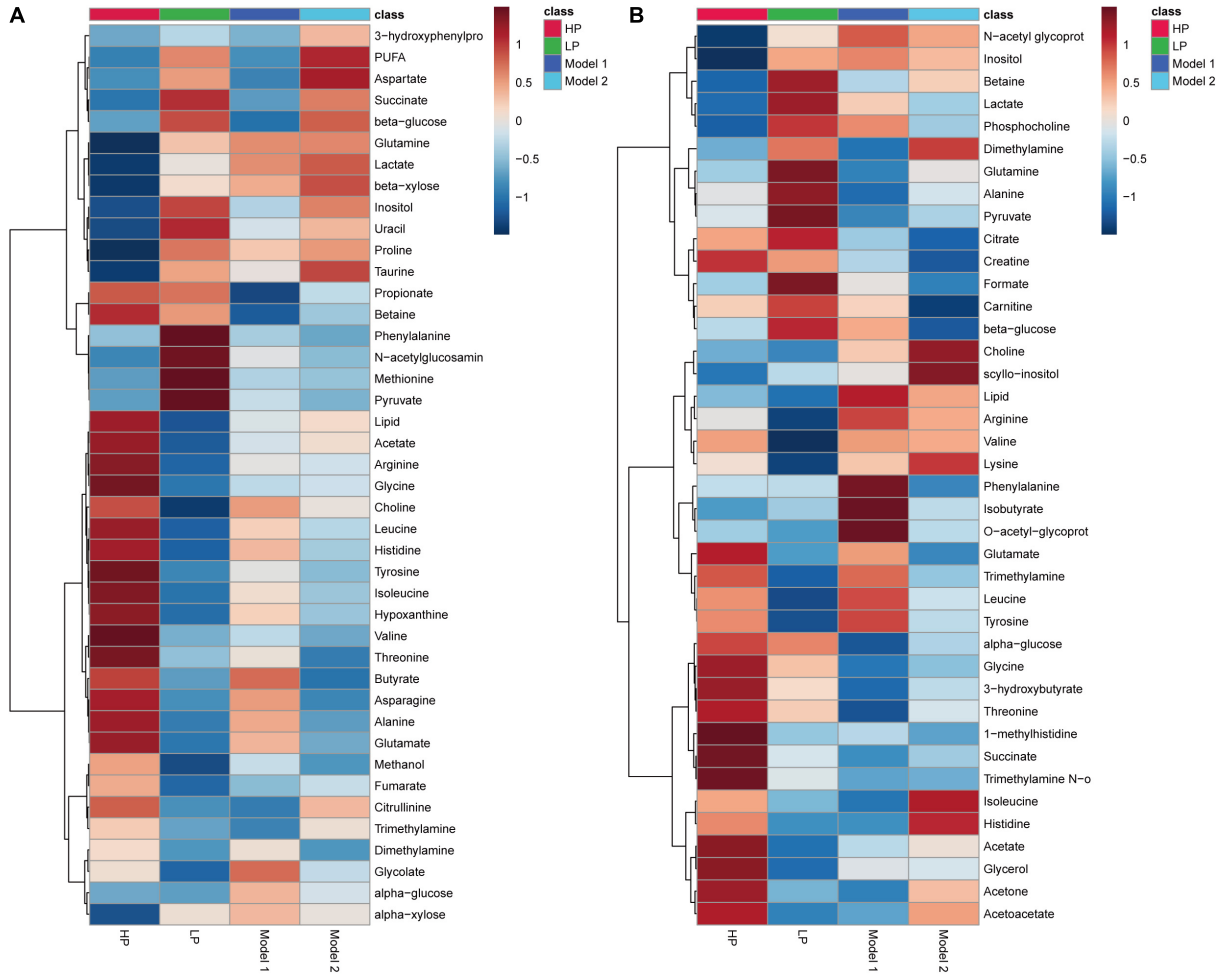


FIGURE 4
Hierarchical clustering heatmap of metabolite concentrations. **(A)** Hierarchical clustering heatmap of metabolite concentrations in rat feces. **(B)** Hierarchical clustering heatmap of metabolite concentrations in rat serum.

et al. found that a brief HP diet for 10 days increased systemic amino acid uptake, but not muscle protein synthesis in older adults (48). Thus, the Model 2 group may have failed to enhance their exercise capacity sufficiently owing to the short duration of the HP intervention, while oxidative stress and inflammation levels were elevated. In a study by Hahn et al., mortality and inflammation levels were reduced in mice subjected to pre-restricted diet followed by free diet compared to those administered free diet for longer periods (49). Protein restriction, a significant cause of anti-aging effect in restricted diets, produced similar results. The members of the Model 1 group, with low protein during the initial period and high protein in the subsequent period, were able to maintain lower levels of oxidative stress and inflammation with enhanced exercise capacity, which would better meet the physiological requirements of the organism for dynamic changes in protein during the natural aging process. As shown in **Figure 1**, Model 1 and Model 2 showed significant differences in health status when their diets were switched from low to high protein at 64.8 and 66.5 years of age, respectively. During a physical examination at 68.3 years of age, Model 1 scored high and Model 2 scored low. This shows that the health status of an organism at this age is susceptible to changes in protein content in food. These results provide more than sufficient support for the validity of the hypotheses in this study. After approximately 50 years of age, there is a smooth change in protein requirement from lower to higher rates that can be beneficial to health. The critical point is estimated to be around 65 years, and a smooth transition is required as drastic changes may be detrimental to health.

It is particularly important to note that this study found that in the period equivalent to the human age of 62.5–68.3 years, the most beneficial way of protein nutrition for a long and healthy life is a gradual change in demand from low to high (Model 1), and simple low and high protein nutrition are not as effective as this. In the reality of several countries, people in that period

10.3389/fnut.2022.1051964

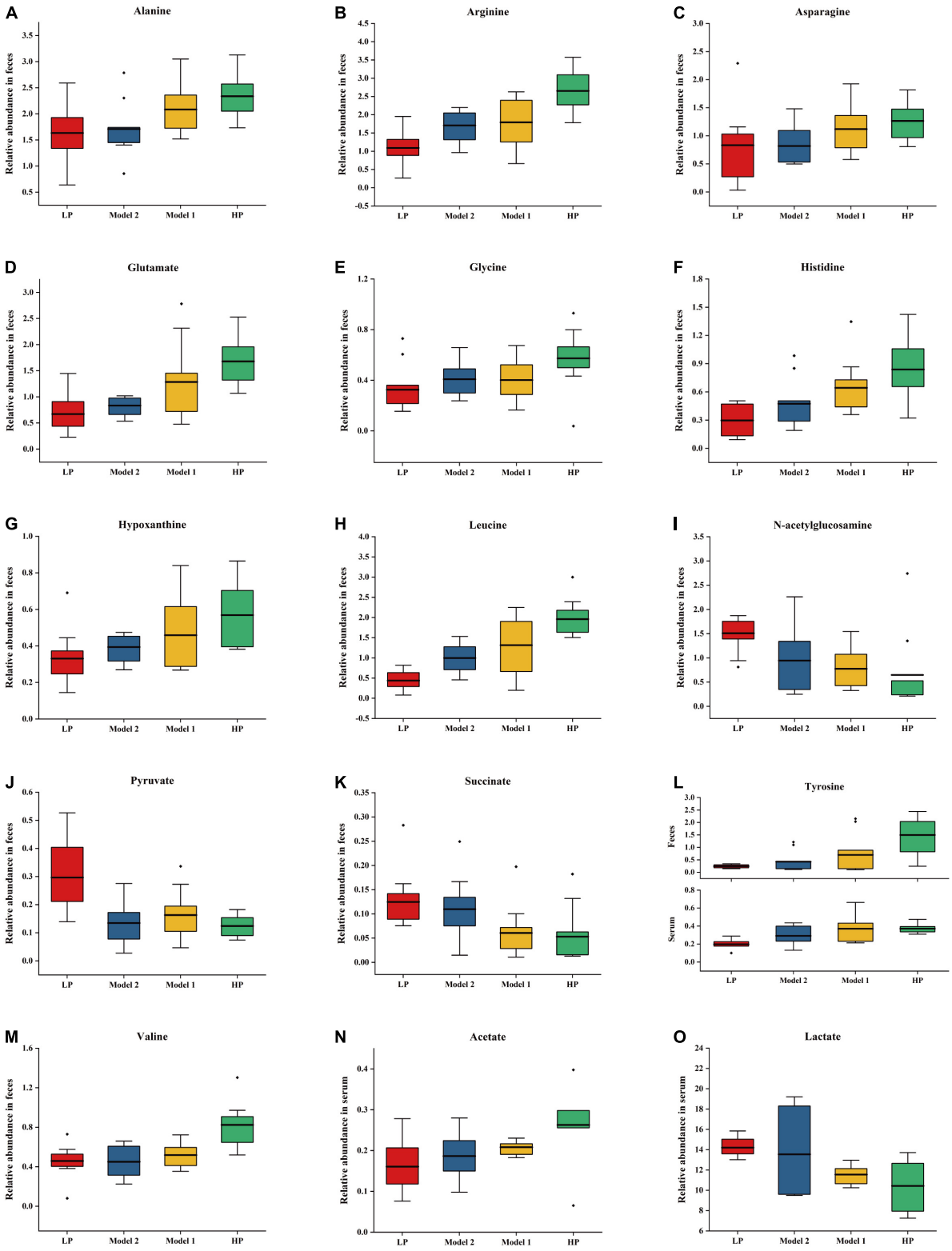


FIGURE 5
Correlation difference metabolite normalized peak area box plots for Model 1, Model 2, chronic low protein, and chronic high protein diet model groups. **(A)** Alanine, **(B)** arginine, **(C)** asparagine, **(D)** glutamate, **(E)** glycine, **(F)** histidine, **(G)** hypoxanthine, **(H)** leucine, **(I)** N-acetylglucosamine, **(J)** pyruvate, **(K)** succinate, **(L)** tyrosine, **(M)** valine, **(N)** acetate, and **(O)** lactate. Data are expressed as mean ± SD.

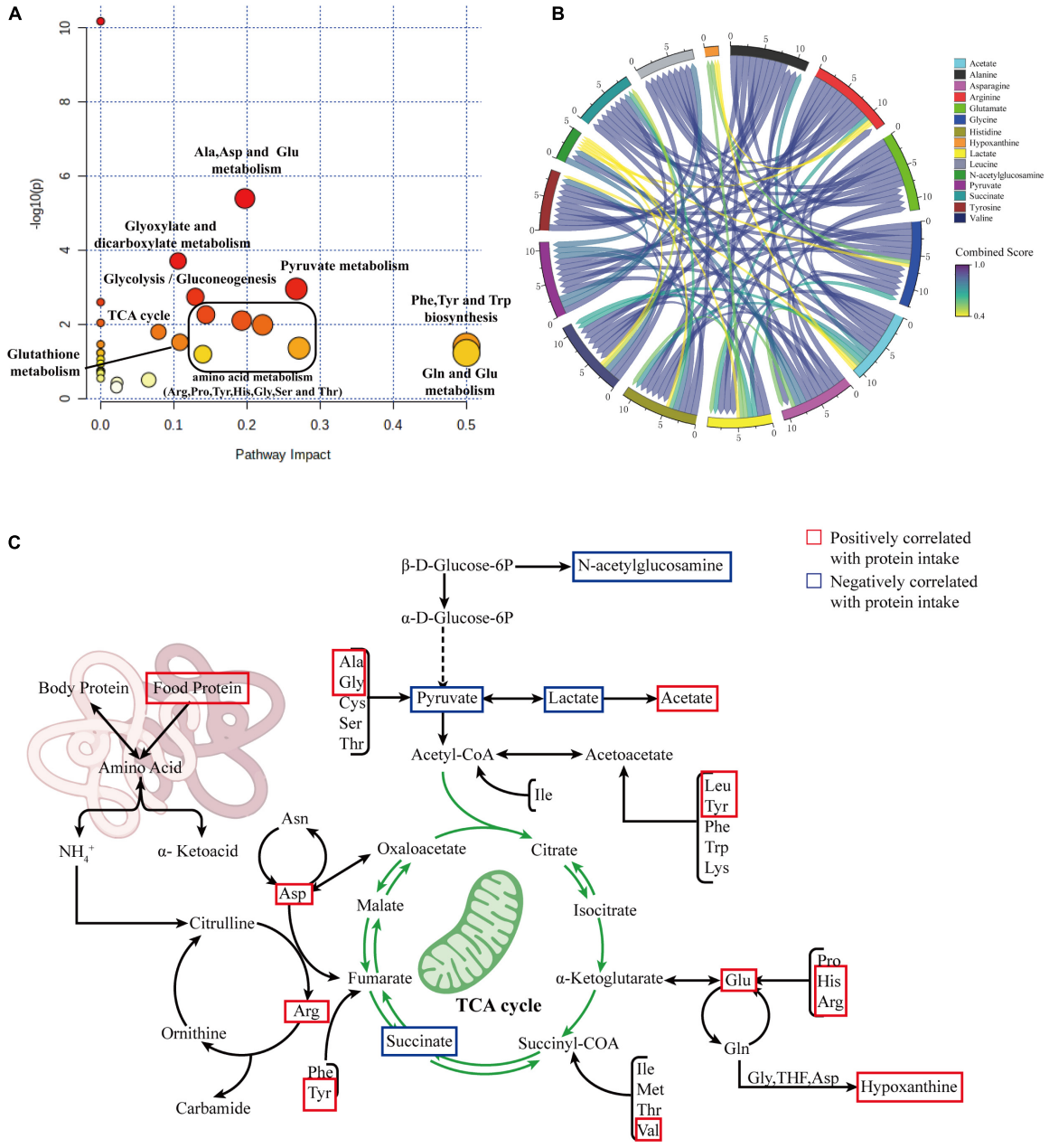


FIGURE 6
(A) Summary plot of pathway analysis of correlated differential metabolites using MetPA. **(B)** Chord diagram showing the relationship between correlated differential metabolites. **(C)** Summary diagram of metabolic pathways associated with protein intake.

have just retired, are financially well-off, still in good health, and have a simple concept of health care, among other reasons, contributing to the excessive protein intake of many people. This will harm many issues such as their pre-retirement performance, healthy longevity, and health care expenses. Therefore, it is considered necessary to further explore protein nutrition in people around the age of menopause to 70 years based on this finding. This is not only a scientific issue, but should also be a far-reaching social issue that needs to be addressed urgently.

Non-targeted fecal and serum metabolomics and metabolites

Next, non-targeted fecal and serum metabolomics were performed in this study to reveal the nutritional effects and mechanisms of action of proteins. Non-targeted fecal and serum metabolomic analyses identified 15 differential metabolites relevant to protein intake. The changes in fecal and serum metabolomic profiles were mainly enriched in amino acid and

carbohydrate metabolism. The intervention with an LP diet in the early years can improve the metabolic profile if followed up with an HP diet in the later years and thus positively impact health according to the appropriately chosen period. A middle-aged LP followed by an elderly HP diet can enhance amino acid metabolism while maintaining carbohydrate metabolism, thus preventing mitochondrial dysfunction and frailty. This metabolomic analysis provides a theoretical basis for the shift from a low-protein to a high-protein nutritional approach in middle-aged and older adults from a metabolic perspective.

Amino acid metabolism

As protein intake increased, fecal levels of leucine, valine, arginine, histidine, glutamate, tyrosine, and glycine increased, positively correlating with protein intake. Contents of stool samples are directly related to dietary patterns and health status, and changes in fecal metabolites can reflect potential mechanisms in the colon-systemic axis of food metabolism (50). Studies have shown that increased levels of amino acids in fecal metabolism increase the risk of developing diseases such as obesity and diabetes during the aging process in middle-aged adults. Levels of branched-chain amino acids, such as leucine and valine, are associated with metabolic syndromes, such as obesity and diabetes mellitus (51). Significant increases in glutamate, leucine, and valine levels have been found in stools of patients (52). Studies in diabetic mice have also found a significant upregulation of amino acids among fecal metabolites (phenylalanine, a precursor of tyrosine metabolism, and aspartate, a precursor of glutamate metabolism) (53). Therefore, increased levels of branched-chain amino acids, glutamate, and tyrosine in feces can harm the health of elderly individuals. Correlation analysis found that the levels of the relevant amino acids decreased in both Model 1 and Model 2 groups after different LP intervention durations in the first period. This implies that, by intervening with an LP diet upfront, the excessive elevation of amino acid levels in the gut can be limited at a later stage, thus reducing the negative effects of an HP diet on the health of the elderly population in terms of metabolic diseases.

In addition, we found an increase in tyrosine, glutamate, and phenylalanine levels in the serum following HP intervention. Serum metabolites indicate nutrient bioavailability and reflect individual metabolic changes (54). Glutamate is central to the processing of the daily protein load, and arginine, ornithine, proline, histidine, and glutamine require conversion to glutamate to enter the metabolic cycle with significant metabolic versatility (55). Glutamate can increase the synthesis of glutamine, which serves as an important component of muscle proteins and contributes to muscle repair and strengthening (56). Studies in adults, pigs, and preterm infants have found that glutamate is metabolized primarily in the gastrointestinal tract and that serum glutamate concentrations return to normal within 2 h, even when high doses of monosodium glutamate are ingested (57). However, during HP dietary intervention in elderly men, the plasma metabolic profile showed significant increase in glutamate levels (58). This may imply that as the body ages, the ability to catabolize glutamate decreases, affecting multiple metabolic pathways, such as gluconeogenesis, carbohydrate metabolism, and glutathione metabolism. Additionally, frailty in the elderly population includes not only a decrease in motor capacity but also a decrease in cognitive ability (59). Tyrosine and phenylalanine play multiple biological roles. Increased protein intake has been shown to increase tyrosine and phenylalanine concentrations in the brain, stimulating the production of catecholamines, which function in active excitatory neurons (60). This process is influenced by the availability of amino acids in the blood. The correlation analysis results from our study showed that tyrosine serum metabolites were positively correlated with protein intake. Serum tyrosine levels in the Model 1 and Model 2 groups increased after later HP intervention, suggesting that the elevated protein intake in the later period enhanced serum amino acid metabolism, which may play a positive role in maintaining young muscle synthesis and cognitive ability in the elderly.

Carbohydrate metabolism

Pyruvate, succinate, and lactate are important components of the carbohydrate metabolism. Among fecal metabolites, pyruvate and succinate levels were negatively correlated with protein intake, and among serum metabolites, lactate level was negatively correlated with protein intake. A significant increase in pyruvate level was found in the fecal and serum metabolites of the LP group. Because an isocaloric dietary intervention was used, the increase in pyruvate, succinate, and lactate levels in the LP group was more likely due to the increased proportion of carbohydrates in the diet. Accelerated TCA cycling following high-carbohydrate intake has been associated with various diseases in the elderly, such as cancer and atherosclerosis (61). However, other reports have demonstrated that reduced pyruvate metabolism and TCA cycle capacity during aging lead to mitochondrial dysfunction (62). It also interferes with ATP production, impairs cellular function, and leads to tissue dysfunction (63). In addition, some inflammatory responses of microglia are associated with dysregulated glucose metabolism (64). Correlation analysis revealed that both Model 1 and Model 2 groups showed a significant increase in pyruvate, succinate, and lactate levels after HP diet following a pre-LP intervention compared to HP group levels. Therefore, it can be hypothesized that a pre-LP dietary intervention can contribute to healthy aging in the elderly population by improving the dysregulation of glucose metabolism and maintaining an optimal level of glucose metabolism after HP dietary intervention.

Intestinal fatty acid metabolism and other metabolic pathways

A significant increase in acetate level was observed in feces of rats following an HP diet, and a significant increase in lactate level was observed in those following an LP diet. It is possible that changes in the proportion of proteins in the diet altered the composition and abundance of intestinal flora. Acetate and lactate in feces are mainly produced by the fermentation of undigested carbohydrates by intestinal microorganisms (65). Acetate accounts for the highest percentage of SCFA produced by the intestinal flora and can regulate intestinal pH and maintain intestinal stability (66). Human and animal models following HP diets have demonstrated an elevation in SCFA (67, 68). HP diets cause an increase in the proportion of proteins that remain incompletely digested after passing through the small intestine, allowing microorganisms to produce more SCFA and other protein fermentation products, mainly using peptides as a carbon source (69). These SCFA and protein fermentation products can regulate protein homeostasis in muscle cells and promote muscle anabolism (70), implying that protein intake can counteract muscle loss in the elderly through the intestinal flora. In the current study, lactate levels in fecal metabolites were significantly increased not only in the LP diet group but also in Model 1 and Model 2 groups. Lactate in the gut is a pro- and anti-inflammatory regulator that reduces inflammation by decreasing damage to cells in the intestinal wall and inhibiting the release of pro-inflammatory cytokines, such as IL-6 (71). This may be an underlying mechanism by which an LP diet upfront and an HP diet later can positively affect intestinal health and reduce inflammation in the elderly population by maintaining intestinal wall integrity.

We also observed a positive correlation between hypoxanthine levels in feces and protein intake. Due to increased protein intake, the amino acids involved in de novo synthesis increased. Gut microbes have an enhanced ability to synthesize purines de novo, thus producing more hypoxanthine (72). The lack of significant increase in hypoxanthine levels in the serum metabolites following an HP diet may be due to the conversion of hypoxanthine to uric acid, prompted by the high activity of xanthine oxidoreductase in rats (73). Many studies have shown that an HP diet leads to an increase in uric acid levels and increases the risk of gout and hyperuricemia in the elderly population (58, 74), which is caused by the abnormal metabolism of uric acid due to decreased renal function in the elderly population (75). This implies that elderly people with renal insufficiency, diabetes, and gout attacks must be more cautious when adopting an HP diet. Compared to the fecal hypoxanthine levels in the HP group, they were reduced in both the Model 1 and Model 2 groups, and the initial LP diet had inhibited excessive uric acid production. This suggests that introducing an LP diet in the first phase and an HP diet in the second phase have a wider range of applicability and should be a more scientific and rational way to promote a long and healthy life than introducing a long-term HP diet to prevent muscle loss in old age.

This study has some limitations: (1) A non-targeted ^{1}H-NMR method for metabolomic analysis was used. To gain a more comprehensive understanding of the overall metabolic process of initial LP diet followed by an HP diet, new methods such as proteomics and lipid metabolomics can be applied in future to investigate the mechanism of anti-aging and effects of such a diet. (2) The indices covered by the comprehensive quantitative evaluation in this study may not fully represent the health status of the organism. In future studies, it will be necessary to include more indices for testing. If the parameters of histological analysis are unified into the system of comprehensive quantitative scoring, more representative data information can be obtained. (3) Only male rats were selected for study in our experiments. Sex differences in aging occur in many animal species, including sex differences in lifespan, onset and progression of age-related decline, and physiological and molecular markers of aging (76). And both the immune system and the aging process in adult mammals are sexually dimorphic, suggesting that aging studies should be stratified according to sex (77, 78). Therefore, to make this model of protein diet based on age-differentiated nutritional strategies applicable to a broader population, it is necessary to include the sex system in future studies. (4) This study was conducted in rats. In subsequent experiments, it is necessary to recruit middle-aged and elderly volunteers for dietary intervention experiments with increased group sizes, decreased intensity of protein level changes, and extended intervention duration. Thus, we can further investigate the effect of protein diets on different ages, nutrition strategies, and the health status of middle-aged and elderly people to provide scientific and technological support for human health and longevity.

Conclusion

Comprehensive quantitative scoring of the overall health of the rats and ^{1}H-NMR-based metabolomic analysis showed that the amount of protein intake, the timing of intake, and the duration of dietary intervention are key factors affecting fitness. Protein supplementation in food should closely match the increased physiological demand for protein that occurs with natural aging of the organism, and a sudden increase in protein intake is detrimental to the health of the organism. Mechanistic studies have found that LP dietary interventions in the middle-age can compensate to some extent for the damage caused by an HP diet in old age, in terms of oxidative damage and inflammation. The later HP diet then helps compensate for the lack of muscle synthesis capacity of the earlier LP diet, thus reducing the risk of protein malnutrition in the elderly population. Fecal and serum metabolomic analyses identified 43 and 40 metabolites, respectively, among which 20 and 13

were identified as differential metabolites, respectively. Eight amino acids and two other substances in feces were significantly positively associated with protein intake, and three substances, including pyruvate, were significantly negatively associated. Serum tyrosine and lactate levels were positively correlated with protein intake, whereas acetate levels were negatively correlated. Metabolic pathway analysis revealed that the LP diet activated carbohydrate metabolism and accelerated the TCA cycle. The HP diet increased energy metabolism more than amino acid metabolism, thus preventing muscle loss in older age groups due to reduced protein synthesis capacity. In summary, the results of combined quantitative scoring and metabolomics analysis supports the hypothesis from different perspectives. After about 50 years of age, there is a change in the nutritional requirements of protein for health from a lower to a higher rate. This study brings together different opinions on protein nutrition strategies and provides an important reference to construct a scientific and rational approach to protein nutrition. This will be significant in helping middle-aged and elderly populations to improve their physical fitness and achieve healthy aging.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary material**, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the Animal Ethics Committee of Guangxi University.

Author contributions

QL, WZ, and YZ: experimental idea conception and experimental design. FS, YL, FZ, XZ, and JL: experimental reagents and materials preparation and experimental investigation. RL and YS: data organization and visualization of experimental results. WZ: full manuscript writing. QL: full review and revision of the manuscript. All authors reviewed the manuscript and approved the final manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (No. 31871802) and Key Research and Development Projects in Guangxi, China (No. AB18221065).

Acknowledgments

We would like to thank all members of our team for their help in behavioral experiments and rat dissection. We would also like to thank Editage (www.editage.cn) for English language editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1051964/full#supplementary-material

References

1. Wang D, Ye J, Shi R, Zhao B, Liu Z, Lin W, et al. Dietary protein and amino acid restriction: Roles in metabolic health and aging-related diseases. *Free Radic Biol Med.* (2022) 178:226–42. doi: 10.1016/j.freeradbiomed.2021.12.009

2. Bhupathiraju SN, Hu FB. Epidemiology of obesity and diabetes and their cardiovascular complications. *Circ Res.* (2016) 118:1723–35. doi: 10.1161/CIRCRESAHA.115.306825

3. Elias MF, Elias PK, Sullivan LM, Wolf PA, D'Agostino RB. Obesity, diabetes and cognitive deficit: The Framingham Heart Study. *Neurobiol Aging.* (2005) 26(Suppl 1):11–6. doi: 10.1016/j.neurobiolaging.2005.08.019

4. Wolf PA, Beiser A, Elias MF, Au R, Vasan RS, Seshadri S. Relation of obesity to cognitive function: Importance of central obesity and synergistic influence of concomitant hypertension. The Framingham Heart Study. *Curr Alzheimer Res.* (2007) 4:111–6. doi: 10.2174/156720507780362263

5. Grandison RC, Piper MD, Partridge L. Amino-acid imbalance explains extension of lifespan by dietary restriction in Drosophila. *Nature.* (2009) 462:1061–4. doi: 10.1038/nature08619

6. Solon-Biet SM, McMahon AC, Ballard J, Ruohonen K, Wu LE, Cogger VC, et al. The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab.* (2020) 31:654. doi: 10.1016/j.cmet.2020.01.010

7. Mair W, Piper MD, Partridge L. Calories do not explain extension of life span by dietary restriction in Drosophila. *PLoS Biol.* (2005) 3:e223. doi: 10.1371/journal.pbio.0030223

8. Longo VD, Antebi A, Bartke A, Barzilai N, Brown-Borg HM, Caruso C, et al. Interventions to slow aging in humans: Are we ready? *Aging Cell.* (2015) 14:497–510. doi: 10.1111/acel.12338

9. Fontana L, Cummings NE, Arriola AS, Neuman JC, Kasza I, Schmidt BA, et al. Decreased consumption of Branched-Chain amino acids improves metabolic health. *Cell Rep.* (2016) 16:520–30. doi: 10.1016/j.celrep.2016.05.092

10. Cummings NE, Williams EM, Kasza I, Konon EN, Schaid MD, Schmidt BA, et al. Restoration of metabolic health by decreased consumption of branched-chain amino acids. *J Physiol.* (2018) 596:623–45. doi: 10.1113/JP275075

11. Mu WC, VanHoosier E, Elks CM, Grant RW. Long-Term effects of dietary protein and Branched-Chain amino acids on metabolism and inflammation in mice. *Nutrients.* (2018) 10:918. doi: 10.3390/nu10070918

12. Pietri P, Stefanadis C. Cardiovascular aging and longevity: JACC State-of-the-Art review. *J Am Coll Cardiol.* (2021) 77:189–204. doi: 10.1016/j.jacc.2020.11.023

13. Sedzikowska A, Szablewski L. Insulin and insulin resistance in Alzheimer's disease. *Int J Mol Sci.* (2021) 22:9987. doi: 10.3390/ijms22189987

14. Kouvari M, D'Cunha NM, Travica N, Sergi D, Zec M, Marx W, et al. Metabolic syndrome, cognitive impairment and the role of diet: A narrative review. *Nutrients.* (2022) 14:333. doi: 10.3390/nu14020333

15. Rodriguez-Manas L, Angulo J, Carnicero JA, El AM, Garcia-Garcia FJ, Sinclair AJ. Dual effects of insulin resistance on mortality and function in non-diabetic older adults: Findings from the Toledo Study of Healthy Aging. *Geroscience.* (2022) 44:1095–108. doi: 10.1007/s11357-021-00384-4

16. Hahn O, Gronke S, Stubbs TM, Ficz G, Hendrich O, Krueger F, et al. Dietary restriction protects from age-associated DNA methylation and induces epigenetic reprogramming of lipid metabolism. *Genome Biol.* (2017) 18:56. doi: 10.1186/s13059-017-1187-1

17. Pignatti C, D'Adamo S, Stefanelli C, Flamigni F, Cetrullo S. Nutrients and pathways that regulate health span and life span. *Geriatrics (Basel).* (2020) 5:95. doi: 10.3390/geriatrics5040095

18. Hanjani NA, Vafa M. Protein restriction, epigenetic diet, intermittent fasting as new approaches for preventing age-associated diseases. *Int J Prev Med.* (2018) 9:58. doi: 10.4103/ijpvm.IJPVM_397_16

19. Brunet A, Rando TA. Interaction between epigenetic and metabolism in aging stem cells. *Curr Opin Cell Biol.* (2017) 45:1–7. doi: 10.1016/j.ceb.2016.12.009

20. Gavrilova NS, Gavrilov LA. Comments on dietary restriction, Okinawa diet and longevity. *Gerontology.* (2012) 58:224–6. doi: 10.1159/000329894

21. Han K, Ma J, Dou J, Hao D, Zhu W, Yu X, et al. A clinical trial of the effects of a dietary pattern on health metrics and fecal metabolites in volunteers with risk of cardiovascular disease. *Front Nutr.* (2022) 9:853365. doi: 10.3389/fnut.2022.853365

22. Wolfe RR. The role of dietary protein in optimizing muscle mass, function and health outcomes in older individuals. *Br J Nutr.* (2012) 108(Suppl 2):S88–93. doi: 10.1017/S0007114512002590

23. Arthur ST, Cooley ID. The effect of physiological stimuli on sarcopenia; Impact of Notch and Wnt signaling on impaired aged skeletal muscle repair. *Int J Biol Sci.* (2012) 8:731–60. doi: 10.7150/ijbs.4262

24. Green CL, Pak HH, Richardson NE, Flores V, Yu D, Tomasiewicz JL, et al. Sex and genetic background define the metabolic, physiologic, and molecular response to protein restriction. *Cell Metab.* (2022) 34:209–26. doi: 10.1016/j.cmet.2021.12.018

25. Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, et al. Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. *Cell Metab.* (2014) 19:407–17. doi: 10.1016/j.cmet.2014.02.006

26. Timmons JF, Hone M, Cogan KE, Duffy O, Egan B. Increased leg strength after concurrent aerobic and resistance exercise training in older adults is augmented by a whole Food-Based high protein diet intervention. *Front Sports Act Living.* (2021) 3:653962. doi: 10.3389/fspor.2021.653962

27. Kim HK, Chijiki H, Fukazawa M, Okubo J, Ozaki M, Nanba T. Supplementation of protein at breakfast rather than at dinner and lunch is effective on skeletal muscle mass in older adults. *Front Nutr.* (2021) 8:797004. doi: 10.3389/fnut.2021.797004

28. Halbesma N, Bakker SJ, Jansen DF, Stolk RP, De Zeeuw D, De Jong PE, et al. High protein intake associates with cardiovascular events but not with loss of renal function. *J Am Soc Nephrol.* (2009) 20:1797–804. doi: 10.1681/ASN.2008060649

29. Sluijs I, Beulens JW, van der ADL, Spijkerman AM, Grobbee DE, van der Schouw YT. Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. *Diabetes Care.* (2010) 33:43–8. doi: 10.2337/dc09-1321

30. Snelson M, Clarke RE, Nguyen TV, Penfold SA, Forbes JM, Tan SM, et al. Long term high protein diet feeding alters the microbiome and increases intestinal permeability, systemic inflammation and kidney injury in mice. *Mol Nutr Food Res.* (2021) 65:e2000851. doi: 10.1002/mnfr.202000851

31. Fontana L, Adelaiye RM, Rastelli AL, Miles KM, Ciamporcero E, Longo VD, et al. Dietary protein restriction inhibits tumor growth in human xenograft models. *Oncotarget.* (2013) 4:2451–61. doi: 10.18632/oncotarget.1586

32. Zhao F, Gao L, Qin X, Du G, Zhou Y. The intervention effect of licorice in d-galactose induced aging rats by regulating the taurine metabolic pathway. *Food Funct.* (2018) 9:4814–21. doi: 10.1039/c8fo00740c

33. Cui CM, Wang CS, Han SS, Yu DY, Zhu L, Jiang P. Impact of a long-term high-fructose diet on systemic metabolic profiles of mice. *FASEB Bioadv.* (2022) 4:560–72. doi: 10.1096/fba.2021-00152

34. Chak CM, Lacruz ME, Adam J, Brandmaier S, Covic M, Huang J, et al. Ageing investigation using Two-Time-Point metabolomics data from KORA and CARLA studies. *Metabolites.* (2019) 9:44. doi: 10.3390/metabo9030044

35. Deelen J, Kettunen J, Fischer K, van der Spek A, Trompet S, Kastenmuller G, et al. A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *Nat Commun.* (2019) 10:3346. doi: 10.1038/s41467-019-11311-9

36. Andreollo NA, Santos EF, Araujo MR, Lopes LR. Rat's age versus human's age: What is the relationship? *Arq Bras Cir Dig.* (2012) 25:49–51. doi: 10.1590/s0102-67202012000100011

37. Seibenhener ML, Wooten MC. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *Jove-J Vis Exp.* (2015) 6:e52434. doi: 10.3791/52434

38. Harro J. Animals, anxiety, and anxiety disorders: How to measure anxiety in rodents and why. *Behav Brain Res.* (2018) 352:81–93. doi: 10.1016/j.bbr.2017.10.016

39. Mei LH, Zheng WX, Zhao ZT, Meng N, Zhang QR, Zhu WJ, et al. A Pilot Study of the Effect of *Lactobacillus casei* obtained from long-lived elderly on blood biochemical, oxidative, and inflammatory markers, and on gut microbiota in young volunteers. *Nutrients.* (2021) 13:3891. doi: 10.3390/nu13113891

40. Liu X, Zhao D, Zhao S, Li Z, Wang Y, Qin X. Deciphering the correlations between aging and constipation by metabolomics and network pharmacology. *Aging.* (2021) 13:3798–818. doi: 10.18632/aging.202340

41. Miyaguti N, Stanisic D, Oliveira S, Dos SG, Manhe BS, Tasic L, et al. Serum and muscle (1)H NMR-Based metabolomics profiles reveal metabolic changes influenced by a maternal Leucine-Rich diet in Tumor-Bearing adult offspring rats. *Nutrients* (2020) 12:2106. doi: 10.3390/nu12072106

42. Zhao D, Liu X, Zhao S, Li Z, Qin X. 1H NMR-Based fecal metabolomics reveals changes in gastrointestinal function of aging rats induced by d-Galactose. *Rejuvenation Res.* (2021) 24:86–96. doi: 10.1089/rej.2020.2352

43. Traylor DA, Gorissen SHM, Phillips SM. Perspective: Protein requirements and optimal intakes in aging: Are we ready to recommend more than the recommended daily allowance? *Adv Nutr.* (2018) 9:171–82. doi: 10.1093/advances/nmy003

44. Deutz NEP, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, et al. Protein intake and exercise for optimal muscle function with aging: Recommendations from the ESPEN Expert Group. *Clin Nutr.* (2014) 33:929–36. doi: 10.1016/j.clnu.2014.04.007

45. Longo VD, Anderson RM. Nutrition, longevity and disease: From molecular mechanisms to interventions. *Cell.* (2022) 185:1455–70. doi: 10.1016/j.cell.2022.04.002

46. Mitchell SE, Delville C, Konstantopedos P, Hurst J, Derous D, Green C, et al. The effects of graded levels of calorie restriction: II. Impact of short term calorie and protein restriction on circulating hormone levels, glucose homeostasis and oxidative stress in male C57BL/6 mice. *Oncotarget.* (2015) 6:23213–37. doi: 10.18632/oncotarget.4003

47. Mitsuishi M, Miyashita K, Muraki A, Tamaki M, Tanaka K, Itoh H. Dietary protein decreases exercise endurance through rapamycin-sensitive suppression of muscle mitochondria. *Am J Physiol Endocrinol Metab.* (2013) 305:E776–84. doi: 10.1152/ajpendo.00145.2013

48. Walrand S, Short KR, Bigelow ML, Sweatt AJ, Hutson SM, Nair KS. Functional impact of high protein intake on healthy elderly people. *Am J Physiol Endocrinol Metab.* (2008) 295:E921–8. doi: 10.1152/ajpendo.90536.2008

49. Hahn O, Drews LF, Nguyen A, Tatsuta T, Gkioni L, Hendrich O, et al. A nutritional memory effect counteracts benefits of dietary restriction in old mice. *Nat Metab.* (2019) 1:1059–73. doi: 10.1038/s42255-019-0121-0

50. Vanden BJ, Marzorati M, Laukens D, Vanhaecke L. Validated high resolution mass Spectrometry-Based approach for metabolomic fingerprinting of the human gut phenotype. *Anal Chem.* (2015) 87:10927–34. doi: 10.1021/acs.analchem.5b02688

51. Siddik M, Shin AC. Recent progress on Branched-Chain amino acids in obesity, diabetes, and beyond. *Endocrinol Metab (Seoul).* (2019) 34:234–46. doi: 10.3803/EnM.2019.34.3.234

52. Palmer ND, Stevens RD, Antinozzi PA, Anderson A, Bergman RN, Wagenknecht LE, et al. Metabolomic profile associated with insulin resistance and conversion to diabetes in the Insulin Resistance Atherosclerosis Study. *J Clin Endocrinol Metab.* (2015) 100:E463–8. doi: 10.1210/jc.2014-2357

53. Zhu Y, Cong W, Shen L, Wei H, Wang Y, Wang L, et al. Fecal metabonomic study of a polysaccharide, MDG-1 from *Ophiopogon japonicus* on diabetic mice based on gas chromatography/time-of-flight mass spectrometry (GC TOF/MS). *Mol Biosyst.* (2014) 10:304–12. doi: 10.1039/c3mb70392d

54. Ulaszewska MM, Weinert CH, Trimigno A, Portmann R, Andres LC, Badertscher R, et al. Nutrimetabolomics: An integrative action for metabolomic analyses in human nutritional studies. *Mol Nutr Food Res.* (2019) 63:e1800384. doi: 10.1002/mnfr.201800384

55. Brosnan JT. Glutamate, at the interface between amino acid and carbohydrate metabolism. *J Nutr.* (2000) 130:988S–90S. doi: 10.1093/jn/130.4.988S

56. Meynial-Denis D. Glutamine metabolism in advanced age. *Nutr Rev.* (2016) 74:225–36. doi: 10.1093/nutrit/nuv052

57. Tome D. The roles of dietary glutamate in the intestine. *Ann Nutr Metab.* (2018) 73(Suppl 5):15–20. doi: 10.1159/000494777

58. Durainayagam B, Mitchell CJ, Milan AM, Zeng N, Sharma P, Mitchell SM, et al. Impact of a high protein intake on the plasma metabolome in elderly males: 10 Week Randomized Dietary Intervention. *Front Nutr.* (2019) 6:180. doi: 10.3389/fnut.2019.00180

59. Hamezah HS, Durani LW, Ibrahim NF, Yanagisawa D, Kato T, Shiino A, et al. Volumetric changes in the aging rat brain and its impact on cognitive and locomotor functions. *Exp Gerontol.* (2017) 99:69–79. doi: 10.1016/j.exger.2017.09.008

60. Fernstrom JD, Fernstrom MH. Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *J Nutr.* (2007) 137:1539S–47S. doi: 10.1093/jn/137.6.1539S

61. Matsuda M, Shimomura I. Increased oxidative stress in obesity: Implications for metabolic syndrome, diabetes, hypertension, dyslipidemia, atherosclerosis, and cancer. *Obes Res Clin Pract.* (2013) 7:e330–41. doi: 10.1016/j.orcp.2013.05.004

62. Baccolo G, Stamerra G, Coppola DP, Orlandi I, Vai M. Mitochondrial metabolism and aging in yeast. *Int Rev Cell Mol Biol.* (2018) 340:1–33. doi: 10.1016/bs.ircmb.2018.05.001

63. Jendrach M, Pohl S, Voth M, Kowald A, Hammerstein P, Bereiter-Hahn J. Morpho-dynamic changes of mitochondria during ageing of human endothelial cells. *Mech Ageing Dev.* (2005) 126:813–21. doi: 10.1016/j.mad.2005.03.002

64. Baik SH, Kang S, Lee W, Choi H, Chung S, Kim JI, et al. A breakdown in metabolic reprogramming causes microglia dysfunction in Alzheimer's disease. *Cell Metab.* (2019) 30:493–507. doi: 10.1016/j.cmet.2019.06.005

65. Yao J, Chen Y, Xu M. The critical role of short-chain fatty acids in health and disease: A subtle focus on cardiovascular disease-NLRP3 inflammasome-angiogenesis axis. *Clin Immunol.* (2022) 238:109013. doi: 10.1016/j.clim.2022.109013

66. Louis P, Scott KP, Duncan SH, Flint HJ. Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol.* (2007) 102:1197–208. doi: 10.1111/j.1365-2672.2007.03322.x

67. Rios-Covian D, Gonzalez S, Nogacka AM, Arboleya S, Salazar N, Gueimonde M, et al. An overview on fecal branched Short-Chain fatty acids along human life and as related with body mass index: Associated dietary and anthropometric factors. *Front Microbiol.* (2020) 11:973. doi: 10.3389/fmicb.2020.00973

68. Yu H, Guo Z, Shen S, Shan W. Effects of taurine on gut microbiota and metabolism in mice. *Amino Acids.* (2016) 48:1601–17. doi: 10.1007/s00726-016-2219-y

69. Aguirre M, Eck A, Koenen ME, Savelkoul P, Budding AE, Venema K. Diet drives quick changes in the metabolic activity and composition of human gut microbiota in a validated in vitro gut model. *Res Microbiol.* (2016) 167:114–25. doi: 10.1016/j.resmic.2015.09.006

70. Prokopidis K, Cervo MM, Gandham A, Scott D. Impact of protein intake in older adults with sarcopenia and obesity: A gut microbiota perspective. *Nutrients.* (2020) 12:2285. doi: 10.3390/nu12082285

71. Parada VD, De la Fuente MK, Landskron G, Gonzalez MJ, Quera R, Dijkstra G, et al. Short chain fatty acids (SCFAs)-Mediated gut epithelial and immune regulation and its relevance for inflammatory bowel diseases. *Front Immunol.* (2019) 10:277. doi: 10.3389/fimmu.2019.00277

72. Hou Y, Hu S, Li X, He W, Wu G. Amino acid metabolism in the liver: Nutritional and physiological significance. *Adv Exp Med Biol.* (2020) 1265:21–37. doi: 10.1007/978-3-030-45328-2_2

73. Furuhashi M. New insights into purine metabolism in metabolic diseases: Role of xanthine oxidoreductase activity. *Am J Physiol Endocrinol Metab.* (2020) 319:E827–34. doi: 10.1152/ajpendo.00378.2020

74. Zhang Y, Chen C, Choi H, Chaisson C, Hunter D, Niu J, et al. Purine-rich foods intake and recurrent gout attacks. *Ann Rheum Dis.* (2012) 71:1448–53. doi: 10.1136/annrheumdis-2011-201215

75. Kanbay M, Jensen T, Solak Y, Le M, Roncal-Jimenez C, Rivard C, et al. Uric acid in metabolic syndrome: From an innocent bystander to a central player. *Eur J Intern Med.* (2016) 29:3–8. doi: 10.1016/j.ejim.2015.11.026

76. Bronikowski AM, Meisel RP, Biga PR, Walters JR, Mank JE, Larschan E, et al. Sex-specific aging in animals: Perspective and future directions. *Aging Cell.* (2022) 21:e13542. doi: 10.1111/acel.13542

77. Sampathkumar NK, Bravo JI, Chen Y, Danthi PS, Donahue EK, Lai RW, et al. Widespread sex dimorphism in aging and age-related diseases. *Hum Genet.* (2020) 139:333–56. doi: 10.1007/s00439-019-02082-w

78. Lu RJ, Wang EK, Benayoun BA. Functional genomics of inflamm-aging and immunosenescence. *Brief Funct Genomics.* (2022) 21:43–55. doi: 10.1093/bfgp/elab009

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 04 January 2023
DOI 10.3389/fnut.2022.1035439

OPEN ACCESS

EDITED BY
Devin Wahl,
Colorado State University,
United States

REVIEWED BY
Qingyu Dou,
Sichuan University, China
Suman Chakrabarty,
West Bengal State University, India

*CORRESPONDENCE
Dongze Wu
✉ dongze_wu@163.com
Lai-shan Tam
✉ lstam@cuhk.edu.hk

SPECIALTY SECTION
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

RECEIVED 02 September 2022
ACCEPTED 05 December 2022
PUBLISHED 04 January 2023

CITATION
Jin YZ, So H, Cerin E, Barnett A, Mubarik S, Hezam KA, Feng XQ, Wang ZY, Huang JJ, Zhong CW, Hayat KH, Wang F, Wu A-M, Xu SW, Zou ZY, Lim L-L, Cai J, Song YM, Tam L-S and Wu DZ (2023) The temporal trend of disease burden attributable to metabolic risk factors in China, 1990–2019: An analysis of the Global Burden of Disease study. *Front. Nutr.* 9:1035439. doi: 10.3389/fnut.2022.1035439

The temporal trend of disease burden attributable to metabolic risk factors in China, 1990–2019: An analysis of the Global Burden of Disease study

Yingzhao Jin[1], Ho So[1], Ester Cerin[2,3], Anthony Barnett[2], Sumaira Mubarik[4], Kamal Hezam[5,6], Xiaoqi Feng[7,8,9], Ziyue Wang[10,11], Junjie Huang[12], Chenwen Zhong[12], Khezar Hayat[13,14], Fang Wang[15], Ai-Min Wu[16], Suowen Xu[17], Zhiyong Zou[18], Lee-Ling Lim[1,19], Jiao Cai[20], Yimeng Song[21], Lai-shan Tam[1]* and Dongze Wu[22,23]*

[1]Department of Medicine and Therapeutics, The Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong, China, [2]Mary MacKillop Institute for Health Research, Australian Catholic University, Melbourne, VIC, Australia, [3]School of Public Health, The University of Hong Kong, Hong Kong, Hong Kong SAR, China, [4]Department of Epidemiology and Biostatistics, Wuhan University, Wuhan, China, [5]Nankai University School of Medicine, Tianjin, China, [6]Department of Microbiology, Faculty of Applied Science, Taiz University, Taiz, Yemen, [7]Faculty of Medicine and Health, School of Population Health, University of New South Wales, Sydney, NSW, Australia, [8]Population Wellbeing and Environment Research Lab (PowerLab), Wollonggong, NSW, Australia, [9]The George Institute for Global Health, Newtown, NSW, Australia, [10]Department of Family Medicine, McGill University, Montreal, QC, Canada, [11]China Centre for Health Development Studies, Peking University, Beijing, China, [12]Jockey Club School of Public Health and Primary Care, The Chinese University of Hong Kong, Hong Kong, China, [13]Institute of Pharmaceutical Sciences, University of Veterinary and Animal Sciences, Lahore, Pakistan, [14]Department of Pharmacy Administration and Clinical Pharmacy, Xi'an Jiaotong University, Xi'an, China, [15]School of Public Health, Xuzhou Medical University, Xuzhou, China, [16]Department of Orthopaedics, Wenzhou Medical University, Wenzhou, China, [17]Department of Endocrinology, University of Science and Technology of China, Hefei, China, [18]Institute of Child and Adolescent Health, Peking University, Beijing, China, [19]Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, [20]Institute for Health and Environment, Chongqing University of Science and Technology, Chongqing, China, [21]School of the Environment, Yale University, New Haven, CT, United States, [22]Department of Rheumatology and Immunology, Sichuan Provincial People's Hospital, University of Electronic Science and Technology of China, Chengdu, China, [23]Chinese Academy of Sciences Sichuan Translational Medicine Research Hospital, Chengdu, China

Background and aims: The disease burden attributable to metabolic risk factors is rapidly increasing in China, especially in older people. The objective of this study was to (i) estimate the pattern and trend of six metabolic risk factors and attributable causes in China from 1990 to 2019, (ii) ascertain its association with societal development, and (iii) compare the disease burden among the Group of 20 (G20) countries.

Methods: The main outcome measures were disability-adjusted life-years (DALYs) and mortality (deaths) attributable to high fasting plasma glucose (HFPG), high systolic blood pressure (HSBP), high low-density lipoprotein (HLDL) cholesterol, high body-mass index (HBMI), kidney dysfunction (KDF), and low bone mineral density (LBMD). The average annual percent change (AAPC) between 1990 and 2019 was analyzed using Joinpoint regression.

Results: For all six metabolic risk factors, the rate of DALYs and death increased with age, accelerating for individuals older than 60 and 70 for DALYs and death, respectively. The AAPC value in rate of DALYs and death were higher in male patients than in female patients across 20 age groups. A double-peak pattern was observed for AAPC in the rate of DALYs and death, peaking at age 20–49 and at age 70–95 plus. The age-standardized rate of DALYs increased for HBMI and LBMD, decreased for HFPG, HSBP, KDF, and remained stable for HLDL from 1990 to 2019. In terms of age-standardized rate of DALYs, there was an increasing trend of neoplasms and neurological disorders attributable to HFPG; diabetes and kidney diseases, neurological disorders, sense organ diseases, musculoskeletal disorders, neoplasms, cardiovascular diseases, digestive diseases to HBMI; unintentional injuries to LBMD; and musculoskeletal disorders to KDF. Among 19 countries of Group 20, in 2019, the age-standardized rate of DALYs and death were ranked fourth to sixth for HFPG, HSBP, and HLDL, but ranked 10th to 15th for LBMD, KDF, and HBMI, despite the number of DALYs and death ranked first to second for six metabolic risk factors.

Conclusions: Population aging continuously accelerates the metabolic risk factor driven disease burden in China. Comprehensive and tight control of metabolic risk factors before 20 and 70 may help to mitigate the increasing disease burden and achieve healthy aging, respectively.

KEYWORDS

disease burden, metabolic risk factors, temporal trend, aging, China

Introduction

Increasing life expectancy has led to a global burden of late-life disease and research has recently been focused on ways of avoiding this trend in the general population (1). In China the period from 1950 to 2019 has seen the total fertility rate decrease from 5.91 to 1.43, yet life expectancy has increased from 49.6 to 74.7 years for men and from 52.6 to 80.8 years for women (2). The summary exposure values of metabolic risk factors increased from 14.90 to 22.14 in China from 1990 to 2019 with an annualized rate of change of 1.37% (3).

Understanding the contributions of metabolic risk factors to disease over time is vital to enabling healthy extended lifespans (4). According to a Global Burden of Disease (GBD) study from 2019, metabolic risk factors include high fasting plasma glucose (HFPG), high systolic blood pressure (HSBP), high low-density lipoprotein (HLDL) cholesterol, high body-mass index (HBMI), kidney dysfunction (KDF), and low bone mineral density (LBMD) (3). Metabolic risk factors have become the leading cause of ischemic heart disease in developing countries (5). The associations between type 2 diabetes with different cardiovascular diseases, including peripheral arterial disease, ischemic stroke, stable angina, heart failure, and non-fatal myocardial infarction have been established (6). Around 31.7% and 23.3% of patients with hypertension had blood pressure below 140/90 mm Hg and below 130/80 mm Hg, contributing to heart attack, stroke, and chronic kidney disease (7). HBMI accounted for 4.0 million deaths globally, more than two-thirds of which were due to cardiovascular disease, and nearly 40% of these occurred in people who were not obese (8). LBMD-associated increase in fracture risk affects virtually all skeletal sites, especially among older women and patients treated with glucocorticoid (9, 10). Occurring in a continuum with acute and chronic kidney disease, people with KDF are 5–10 times more likely to die prematurely, largely due to cardiovascular disease and cancer (11, 12). The principal target of lipid-modification therapies is to lower LDL cholesterol to prevent cardiovascular death, although recent research focuses on triglyceride-rich lipoproteins in addition to LDL as the causal risk factor for atherosclerosis (13).

Over the past three decades, the role of metabolic aging in extending a healthy lifespan has been increasingly acknowledged with population aging (14). Available evidence supports the idea that decreased nutrient signaling extends longevity and anabolic signaling accelerates aging (15). Previous study has investigated the mortality, morbidity, and risk factors in China (16). Therefore, our study further analyzed the disease burden of metabolic risk factors in the era of population aging. The Group of 20 (G20) may provide significant research insights that are highly relevant to the Chinese context, as the mean

population age in China was lower than most countries in G20 (2).

The objectives of this study were to (1) investigate the pattern of disease burden driven by six metabolic risk factors disaggregated by age and sex, (2) characterize the temporal trend of the six metabolic risk factors, (3) ascertain the temporal trend of metabolic risk attributable cause, (4) determine the association between societal development and metabolic risk factors, and (5) compare metabolic risk factors in China with those of G20 countries.

Methods

Data sources and data extraction

Global Burden of Disease study (GBD) 2019 was established to quantify the health loss caused by diseases, injuries, and risk factors, including 369 diseases, injuries, and 87 risk factors [including six metabolic risk factors–HFPG, HSBP, HLDL, HBMI, KDF, and LBMD (Case definition in Supplementary material)] (3, 17). This study was produced as part of the GBD Collaborator Network and in accordance with the GBD Protocol (IHME ID. 1775-GBD2019-012021). The study collected original data from the Global Health Data Exchange (GHDx), including age-sex-year disability adjusted life years (DALYs), years lived with disability (YLDs), years of life lost (YLLs), and death of six metabolic risk factors in terms of absolute number, age-standardized rate, and crude rate per 100,000 population.

Inclusion and exclusion criteria

According to the comparative risk assessment conceptual framework, the GBD 2019 study established a causal web of hierarchically organized risks or causes that contributed to health outcomes. A set of behavioral, environmental, occupational, and metabolic risk factors-outcome pairs were constructed based on evidence rules. The study included 23, 66, and 61 metabolic risk factor-level 2, 3, and 4 outcome pairs, and 108 risk-most detailed outcome pairs (The GBD metabolic risk factor attributable cause hierarchy in Supplementary material) (3). The study excluded GBD behavioral, environmental, and occupational risk factors attributable to cause hierarchy.

Population attributable fraction and socio-demographic index

The population attributable fractions (PAFs) were used to quantify the contribution of risk factors to the burden of disease (Estimation of six metabolic risk factors in Supplementary material) (18). The sociodemographic index (SDI) is a composite indicator of socio-demographic development status, which is strongly correlated with health outcomes. It is the geometric mean of 0 to 1 indices of total fertility rate in those under 25 years old, mean education for those aged 15 years or older, and lag-distributed income per capita. The national SDIs for China between 1990 and 2019 ranged from 0.433 to 0.686 (17).

Statistical analysis

The number and rate of DALYs, YLDs, YLLs, and deaths with 95% uncertainty intervals (UIs) of the six metabolic risk factors were reported according to age and sex from 1990 to 2019. Age-standardized rates of DALYs and death were plotted against SDI between 1990 and 2019 with a simple correlation. Temporal trend changes were determined using a Joinpoint regression model. Average annual percent change (AAPC) was calculated for the entire period analyzed, and annual percent change (APC) was calculated for each segmented line regression. The temporal trends were defined according to the statistical significance of the AAPC compared to zero. Any AAPC or APC with a 95% CI overlapping with zero was considered stable. All statistical analyses were performed using Joinpoint Regression Program (version 4.8.0.1, Statistical Methodology and Applications Branch, Surveillance Research Program, National Cancer Institute), with P-values <0.05 considered statistically significant.

Results

Trend of disease burden driven by six metabolic risk factors according to age and year

We first addressed how metabolic risk factor driven disease burden changed with increasing age. Overall, people aged 65–69 and 80–84 years had the highest number of DALYs and deaths, respectively (Figures 1A, B). The rate of DALYs and death increased with age, especially for people older than 60 and 70 for DALYs and deaths, respectively (Figures 1C, D). Notably, the disease burden attributable to KDF and LBMD increased rapidly with age (Supplementary Figures 1E, F, 2E, F). The disease burden was higher in male patients than female patients across different age groups for each (Supplementary Figures 1A–F, 2A–F) and all of the metabolic risk factors (Figures 1E–H). Besides, a double-peak pattern was observed for AAPC in the rate of DALYs, deaths, and YLLs, peaking at age 20 to 49 and at age 70 to 95 plus (Figures 1E, G, H); while a trumpet pattern was found for AAPC in the rate of YLDs (Figure 1F). A double-peak

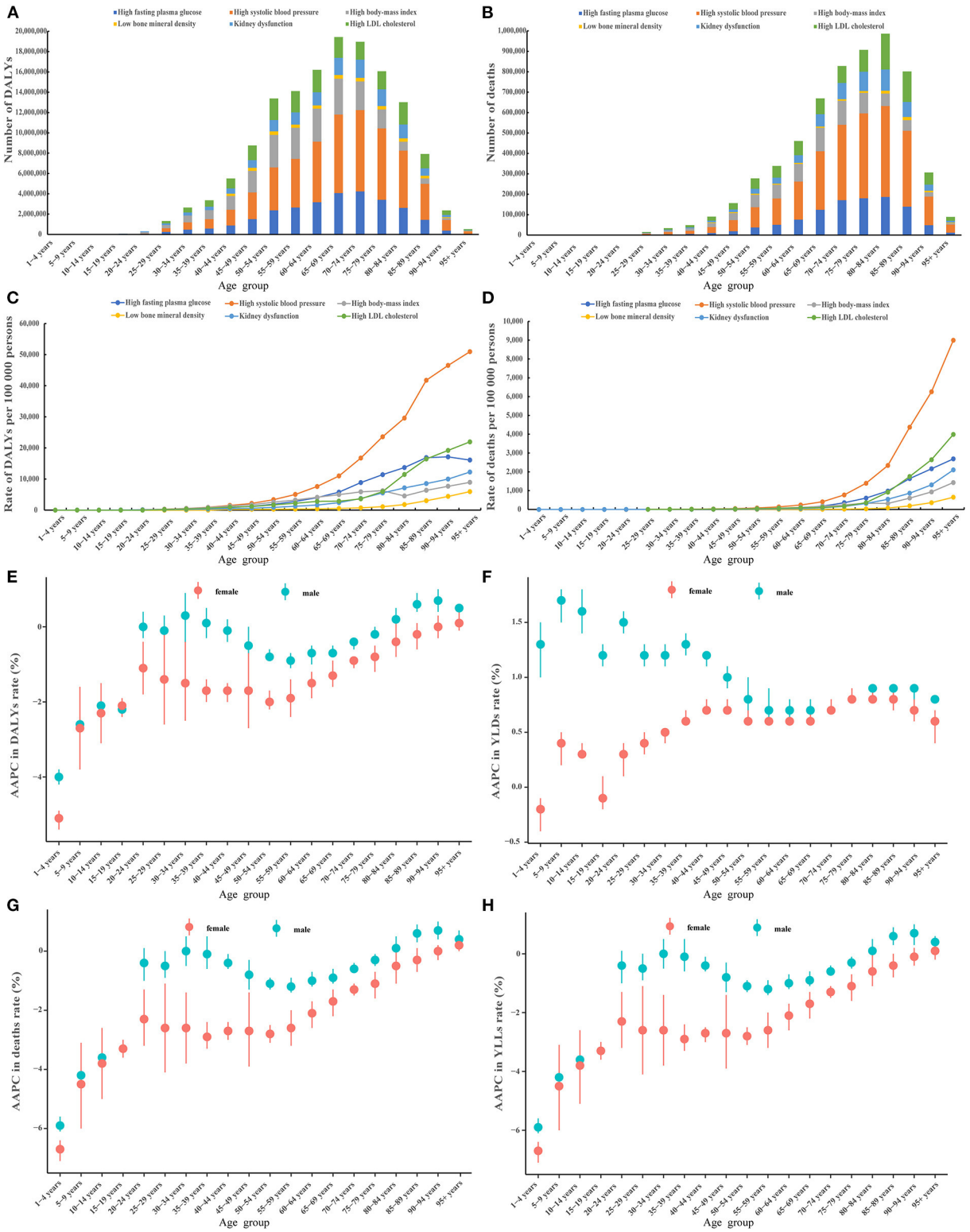


FIGURE 1
Cross-sectional and longitudinal trend of the disease burden attributable to six metabolic risk factors throughout the human lifespan. Number of DALYs attributable to six metabolic risk factors across 20 age groups in 2019 **(A)**, number of deaths attributable to six metabolic risk factors across 20 age groups in 2019 **(B)**, rate of DALYs attributable to six metabolic risk factors across 20 age groups in 2019 **(C)**, rate of deaths attributable to six metabolic risk factors across 20 age groups in 2019 **(D)**, AAPC in the rate of DALYs attributable to metabolic risk factors across
(Continued)

FIGURE 1 (Continued)
20 age groups, 1990–2019 **(E)**, AAPC in the rate of YLDs attributable to metabolic risk factors across 20 age groups, 1990–2019 **(F)**, AAPC in the rate of deaths attributable to metabolic risk factors across 20 age groups, 1990–2019 **(G)**, AAPC in the rate of YLLs attributable to metabolic risk factors across 20 age groups, 1990–2019 **(H)**. AAPC, average annual percent change; DALYs, disability-adjusted life years; YLDs, years lived with a disability; YLLs, years of life lost.

pattern was also observed for AAPC in the rate of HFPG, HLDL, HSBP (Supplementary Figures 1B–D, 2B–D).

We further determined how the disease burden changed with age. A strong increasing trend was found across the 16 age groups, especially in people older than 60 (Supplementary Figure 3). Amongst all subjects aged 15 to 94, individuals aged 60–74 contributed the greatest numbers of DALYs and the 75–94 age group contributed the greatest number of deaths (Supplementary Figures 4, 5).

We next ascertained if the gender differences in the six metabolic risk factors existed. The numbers and age-standardized rate of DALYs and deaths were higher in male than female patients for five out of six metabolic risk factors (except LBMD) (Supplementary Figure 6, Supplementary Table 1). The age-standardized rate of YLDs was higher in male than female patients for five out of six metabolic risk factors (except HFPG), while the age-standardized rate of YLLs was higher in female than male patients for all six metabolic risk factors (Supplementary Figure 7).

Temporal trend of metabolic risk factors from 1990 to 2019

From 1990 to 2019, the number of DALYs and deaths attributable to the six metabolic risk factors showed rapid growth, with the highest rate for HBMI (DALYs: 4.0; death: 4.2) with no sex-based difference (Table 1, Supplementary Figure 8, Supplementary Table 1). The age standardized rate of DALYs increased for HBMI (1.28) and LBMD (0.18), decreased for HFPG (−0.41), HSBP (−0.89), KDF (−0.61), and remained stable for HLDL. The age standardized rate of death increased for HBMI (1.03), LBMD (0.38), HLDL (0.45), decreased for HSBP (−0.78) and remained stable for HFPG (−0.41) and KDF (−0.10) (Table 1, Supplementary Figure 8). A sex difference was identified in the trend of the age-standardized rate of DALYs for HFPG and HLDL, the age-standardized rate of death for HFPG, HSBP, KDF, and HLDL (Supplementary Table 1).

For the 22 level 2 causes, the number of DALYs and death increased from 1990 to 2019, with the highest speed in HBMI attributable neurological disorders (DALYs: 6.7; death: 6.8) (Table 2). In terms of age-standardized rate of DALYs, there was an increasing trend of neoplasms and neurological disorders attributable to HFPG, diabetes and kidney diseases, neurological disorders, sense organ diseases, musculoskeletal disorders, neoplasms, cardiovascular diseases, digestive diseases attributable to HBMI, unintentional injuries attributable to LBMD, and musculoskeletal disorders attributable to KDF (Table 2). In terms of age-standardized rate of death, there was an increasing trend of neoplasms attributable to HFPG, cardiovascular diseases attributable to HSBP, neurological disorders, diabetes and kidney diseases, neoplasms, cardiovascular diseases attributable to HBMI, unintentional injuries attributable to LBMD (Table 2). A similar trend was also observed in level 3, level 4, and detailed causes (Supplementary Tables 2–4).

Temporal trend of the effect of metabolic risk factors on cause from 1990 to 2019

In 2019, in terms of age-standardized rate of DALYs, HBMI contributed to 29.4, 15.1, 3.7% of diabetes and kidney diseases, cardiovascular diseases, neoplasms, HFPG to 73.8, 14.5, 2.8, 2.5% of diabetes and kidney diseases, cardiovascular diseases, neoplasms, neurological disorders. HLDL contributed to 21.3% of cardiovascular diseases, HSBP to 54.1%, 21.4% of cardiovascular diseases, diabetes and kidney diseases, KDF to 38.3%, 8.0% of diabetes and kidney diseases, cardiovascular diseases, and LBMD to 10.3%, 7.2% of unintentional injuries, transport injuries (Supplementary Table 5). Similar results were found for the contribution of the six metabolic risk factors to disease burden in terms of age-standardized rate of death (Supplementary Table 5).

Analysis of the PAF of age standardized DALYs rate indicated a significant increasing contribution of HFPG to neoplasms (2.1), respiratory infections and tuberculosis (0.7), cardiovascular diseases (0.7), neurological disorders (0.5), diabetes and kidney diseases (0.4), increasing contribution of HSBP to cardiovascular diseases (0.5), diabetes and kidney diseases (0.3), increasing contribution of HBMI to digestive diseases (4.0), chronic respiratory diseases (3.8), diabetes and kidney diseases (3.0), neurological disorders (2.8), musculoskeletal disorders (2.5), sense organ diseases (2.5), cardiovascular diseases (2.3), neoplasms (2.2), increasing contribution of LBMD to unintentional injuries (3.4), self-harm and interpersonal violence (2.0), transport injuries (1.0), increasing contribution of HLDL to cardiovascular diseases (1.4), increasing contribution of KDF to cardiovascular diseases (1.0) and musculoskeletal disorders (1.5), but a significant decreasing contribution to diabetes and kidney diseases (−0.5) (Table 3, Supplementary Figure 9). Analysis of PAF of age

TABLE 1 Trends in the number and age-standardized rate of DALYs and death attributable to six metabolic risk factors for both sexes in China, 1990–2019.

		1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)
DALYs	Number (95% UI)						
	High fasting plasma glucose	13,423,677 (10,777,527–16,387,796)	28,228,439 (22,052,698–35,375,022)	2.5 (2.1–2.9)	2.8 (2.2–3.4)	3.7 (2.9–4.6)	1.1 (0.6–1.7)
	High systolic blood pressure	29,372,404 (24,312,404–34,731,124)	54,441,615 (45,474,002–63,689,070)	2.1 (2.0–2.3)	1.6 (1.5–1.7)	2.7 (2.4–3.0)	1.8 (1.8–1.9)
	High body-mass index	7,876,348 (2,002,230–16,754,458)	24,830,041 (11,788,976–40,545,899)	4.0 (3.9–4.2)	3.9 (3.8–4.0)	4.4 (3.9–4.9)	3.6 (3.5–3.7)
	Low bone mineral density	1,339,535 (1,100,875–1,640,114)	3,320,275 (2,594,480–4,004,265)	3.4 (3.2–3.5)	3.4 (3.2–3.5)	3.4 (3.2–3.5)	3.4 (3.2–3.5)
	Kidney dysfunction	7,678,849 (6,630,862–8,750,551)	13,353,909 (11,150,932–15,771,793)	2.0 (1.8–2.2)	1.3 (1.1–1.5)	2.9 (2.4–3.3)	1.5 (1.0–1.9)
	High LDL cholesterol	8,889,099 (7,139,095–11,088,753)	19,813,962 (15,205,317–25,139,359)	2.8 (2.7–3.0)	2.0 (1.9–2.1)	3.8 (3.5–4.1)	2.2 (1.9–2.5)
	Age standardized rate per 100,000 population (95% UI)						
	High fasting plasma glucose	1,605 (1,283–1,965)	1,452 (1,139–1,832)	−0.4 (−0.8 to 0.0)	0.1 (−0.4 to 0.7)	0.8 (0.0–1.6)	−2.1 (−2.6 to −1.6)
	High systolic blood pressure	3,672 (3,073–4,301)	2,844 (2,392–3,321)	−0.9 (−1.0 to −0.8)	−1.3 (−1.4 to −1.3)	−0.3 (−0.6 to 0)	−1.4 (−1.5 to −1.3)
	High body-mass index	852 (214–1,834)	1,231 (578–2,023)	1.3 (1.1–1.4)	1.1 (1.0–1.2)	1.6 (1.2–1.9)	1.0 (0.8–1.2)
	Low bone mineral density	168 (138–204)	177 (138–214)	0.2 (0.0–0.3)	−0.4 (−0.8 to 0.0)	0.7 (0.6–0.8)	0.2 (0.0–0.4)
	Kidney dysfunction	863 (747–995)	709 (593–832)	−0.6 (−0.9 to −0.4)	−1.2 (−1.5 to −0.9)	0.5 (0.2–0.8)	−1.5 (−2.0 to−0.9)
	High LDL cholesterol	1,046 (826–1,346)	1,052 (800–1,345)	0.0 (−0.1 to 0.1)	−0.7 (−0.8 to −0.7)	1.2 (0.8–1.5)	−0.8 (−0.8 to −0.7)
Death	Number (95% UI)						
	High fasting plasma glucose	450,809 (343,516–575,374)	1,067,554 (793,048–1,442,201)	2.9 (2.3–3.4)	3.3 (2.9–3.7)	4.2 (2.9–5.6)	1.1 (0.4–1.8)
	High systolic blood pressure	1,222,195 (1,017,782–1,445,891)	2,599,879 (2,138,699–3,082,235)	2.6 (2.4–2.8)	1.7 (1.6–1.8)	3.4 (3.0–3.9)	2.2 (2.1–2.3)
	High body-mass index	234,998 (58,375–513,815)	764,698 (333,163–1,310,557)	4.2 (3.9–4.4)	3.7 (3.6–3.9)	4.4 (3.9–4.9)	4.0 (3.7–4.4)
	Low bone mineral density	32,002 (26,081–42,555)	89,857 (58,927–110,423)	4.3 (3.9–4.7)	4.3 (3.9–4.7)	4.3 (3.9–4.7)	4.3 (3.9–4.7)
	Kidney dysfunction	248,344 (210,134–288,765)	573,537 (467,902–690,324)	3.0 (2.7–3.3)	1.8 (1.7–2.0)	4.4 (3.7–5.1)	2.2 (1.8–2.6)
	High LDL cholesterol	317,060 (244,158–413,836)	915,983 (647,993–1,239,382)	3.7 (3.6–3.9)	2.4 (2.3–2.5)	5.3 (4.9–5.7)	3.0 (2.8–3.1)
	Age standardized rate per 100,000 population (95% UI)						
	High fasting plasma glucose	67 (51–89)	62 (45–85)	−0.4 (−0.9–0.1)	0.4 (0.1–0.7)	0.9 (0.0–1.9)	−2.5 (−3.8 to −1.3)
	High systolic blood pressure	191 (160–226)	153 (126–182)	−0.8 (−0.9 to −0.7)	−1.3 (−1.4 to −1.2)	0.0 (−0.3–0.4)	−1.5 (−1.6 to −1.4)

(Continued)

TABLE 1 (Continued)

		1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)
	High body-mass index	30 (7–67)	41 (18–71)	1.0 (0.8–1.3)	0.8 (0.6–1.0)	1.4 (0.7–2.0)	0.6 (0.5–0.8)
	Low bone mineral density	5 (4–7)	6 (4–7)	0.4 (0.2–0.6)	−0.7 (−0.9 to −0.5)	3.1 (2.7–3.4)	−1.3 (−1.3 to −1.2)
	Kidney dysfunction	36 (30–42)	34 (27–41)	−0.1 (−0.4 to 0.2)	−0.9 (−1.1 to −0.6)	1.4 (0.5–2.2)	−1.3 (−1.6 to −1.1)
	High LDL cholesterol	49 (35–68)	56 (38–77)	0.4 (0.3–0.6)	−0.3 (−0.4 to −0.2)	2.1 (1.7–2.4)	−0.9 (−1 to −0.8)

AAPC, average annual percent changes; APC, annual percent change; DALYs, disability-adjusted life years; UI, uncertainty interval; CI, confidence interval.

standardized death rate also showed a similar trend (Table 3, Supplementary Figure 10).

Association between metabolic risk factor and SDI

There was a positive correlation between the SDI level and the number of six metabolic risk factors attributable DALYs, YLDs, deaths, and YLLs (Supplementary Table 6, Supplementary Figure 11). The SDI level showed a positive correlation with the age-standardized rate of DALYs for HBMI, LBMD, and HLDL, but a negative correlation with DALYs for HSBP and KDF. The SDI level showed a positive correlation with the age-standardized rate of deaths for HBMI, KDF, and HLDL, but a negative correlation with HSBP. The SDI level was positively correlated with the age-standardized rate of YLDs for all six metabolic risk factors except LBMD. The SDI level was positively correlated with the age-standardized rate of deaths YLLs for HBMI, and HLDL, but was negatively correlated with HFPG, HSBP, and KDF (Supplementary Table 6, Supplementary Figure 11).

Overall burden ranking in Group of 20 (G20) countries

Among 19 countries of Group 20, in 2019, China ranked 1^{st}, 1^{st}, 1^{st}, 2^{nd}, 2^{nd}, 2^{nd} for HSBP, HBMI, HLDL, HFPG, LBMD, KDF in terms of number of DALYs and ranked 1^{st}, 1^{st}, 1^{st}, 1^{st}, 2^{nd}, 2^{nd} for HSBP, HBMI, HLDL, KDF, HFPG, LBMD in terms of number of deaths, respectively (Figure 2, Supplementary Table 7). The age-standardized rate of DALYs for HFPB, HSBP, HLDL, LBMD, KDF, HBMI ranked 4^{th}, 6^{th}, 6^{th}, 10^{th}, 11^{th}, 15^{th}, respectively (Figure 2, Supplementary Table 7). The age standardized rate of death for LBMD, HSBP, HLDL, KDF, HFPG, HBMI ranked 4^{th}, 5^{th}, 5^{th}, 10^{th}, 12^{th}, 15^{th}, respectively (Figure 2, Supplementary Table 7).

Discussion

Our findings support the view that mitigation of metabolic risk factors provides a unique opportunity to achieve better health in the future. At first, 36 independent drivers of global health, especially 5 metabolic risk factors, were expected to deteriorate without comprehensive management, although most of them were forecast to improve by 2040 (19). In China, the population aged >65 is increasing rapidly and those aged >65 and >80 may reach 400 million and 150 million, respectively, before 2050 (20). Most importantly, our data support the view that risk factor driven disease burdens increased with age, reaching a peak for individuals aged >60.

Our results call for early and tight control of metabolic risk factors starting before the first peak (age 20–49) targeting adolescence. As a pivotal point in the life course characterized by openness to change, adolescence offers a unique window of opportunity to promote the adoption of a healthy lifestyle (diet and physical activity). However, this window of opportunity has largely been overlooked in behavior and policy research (21). Growing up in an era of "toxic" food environment, the current generation of adolescents may face unprecedented disease burdens from metabolic risk factors in their later life (22). The pace of change in the nutritional habits of adolescents poses a great threat to their health. National and individual efforts should identify key meanings and context of their food choices and seek to improve their food environments and choices by harnessing widely shared adolescent values that go beyond nutrition or health (23). Our findings also provided us an impetus to pharmacologically target key metabolic pathways linked to longevity before the second peak factors with the hope of delaying aging and ameliorating age-related diseases (24). On the one hand, a hallmark of aging is metabolism dysfunction, especially glucose homeostasis, negatively regulating energy metabolism and ultimately increasing the organism's susceptibility to disease. On the other hand, metabolic dysfunction occurs increasingly with age, including modulation of mitochondrial function, a decline in insulin sensitivity, and alterations in substrate utilization,

TABLE 2 Trends in the number and age-standardized rate of DALYs and death for level 2 causes attributable to six metabolic risk factors in China, 1990–2019.

	Number (95% UI)						Age standardized rate per 100,000 population (95% UI)					
	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)
DALYs												
High fasting plasma glucose												
Neoplasms	603,435 (152,503–1,309,632)	1,955,214 (496,063–4,292,456)	4.1 (3.9–4.3)	4.1 (3.8–4.3)	5.7 (5.4–6.0)	2.6 (2.1–3.1)	70 (18–151)	95 (24–210)	1.0 (0.8–1.3)	1.4 (1.1–1.7)	2.8 (2.4–3.1)	−0.9 (−1.5 to −0.3)
Cardiovascular diseases	6,657,471 (4,752,045–9,121,111)	13,585,849 (9,679,707–18,867,220)	2.4 (1.8–3.0)	3.1 (2.2–4.0)	3.6 (2.4–4.7)	0.5 (−0.3 to 1.4)	856 (611–1,173)	715 (502–1,022)	−0.7 (−1.3 to −0.2)	0.3 (−0.6 to 1.2)	0.7 (−0.5 to 2.0)	−3.1 (−3.9 to −2.3)
Neurological disorders	124,438 (18,782–445,419)	442,695 (73,488–1,425,147)	4.4 (4.2–4.6)	4.8 (4.4–5.2)	5.6 (5.1–6.0)	2.9 (2.7–3.2)	24 (4–85)	27 (4–89)	0.4 (0.2–0.6)	1.2 (0.8–1.6)	1.7 (1.3–2.1)	−1.6 (−1.8 to −1.3)
Sense organ diseases	31,679 (7,189–76,325)	84,914 (18,868–200,923)	3.5 (3.3–3.7)	5.9 (5.5–6.3)	3.5 (3.2–3.8)	1.5 (1.1–1.9)	5 (1–12)	5 (1–11)	−0.2 (−0.4 to 0.1)	2.2 (1.8–2.6)	−0.3 (−0.6 to 0.0)	−2.4 (−2.7 to −2.0)
Respiratory infections and tuberculosis	389,268 (234,926–568,709)	125,538 (73,808–183,637)	−3.9 (−4.3 to −3.5)	−3.9 (−4.8 to −2.9)	−4.0 (−4.7 to −3.3)	−3.8 (−4.2 to −3.5)	42 (25–62)	6 (4–9)	−6.4 (−6.8 to −6.1)	−6.4 (−7.3 to −5.5)	−7.1 (−7.8 to −6.5)	−6.2 (−6.5 to −5.9)
Diabetes and kidney diseases	5,617,387 (4,687,144–6,682,997)	12,034,230 (9,908,708–14,504,239)	2.6 (2.5–2.8)	2.6 (2.5–2.7)	3.4 (3.0–3.8)	1.9 (1.8–2.0)	608 (510–718)	604 (499–726)	0.0 (−0.1 to 0.1)	0.1 (0.0–0.1)	0.7 (0.4–1.1)	−0.8 (−0.9 to −0.8)
High systolic blood pressure												
Cardiovascular diseases	27,793,037 (22,943,586–33,182,723)	51,069,180 (42,484,465–59,828,159)	2.1 (2.0–2.2)	1.5 (1.5–1.6)	2.6 (2.3–2.9)	1.8 (1.7–1.9)	3,491 (2,894–4,115)	2,670 (2,232–3,124)	−0.9 (−1.1 to −0.8)	−1.4 (−1.5 to −1.3)	−0.4 (−0.7 to 0.0)	−1.5 (−1.5 to −1.4)
Diabetes and kidney diseases	1,579,368 (1,317,964–1,870,237)	3,372,435 (2,783,861–4,004,996)	2.7 (2.5–3.0)	2.2 (1.9–2.5)	3.7 (3.4–3.9)	2.1 (1.5–2.7)	182 (153–211)	174 (144–206)	−0.1 (−0.3 to 0.2)	−0.7 (−0.9 to −0.4)	1.0 (0.7–1.4)	−0.9 (−1.5 to −0.2)
High body-mass index												
Neoplasms	885,448 (194,534–2,154,883)	2,669,799 (1,094,208–4,839,179)	3.9 (3.7–4.1)	5.0 (4.9–5.1)	2.6 (2.4–2.9)	3.9 (3.3–4.5)	94 (21–227)	128 (53–233)	1.1 (0.9–1.2)	2.3 (2.2–2.3)	−0.2 (−0.4 to 0.0)	1.1 (0.6–1.6)
Cardiovascular diseases	5,327,955 (1,329,819–11,290,734)	15,004,139 (6,947,157–24,943,213)	3.6 (3.5–3.8)	3.5 (3.3–3.6)	3.9 (3.6–4.2)	3.3 (3.1–3.4)	577 (142–1,251)	747 (344–1,254)	0.9 (0.7–1.1)	0.6 (0.4–0.9)	1.1 (0.6–1.6)	0.6 (0.5–0.7)

(Continued)

10.3389/fnut.2022.1035439

TABLE 2 (Continued)

	Number (95% UI)						Age standardized rate per 100,000 population (95% UI)					
	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)
Chronic respiratory diseases	98,457 (25,467–228,184)	175,325 (75,052–320,742)	2.1 (1.9–2.2)	2.1 (1.9–2.3)	−0.3 (−0.5 to 0.0)	4.4 (3.9–4.9)	11 (3–26)	10 (5–19)	−0.1 (−0.3 to 0.2)	−0.5 (−0.7 to −0.3)	−2.4 (−2.9 to −1.9)	2.8 (2.3–3.2)
Digestive diseases	139,950 (32,648–342,137)	384,258 (158,489–746,027)	3.5 (3.4–3.6)	3.0 (2.9–3.1)	3.9 (3.7–4.0)	3.4 (3.3–3.5)	15 (4–37)	19 (8–37)	0.8 (0.7–0.9)	0.1 (0.0–0.3)	1.0 (0.8–1.1)	1.1 (0.9–1.3)
Neurological disorders	72,969 (8,730–252,707)	477,209 (101,059–1,388,766)	6.7 (6.6–6.8)	6.1 (6.0–6.2)	7.2 (7.0–7.4)	6.7 (6.5–6.8)	13 (2–46)	28 (6–82)	2.7 (2.6–2.8)	2.4 (2.3–2.5)	3.1 (3.0–3.3)	2.4 (2.3–2.5)
Musculoskeletal disorders	273,363 (58,598–684,499)	1,133,691 (431,660–2,264,916)	5.0 (4.9–5.1)	3.4 (3.1–3.6)	6.6 (6.5–6.6)	5.0 (4.9–5.1)	28 (6–70)	55 (21–110)	2.3 (2.2–2.4)	0.8 (0.4–1.2)	3.5 (3.5–3.6)	2.4 (2.3–2.5)
Sense organ diseases	9,122 (1,861–23,630)	47,688 (15,902–101,631)	5.9 (5.7–6.2)	7.2 (6.6–7.8)	4.7 (4.6–4.8)	5.2 (4.7–5.8)	1 (0–3)	3 (1–5)	2.4 (2.2–2.5)	3.8 (3.4–4.2)	1.3 (1.2–1.3)	1.5 (1.1–1.8)
Diabetes and kidney diseases	1,069,083 (291,743–2,209,261)	4,937,932 (2,514,211–7,732,009)	5.4 (5.3–5.5)	5.1 (5.0–5.3)	6.9 (6.6–7.2)	4.2 (4.1–4.3)	113 (30–236)	240 (122–379)	2.7 (2.5–2.8)	2.3 (2.2–2.5)	4.0 (3.6–4.4)	1.6 (1.5–1.7)
Low bone mineral density												
Transport injuries	617,844 (498,088–816,274)	1,274,510 (1,013,062–1,507,922)	2.5 (2.3–2.7)	2.8 (2.4–3.2)	4.8 (4.5–5.1)	−0.4 (−0.8 to 0.0)	67 (54–88)	62 (50–74)	−0.3 (−0.4 to −0.1)	−0.2 (−0.5 to 0.0)	1.4 (1.3–1.6)	−2.3 (−2.5 to −2.2)
Unintentional injuries	699,578 (569,079–854,117)	2,015,682 (1,477,479–2,535,537)	3.7 (3.6–3.8)	2.5 (2.3–2.6)	3.4 (3.3–3.5)	5.4 (5.3–5.5)	99 (81–120)	113 (82–143)	0.5 (0.3–0.6)	−0.6 (−0.7 to −0.5)	0.2 (−0.2 to 0.5)	1.9 (1.6–2.1)
Self-harm and interpersonal violence	22,113 (17,657–27,191)	30,084 (22,865–38,560)	1.1 (0.9–1.2)	1.0 (1.0–1.1)	1.3 (0.9–1.8)	0.9 (0.6–1.2)	3 (2–3)	1 (1–2)	−1.9 (−2.0 to −1.8)	−2.0 (−2.1 to −1.9)	−1.6 (−1.8 to −1.4)	−1.9 (−2.2 to −1.7)
Kidney dysfunction												
Cardiovascular diseases	3,669,169 (2,953,636–4,473,079)	7,475,542 (5,796,532–9,391,024)	2.5 (2.2–2.9)	1.5 (1.2–1.8)	4.1 (3.2–5.0)	1.7 (1.2–2.1)	451 (363–551)	395 (304–495)	−0.4 (−0.7 to −0.2)	−1.3 (−1.6 to −1)	1.2 (0.9–1.5)	−1.6 (−2.2 to −0.9)
Musculoskeletal disorders	12,654 (7,655–18,640)	46,523 (28,108–68,591)	4.6 (4.5–4.8)	1.6 (1.4–1.7)	6.8 (6.7–6.9)	5.5 (5.1–5.9)	2 (1–2)	2 (1–4)	1.3 (1.1–1.5)	−1.4 (−1.6 to −1.2)	3.6 (3.4–3.7)	1.9 (1.4–2.3)

(Continued)

10.3389/fnut.2022.1035439

TABLE 2 (Continued)

	Number (95% UI)						Age standardized rate per 100,000 population (95% UI)					
	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)
Diabetes and kidney diseases	3,997,026 (3,531,282–4,458,760)	5,831,843 (4,992,206–6,645,333)	1.4 (1.0–1.7)	1.1 (0.8–1.4)	1.8 (1.5–2.0)	1.1 (0.1–2.2)	410 (364–457)	312 (268–354)	−0.9 (−1.2 to −0.5)	−1.1 (−1.3 to −0.8)	−0.4 (−0.6 to −0.1)	−1.3 (−2.4 to −0.3)
High LDL cholesterol												
Cardiovascular diseases	8,889,099 (7,139,095–11,088,753)	19,813,962 (15,205,317–25,139,359)	2.8 (2.7–3.0)	2.0 (1.9–2.1)	3.8 (3.5–4.1)	2.2 (1.9–2.5)	1,046 (826–1,346)	1,052 (800–1,345)	0.0 (−0.1 to 0.1)	−0.7 (−0.8 to −0.7)	1.2 (0.8–1.5)	−0.8 (−0.8 to −0.7)
Death												
High fasting plasma glucose												
Neoplasms	24,887 (6,343–53,939)	90,655 (23,078–197,161)	4.5 (4.3–4.8)	4.8 (4.4–5.1)	6.3 (5.9–6.7)	2.7 (2.0–3.3)	3 (1–7)	5 (1–10)	1.2 (1.0–1.5)	1.9 (1.6–2.3)	2.9 (2.5–3.3)	−1.0 (−1.7 to −0.3)
Cardiovascular diseases	298,051 (208,303–414,537)	700,341 (481,073–1,026,949)	2.9 (2.2–3.5)	3.8 (2.7–4.8)	4.6 (3.0–6.2)	0.3 (−0.4 to 1.1)	47 (33–69)	41 (28–62)	−0.6 (−1.3 to 0.1)	0.6 (−0.5 to 1.7)	0.8 (−1.0 to 2.5)	−3.5 (−4.2 to −2.8)
Neurological disorders	6,521 (629–26,296)	23,988 (2,536–94,088)	4.6 (4.3–4.8)	4.6 (4.5–4.7)	6.2 (5.7–6.7)	3.0 (2.5–3.5)	2 (0–7)	2 (0–7)	0.1 (−0.1 to 0.3)	0.5 (0.4–0.7)	1.7 (1.4–2.1)	−2.0 (−2.2 to −1.7)
Respiratory infections and tuberculosis	12,665 (7,319–19,035)	3,646 (2,056–5,638)	−4.3 (−4.7 to −3.8)	−3.9 (−5.0 to −2.8)	−5.3 (−6.1 to −4.5)	−4.0 (−4.4 to −3.7)	2 (1–2)	0 (0–0)	−7.0 (−7.4 to −6.6)	−6.2 (−7.1 to −5.3)	−8.3 (−9.0 to −7.5)	−7.0 (−7.3 to −6.7)
Diabetes and kidney diseases	108,685 (95,113–124,011)	248,925 (211,404–287,553)	2.9 (2.7–3.1)	3.0 (2.7–3.2)	2.8 (2.3–3.2)	2.8 (2.6–2.9)	14 (12–16)	14 (12–16)	−0.1 (−0.3 to 0.1)	0.2 (0.0–0.4)	0.0 (−0.7 to 0.6)	−0.6 (−0.7 to −0.5)
High systolic blood pressure												
Cardiovascular diseases	1,172,880 (972,201–1,384,099)	2,471,920 (2,026,048–2,942,576)	2.6 (2.4–2.8)	1.7 (1.6–1.8)	3.4 (2.9–3.9)	2.2 (2.1–2.3)	184 (153–218)	146 (120–174)	−0.8 (−1.0 to −0.7)	−1.3 (−1.4 to −1.2)	0.0 (−0.4 to 0.4)	−1.5 (−1.6 to −1.4)
Diabetes and kidney diseases	49,315 (41,426–58,188)	127,960 (105,241–151,102)	3.3 (3.2–3.5)	2.4 (2.1–2.7)	4.3 (4.0–4.6)	2.9 (2.8–3.0)	7 (6–8)	7 (6–9)	0.1 (−0.1 to 0.2)	−0.5 (−0.6 to −0.4)	1.1 (0.7–1.5)	−0.9 (−1.0 to −0.7)
High body-mass index												
Neoplasms	30,067 (6,567–73,656)	100,442 (41,168–185,335)	4.3 (4.2–4.4)	5.2 (5.0–5.3)	3.2 (3.1–3.2)	4.4 (4.2–4.7)	4 (1–9)	5 (2–9)	1.2 (0.9–1.5)	2.3 (1.9–2.7)	0.2 (0.0–0.3)	1.1 (0.3–1.9)

(Continued)

10.3389/fnut.2022.1035439

TABLE 2 (Continued)

	Number (95% UI)						Age standardized rate per 100,000 population (95% UI)					
	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2009–2019)
Cardiovascular diseases	178,080 (43,400–389,585)	549,540 (242,018–949,280)	3.9 (3.7–4.2)	3.4 (3.2–3.5)	4.4 (3.8–4.9)	3.7 (3.6–3.8)	22 (5–50)	29 (13–52)	0.9 (0.7–1.1)	0.4 (0.2–0.5)	1.3 (0.9–1.8)	0.6 (0.5–0.7)
Chronic respiratory diseases	2,514 (549–6,524)	3,270 (1,235–6,227)	0.9 (0.5–1.3)	1.6 (1.5–1.7)	−0.8 (−1.7 to 0.2)	1.8 (1.6–2.1)	0 (0–1)	0 (0–0)	−2.4 (−2.7 to −2.2)	−1.4 (−1.6 to −1.3)	−4.1 (−4.9 to −3.2)	−1.9 (−2.1 to −1.8)
Digestive diseases	1,846 (428–4,213)	3,053 (1,235–5,627)	1.7 (1.3–2.1)	1.6 (0.6–2.5)	0.2 (−0.2 to 0.5)	3.3 (2.8–3.8)	0 (0–1)	0 (0–0)	−1.4 (−1.8 to −1.1)	−1.1 (−1.9 to −0.2)	−2.7 (−3 to −2.5)	−0.6 (−1.0 to −0.2)
Neurological disorders	3,531 (290–13,782)	23,787 (3,264–78,659)	6.8 (6.7–6.9)	5.8 (5.7–5.9)	7.6 (7.3–7.8)	6.8 (6.6–7.0)	1 (0–3)	2 (0–6)	2.3 (2.2–2.5)	1.7 (1.4–2.0)	3.1 (2.9–3.3)	2.1 (1.9–2.2)
Diabetes and kidney diseases	18,961 (5,077–39,582)	84,607 (39,544–139,620)	5.3 (5.0–5.5)	5.1 (4.9–5.4)	5.6 (4.9–6.3)	4.8 (4.7–5.0)	2 (1–5)	4 (2–7)	2.2 (1.9–2.4)	2.3 (2.0–2.5)	2.6 (1.8–3.3)	1.3 (1.1–1.5)
Low bone mineral density												
Transport injuries	15,250 (12,207–21,550)	29,232 (23,067–35,070)	2.3 (2.1–2.4)	2.7 (2.4–3.1)	5.0 (4.8–5.2)	−1.3 (−1.6 to −1.0)	2 (1–2)	1 (1–2)	−0.6 (−0.7 to −0.5)	−0.1 (−0.3 to 0.2)	1.6 (1.5–1.8)	−3.9 (−4.1 to −3.7)
Unintentional injuries	16,302 (13,347–21,271)	60,259 (32,787–76,443)	4.6 (4.2–5.0)	2.1 (1.7–2.5)	7.8 (6.9–8.7)	4.0 (3.8–4.2)	3 (3–4)	4 (2–5)	0.8 (0.5–1.0)	−1.1 (−1.3 to −0.9)	3.9 (3.4–4.3)	−0.3 (−0.6 to 0.1)
Self-harm and interpersonal violence	449 (367–527)	366 (296–447)	−0.7 (−0.9 to −0.5)	0.1 (0.0–0.2)	−0.3 (−0.7 to 0.1)	−1.8 (−2.1 to −1.5)	0 (0–0)	0 (0–0)	−3.7 (−3.9 to −3.6)	−2.9 (−3.0 to −2.8)	−3.4 (−3.8 to −3.0)	−4.7 (−4.9 to −4.4)
Kidney dysfunction												
Cardiovascular diseases	149,737 (119,076–184,749)	376,811 (285,378–480,070)	3.3 (2.9–3.6)	1.9 (1.5–2.3)	5.2 (4.8–5.6)	2.3 (1.5–3.1)	23 (18–29)	23 (17–29)	0.0 (−0.3 to 0.3)	−0.9 (−1.1 to −0.7)	2.0 (1.3–2.8)	−1.7 (−2.2 to −1.3)
Diabetes and kidney diseases	98,607 (86,800–111,078)	196,726 (168,241–224,684)	2.4 (2.3–2.5)	1.7 (1.5–1.8)	2.9 (2.6–3.2)	2.3 (2.2–2.4)	13 (12–15)	11 (10–13)	−0.5 (−0.6 to −0.3)	−0.8 (−0.9 to −0.7)	0.2 (−0.3 to 0.6)	−1.2 (−1.3 to −1.0)
High LDL cholesterol												
Cardiovascular diseases	317,060 (244,158–413,836)	915,983 (647,993–1,239,382)	3.7 (3.6–3.9)	2.4 (2.3–2.5)	5.3 (4.9–5.7)	3.0 (2.8–3.1)	49 (35–68)	56 (38–77)	0.4 (0.3–0.6)	−0.3 (−0.4 to −0.2)	2.1 (1.7–2.4)	−0.9 (−1.0 to −0.8)

AAPC, average annual percent changes; APC, annual percent change; DALYs, disability-adjusted life years; UI, uncertainty interval; CI, confidence interval.

10.3389/fnut.2022.1035439

TABLE 3 Trends in the population attributable fraction on the age-standardized rate of DALYs and death of level 2 causes attributable to six metabolic risk factors, 1990–2019.

	Age standardized attributable burden (per 100,000)					
	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2010–2019)
DALYs						
High fasting plasma glucose						
Cardiovascular diseases	11.5 (8.4–15.6)	14.5 (10.6–20.2)	0.7 (0.1–1.2)	1.9 (1.1–2.8)	1.7 (0.8–2.7)	−1.3 (−2.4 to −0.2)
Diabetes and kidney diseases	66.2 (62.9–69.9)	73.8 (70.4–77.5)	0.4 (0.3–0.4)	0.6 (0.5–0.7)	0.4 (0.3–0.5)	0.2 (0.0–0.3)
Neoplasms	1.5 (0.4–3.2)	2.8 (0.7–5.9)	2.1 (1.8–2.4)	2.0 (1.4–2.6)	4.4 (3.8–4.9)	0.1 (−0.2 to 0.5)
Neurological disorders	2.2 (0.4–6.1)	2.5 (0.4–7.0)	0.5 (0.3–0.7)	1.1 (0.8–1.4)	1.9 (1.6–2.1)	−1.4 (−1.6 to −1.1)
Respiratory infections and tuberculosis	1.0 (0.6–1.4)	1.2 (0.7–1.7)	0.7 (0.3–1.0)	−1.4 (−1.7 to −1.1)	3.1 (2.4–3.7)	−0.3 (−1.0 to 0.5)
Sense organ diseases	0.6 (0.1–1.3)	0.5 (0.1–1.2)	0.0 (−0.3 to 0.2)	2.0 (1.5–2.4)	0.1 (−0.2 to 0.4)	−2.0 (−2.4 to −1.6)
High systolic blood pressure						
Cardiovascular diseases	47.1 (40.8–53.1)	54.1 (47.6–60.5)	0.5 (0.5–0.5)	0.3 (0.3–0.4)	0.8 (0.8–0.9)	0.3 (0.3–0.3)
Diabetes and kidney diseases	19.8 (16.9–22.8)	21.4 (17.8–24.9)	0.3 (0.1–0.5)	−0.2 (−0.3 to 0.0)	0.8 (0.7–1.0)	0.0 (−0.6 to 0.6)
High body-mass index						
Cardiovascular diseases	7.8 (1.9–16.7)	15.1 (7.3–24.6)	2.3 (2.2–2.4)	2.3 (2.1–2.5)	2.2 (2.1–2.4)	2.5 (2.4–2.5)
Chronic respiratory diseases	0.3 (0.1–0.7)	0.8 (0.4–1.4)	3.8 (3.5–4.1)	2.4 (2.1–2.8)	2.6 (2.3–2.8)	6.5 (5.7–7.2)
Diabetes and kidney diseases	12.3 (3.4–25)	29.4 (15.8–43.5)	3.0 (2.9–3.2)	2.8 (2.7–3.0)	3.7 (3.6–3.8)	2.6 (2.3–3.0)
Digestive diseases	1.1 (0.3–2.7)	3.6 (1.5–6.5)	4.0 (3.9–4.1)	3.6 (3.3–3.8)	4.5 (4.3–4.6)	3.9 (3.8–4.0)
Musculoskeletal disorders	1.7 (0.4–3.9)	3.5 (1.4–6.3)	2.5 (2.5–2.6)	1.8 (1.7–2.0)	3.3 (3.3–3.3)	2.4 (2.3–2.5)
Neoplasms	2.0 (0.5–4.8)	3.7 (1.6–6.7)	2.2 (2.1–2.3)	2.8 (2.8–2.9)	1.5 (1.3–1.7)	2.5 (2.3–2.6)
Neurological disorders	1.2 (0.2–3.8)	2.6 (0.6–6.8)	2.8 (2.7–2.9)	2.3 (2.2–2.4)	3.3 (3.0–3.6)	2.7 (2.5–2.9)
Sense organ diseases	0.1 (0.0–0.4)	0.3 (0.1–0.6)	2.5 (2.3–2.7)	3.5 (3.0–4.0)	1.8 (1.7–1.9)	1.7 (1.3–2.0)
Low bone mineral density						
Self-harm and interpersonal violence	0.2 (0.2–0.3)	0.4 (0.3–0.5)	2.0 (1.7–2.3)	0.9 (0.5–1.3)	3.6 (3.1–4.0)	1.0 (0.6–1.4)
Transport injuries	5.4 (4.6–5.9)	7.2 (6.1–7.9)	1.0 (0.8–1.2)	−0.2 (−0.4 to 0.1)	2.2 (1.7–2.7)	1.1 (0.9–1.2)
Unintentional injuries	4.2 (3.5–4.9)	10.3 (8.6–11.7)	3.4 (3.1–3.7)	2.6 (2.3–2.9)	2.8 (2.6–3.1)	4.6 (4.0–5.3)

(Continued)

TABLE 3 (Continued)

	Age standardized attributable burden (per 100,000)					
	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2010–2019)
Kidney dysfunction						
Cardiovascular diseases	6.1 (5.0–7.2)	8.0 (6.4–9.7)	1.0 (0.9–1.1)	0.3 (0.2–0.5)	2.5 (2.4–2.7)	0.1 (−0.1 to 0.3)
Diabetes and kidney diseases	44.8 (40.8–48.6)	38.3 (33.2–42.9)	−0.5 (−0.7 to −0.3)	−0.6 (−0.8 to −0.4)	−0.7 (−0.9 to −0.5)	−0.3 (−0.7 to 0.2)
Musculoskeletal disorders	0.1 (0.1–0.1)	0.2 (0.1–0.2)	1.5 (1.4–1.6)	−0.3 (−0.4 to −0.3)	3.2 (3.1–3.3)	1.7 (1.3–2.1)
High LDL cholesterol						
Cardiovascular diseases	14.1 (11.4–17.6)	21.3 (16.7–26.9)	1.4 (1.4–1.5)	0.9 (0.8–1.0)	2.3 (2.3–2.4)	1.0 (0.9–1.1)
Death						
High fasting plasma glucose						
Cardiovascular diseases	12.3 (8.6–17.4)	14.9 (10.3–21.9)	0.5 (0.0–1.1)	2.0 (1.2–2.9)	1.6 (0.5–2.6)	−1.6 (−2.7 to −0.5)
Diabetes and kidney diseases	60.4 (57.2–63.3)	64.8 (61.7–67.7)	0.3 (0.2–0.3)	0.6 (0.5–0.7)	0.0 (0.0–0.1)	0.2 (0.1–0.3)
Neoplasms	1.9 (0.5–4.0)	3.3 (0.9–7.0)	1.9 (1.7–2.2)	2.3 (1.7–2.9)	3.7 (3.2–4.3)	−0.1 (−0.4 to 0.3)
Neurological disorders	4.8 (0.9–12.0)	5.4 (1.1–13.2)	0.4 (0.1–0.6)	1.0 (0.6–1.5)	1.8 (1.3–2.2)	−1.6 (−1.9 to −1.2)
Respiratory infections and tuberculosis	1.8 (1.0–2.8)	1.2 (0.7–1.8)	−1.5 (−1.9 to −1.2)	−2.0 (−2.7 to −1.2)	−0.6 (−1.2 to −0.1)	−2.4 (−3.0 to −1.7)
High systolic blood pressure						
Cardiovascular diseases	48.2 (41.5–55.3)	52.7 (45.1–60.7)	0.3 (0.3–0.3)	0.0 (−0.1 to 0.0)	0.6 (0.6–0.7)	0.3 (0.3–0.4)
Diabetes and kidney diseases	30.9 (27.5–34.2)	34.8 (31.1–37.8)	0.4 (0.3–0.5)	−0.1 (−0.2 to 0.0)	1.1 (1.0–1.2)	0.0 (−0.2 to 0.2)
High body-mass index						
Cardiovascular diseases	5.9 (1.4–13.3)	10.5 (4.7–18.2)	2.0 (2.0–2.1)	1.7 (1.5–1.8)	1.9 (1.8–2.0)	2.5 (2.5–2.6)
Chronic respiratory diseases	0.2 (0.0–0.4)	0.3 (0.1–0.5)	1.7 (1.6–1.9)	1.3 (1–1.5)	0.9 (0.7–1.1)	2.7 (2.5–2.9)
Diabetes and kidney diseases	9.9 (2.6–21.1)	20.5 (9.9–33.4)	2.5 (2.4–2.6)	2.6 (2.5–2.7)	2.7 (2.5–2.9)	2.3 (2.2–2.4)
Digestive diseases	0.7 (0.2–1.7)	1.3 (0.5–2.3)	1.9 (1.8–2.1)	2.3 (1.9–2.7)	0.7 (0.6–0.8)	3.0 (2.8–3.1)
Neoplasms	2.0 (0.5–4.9)	3.5 (1.5–6.4)	2.0 (1.9–2.0)	2.7 (2.6–2.7)	1.1 (1.0–1.3)	2.3 (2.1–2.4)
Neurological disorders	2.5 (0.4–7.4)	5.3 (1.4–12.5)	2.6 (2.5–2.7)	2.1 (1.9–2.3)	3.0 (2.9–3.2)	2.7 (2.6–2.8)
Low bone mineral density						
Self-harm and interpersonal violence	0.2 (0.2–0.3)	0.2 (0.2–0.3)	0.2 (0.0–0.5)	0.2 (−0.2 to 0.6)	0.9 (0.6–1.1)	−0.7 (−1.2 to −0.3)
Transport injuries	8.2 (7.1–8.8)	9.8 (8.4–10.6)	0.6 (0.3–0.8)	−0.3 (−0.5 to −0.1)	1.4 (0.8–2.0)	0.3 (−0.1 to 0.8)

(Continued)

TABLE 3 (Continued)

	Age standardized attributable burden (per 100,000)					
	1990	2019	AAPC (95% CI)	APC (1990–1999)	APC (2000–2009)	APC (2010–2019)
Unintentional injuries	9.1 (7.7–10.9)	19.9 (14.0–23.4)	2.8 (1.8–3.7)	1.1 (0.1–2.1)	5.1 (2.2–8.0)	2.4 (1.8–2.9)
Kidney dysfunction						
Cardiovascular diseases	6.0 (4.7–7.3)	8.2 (6.2–10.2)	1.1 (1–1.2)	0.4 (0.3–0.6)	2.7 (2.6–2.9)	0.2 (−0.1 to 0.5)
Diabetes and kidney diseases	55.4 (52.8–57.3)	53.3 (52.1–54.6)	−0.2 (−0.2 to −0.1)	−0.4 (−0.5 to −0.3)	0.1 (0.1 to 0.2)	−0.2 (−0.3 to −0.2)
High LDL cholesterol						
Cardiovascular diseases	12.8 (9.4–17.3)	20.2 (14.3–27.4)	1.6 (1.5–1.6)	1.0 (0.9–1.1)	2.8 (2.7–2.9)	0.9 (0.8–1.0)

AAPC, average annual percent change; APC, annual percent change; DALYs, disability-adjusted life years; CI, confidence interval.

which are associated with obesity, hyperglycemia, dyslipidemia, and insulin resistance (24).

These findings highlighted that male gender is a biological variable due to higher disease burden attributable to metabolic risk factors across all age groups. Free testosterone in men may be associated with a risk for major adverse cardiovascular events as men with lower sex hormone–binding globulin concentrations have a higher risk for myocardial infarction (25). The greater increase in disease burden among males will require more attention from health systems. Our data also support the view that menopause transition contributes to the increase in metabolic disease burden of old age (26), based on the fact that the AAPC in the rate of YLDs decreased for male patients but increased for female patients from 1 to 49 years before reaching a plateau. The reported findings underline the potential benefits of monitoring women's health during midlife and emphasize the critical window for early intervention strategies. Postmenopausal women, especially those younger than 60, should be fully advised on the benefits and harms of different types and timing of hormone therapy (27).

Our data imply that an integrative metabolic strategy should prioritize HBMI and LBMD due to the increased age-standardized rates of DALYs and death. Maintaining a BMI of 20.0–25.0 kg/m^2 can help minimize all-cause mortality, and a minimum of 150 min weekly leisure moderate to vigorous physical activity was associated with the most health benefits (28, 29). Phentermine–topiramate and glucagon-like peptide-1 receptor agonists (GLP-1RA), particularly semaglutide, seem to be the best drugs for controlling weight in overweight and obese adults (30). Men and women with LBMD, consistent with osteoporosis or osteopenia, showed a significantly increased risk of fractures and mortality (31). Nonetheless, indiscriminate use of vitamin D supplementation to prevent fracture and osteoporosis seems should be avoided (32). Treatment with bisphosphonates or denosumab, and teriparatide or abaloparatide should be considered for individuals at high and imminent risk of fracture, respectively (33).

Integrative management in old individuals often requires the use of multiple drugs to treat concurrent dyslipidemia, diabetes, and hypertension, and those with metabolic syndrome should be tightly managed. Anti-hypertensive treatment targeting systolic blood-pressure 110–130 mmHg rather than 130–150 mmHg led to fewer incidences of cardiovascular events in Chinse older patients with hypertension (34). Compared with the gold standard angiotensin-converting enzyme inhibitor (enalapril) an angiotensin receptor-neprilysin inhibitor (sacubitril/valsartan) not only decreases the risk of cardiovascular death or heart failure hospitalization but also improves symptoms in patients with chronic heart failure with reduced ejection fraction (35). Clinical trials exploring the cardiovascular and renal outcomes of GLP-1RA and sodium-glucose cotransporter-2 inhibitors (SGLT2i) not only change the treatment paradigm of diabetes but also support a more holistic approach beyond glycemic control which emphasize on cardiac and reno-protective effects (36). Although statins, ezetimibe, and PCSK9 inhibitors are the standard of care for coronary artery disease, many novel LDL cholesterol–lowering drugs, such as inclisiran recently approved by the FDA, may transform the care of patients who are at risk of life-threatening coronary events (37, 38). KDF is harmful but treatable if individuals at risk are identified at an early stage. More individuals than ever before are experiencing KDF and nephrosclerosis-age-associated histologic changes, as observed in 2.7, 58, and 73% of biopsies from donors aged <30, 60–69,70 plus, respectively (39). There is partial but limited success for kidney diseases treated with immunosuppressive agents, antihypertensives, and diuretics. Sodium-glucose co-transporter-2 (SGLT2) inhibition should be used as a foundational therapy for chronic kidney disease as SGLT2 inhibitors might substantially slow the progression of chronic kidney disease in people with type 2

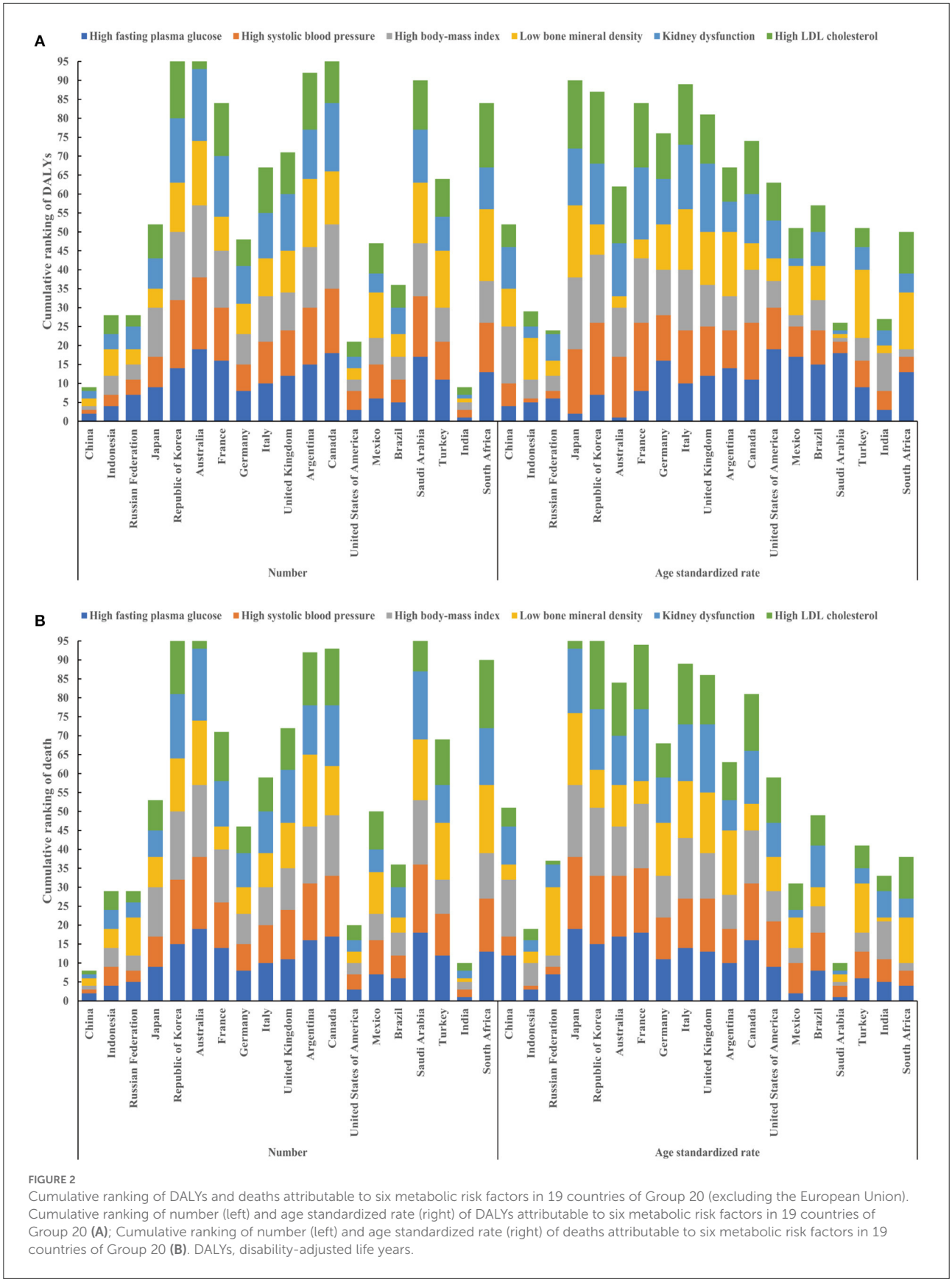


FIGURE 2
Cumulative ranking of DALYs and deaths attributable to six metabolic risk factors in 19 countries of Group 20 (excluding the European Union). Cumulative ranking of number (left) and age standardized rate (right) of DALYs attributable to six metabolic risk factors in 19 countries of Group 20 **(A)**; Cumulative ranking of number (left) and age standardized rate (right) of deaths attributable to six metabolic risk factors in 19 countries of Group 20 **(B)**. DALYs, disability-adjusted life years.

diabetes (40, 41). New candidate therapeutic drugs targeting the glomerular filtration barrier may help to correct defects within or between cells of the glomerular filtration barrier and maintain its integrity (42). Most importantly, a mechanistic link has been observed among obesity, kidney dysfunction, and hypertension (43). Namely, excessive adiposity, which is a major driver of kidney diseases and prolonged obesity, and progressive renal injury, often leads to the development of treatment-resistant hypertension.

Our findings identified the precise diseases and risk factors that are most in need of attention. For example, our data showed that KDF-driven musculoskeletal disorders at level 2 cause and gout at level 3 cause. We also ascertained the trend of the contribution of risk factors, which may help formulate feasible and quantitative health policy. Analysis of population-attributable fractions indicated the increasing contribution of HSBP to cardiovascular diseases and of HFPG to neoplasms. A 5-mmHg decrement in systolic blood pressure decreases major cardiovascular events by ∼10%, irrespective of previous cardiovascular disease (44). The risk of incident colorectal cancer associated with HFPG may help to identify people at high risk for future colorectal cancer (45). In addition, the evolutionary pattern of disability and death with societal development, as measured by the SDI, may help prioritize metabolic factors while formulating health policy. In addition to physical equipment, the government may also need to improve the availability of phentermine–topiramate and GLP-1 receptor agonists in adults who are overweight and obese as there is an increasing trend for HBMI and HLDL with the increase of SDI (46). In addition to the early detection of chronic kidney disease and interventions aiming to reduce urine protein excretion, more efforts are needed to delay and recover the progression of kidney function because there is increasing disability and decreasing death for KDF with the increase of SDI (47).

Our data revealed that China was the largest contributor to disease burden due to metabolic risk factors among 19 G20 countries. This presents a public health challenge and an opportunity for China to improve future health worldwide. Systematic management of individuals with metabolic syndromes must be stressed again as the age-standardized rate of DALYs and death in China ranked fourth to sixth among G20 countries for HFPG, HSBP, and HLDL. Although the age-standardized rate of DALYs and death in China ranked 10th to 15th for LBMD, KDF, and HBMI, this does not automatically imply that these indices are well-controlled. Since China gained more increments in life expectancy compared to other G20 countries, which may have led to a lower disease burden from age-related LBMD, KDF, and HBMI in the past three decades (2).

These findings are not entirely surprising if one follows the concept that metabolic aging is remolding future health structures. At the same time, these findings are interesting as they foster the concept of prevention always triumphs over remediation during adolescence and integrative strategy for key risk factors and individuals. Nonetheless, these findings are subject to two major limitations. First, the study did not include urban-rural and province-level data, which may be useful to formulate provincial-, urban-, and rural-specific health policies. Second, the study is subject to the same limitation as the GBD 2019 studies (2, 3).

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

This manuscript was produced as part of the GBD Collaborator Network in accordance with the GBD Protocol (IHME ID. 1775-GBD2019-012021). Data released from the Global Health Data Exchange query did not require informed patient consent. This study used an anonymized publicly available data set with no identifiable information about the survey participants.

Author contributions

JC, KHH, YZJ, L-LL, SM, HS, A-MW, and SWX provided data or critical feedback on data sources. YZJ and A-MW developed methods of computational machinery and managed the estimation or publication process. AB, JC, EC, XQF, KHH, KAH, YZJ, L-LL, SM, FW, ZYW, DZW, A-MW, SWX, and ZYZ provided critical feedback on methods or results. AB, JC, EC, KAH, JJH, YZJ, L-LL, YMS, L-ST, FW, ZYW, DZW, A-MW, and CWZ drafted the work or revising it critically for important intellectual content. All authors contributed to the article and approved the submitted version.

Funding

The Global Burden of Disease study was funded by the Bill and Melinda Gates Foundation. The funders had no role in study design, data collection, and analysis, nor the decision to publish or preparation of the manuscript.

Acknowledgments

We thank the staff at the Institute for Health Metrics and Evaluation and its collaborators who prepared publicly available and impactful data.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1035439/full#supplementary-material

References

1. Partridge L, Deelen J, Slagboom PE. Facing up to the global challenges of ageing. *Nature.* (2018) 561:45–56. doi: 10.1038/s41586-018-0457-8

2. Collaborators GBDD. Global age-sex-specific fertility, mortality, healthy life expectancy (HALE), and population estimates in 204 countries and territories, 1950-2019: a comprehensive demographic analysis for the Global Burden of Disease Study 2019. *Lancet.* (2020) 396:1160–203. doi: 10.1016/S0140-6736(20)30977-6

3. GBD 2019 Risk Factors Collaborators. Global burden of 87 risk factors in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet.* (2020) 396:1223–49. doi: 10.1016/S0140-6736(20)30752-2

4. Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E. From discoveries in ageing research to therapeutics for healthy ageing. *Nature.* (2019) 571:183–92. doi: 10.1038/s41586-019-1365-2

5. Wang W, Hu M, Liu H, Zhang X, Li H, Zhou F, et al. Global Burden of Disease Study 2019 suggests that metabolic risk factors are the leading drivers of the burden of ischemic heart disease. *Cell Metab.* (2021) 33:1943–56. doi: 10.1016/j.cmet.2021.08.005

6. Shah AD, Langenberg C, Rapsomaniki E, Denaxas S, Pujades-Rodriguez M, Gale CP, et al. Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1.9 million people. *Lancet Diabetes Endocrinol.* (2015) 3:105–13. doi: 10.1016/S2213-8587(14)70219-0

7. Brouwers S, Sudano I, Kokubo Y, Sulaica EM. Arterial hypertension. *Lancet.* (2021) 398:249–61. doi: 10.1016/S0140-6736(21)00221-X

8. Collaborators G2O, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, et al. Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med.* (2017) 377:13–27. doi: 10.1056/NEJMoa1614362

9. Black DM, Rosen CJ. Clinical practice. Postmenopausal osteoporosis. *N Engl J Med.* (2016) 374:254–62. doi: 10.1056/NEJMcp1513724

10. Chotiyarnwong P, McCloskey EV. Pathogenesis of glucocorticoid-induced osteoporosis and options for treatment. *Nat Rev Endocrinol.* (2020) 16:437–47. doi: 10.1038/s41574-020-0341-0

11. Ronco C, Bellomo R, Kellum JA. Acute kidney injury. *Lancet.* (2019) 394:1949–64. doi: 10.1016/S0140-6736(19)32563-2

12. Webster AC, Nagler EV, Morton RL, Masson P. Chronic kidney disease. *Lancet.* (2017) 389:1238–52. doi: 10.1016/S0140-6736(16)32064-5

13. Libby P. The changing landscape of atherosclerosis. *Nature.* (2021) 592:524–33. doi: 10.1038/s41586-021-03392-8

14. Hofmann SM, Landgraf R. Research in metabolic ageing - a tale of mice versus humans? *Nat Rev Endocrinol.* (2022) 18:7–8. doi: 10.1038/s41574-021-00597-9

15. Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. *Cell.* (2013) 153:1194–217. doi: 10.1016/j.cell.2013.05.039

16. Zhou M, Wang H, Zeng X, Yin P, Zhu J, Chen W, et al. Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet.* (2019) 394:1145–58. doi: 10.1016/S0140-6736(19)30427-1

17. Diseases GBD, Injuries C. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet.* (2020) 396:1204–22. doi: 10.1016/S0140-6736(20)30925-9

18. Brooks-Pollock E, Danon L. Defining the population attributable fraction for infectious diseases. *Int J Epidemiol.* (2017) 46:976–82. doi: 10.1093/ije/dyx055

19. Foreman KJ, Marquez N, Dolgert A, Fukutaki K, Fullman N, McGaughey M, et al. Forecasting life expectancy, years of life lost, and all-cause and cause-specific mortality for 250 causes of death: reference and alternative scenarios for 2016-40 for 195 countries and territories. *Lancet.* (2018) 392:2052–90. doi: 10.1016/S0140-6736(18)31694-5

20. Fang EF, Scheibye-Knudsen M, Jahn HJ, Li J, Ling L, Guo H, et al. A research agenda for aging in China in the 21st century. *Ageing Res Rev.* (2015) 24(Pt B):197–205. doi: 10.1016/j.arr.2015.08.003

21. Hargreaves D, Mates E, Menon P, Alderman H, Devakumar D, Fawzi W, et al. Strategies and interventions for healthy adolescent growth, nutrition, and development. *Lancet.* (2021) 399:198–210. doi: 10.1016/S0140-6736(21)01593-2

22. Norris SA, Frongillo EA, Black MM, Dong Y, Fall C, Lampl M, et al. Nutrition in adolescent growth and development. *Lancet.* (2021) 399:172–84. doi: 10.1016/S0140-6736(21)01590-7

23. Neufeld LM, Andrade EB, Suleiman AB, Barker M, Beal T, Blum LS, et al. Food choice in transition: adolescent autonomy, agency, and the food environment. *Lancet.* (2021) 399:185–97. doi: 10.1016/S0140-6736(21)01687-1

24. Finkel T. The metabolic regulation of aging. *Nat Med.* (2015) 21:1416–23. doi: 10.1038/nm.3998

25. Yeap BB, Marriott RJ, Antonio L, Raj S, Dwivedi G, Reid CM, et al. Associations of serum testosterone and sex hormone-binding globulin with incident cardiovascular events in middle-aged to older men. *Ann Intern Med.* (2021) 175:159–70. doi: 10.7326/M21-0551

26. Khoudary SRE, Aggarwal B, Beckie TM, Hodis HN, Johnson AE, Langer RD, et al. Menopause transition and cardiovascular disease risk: implications for timing of early prevention: a scientific statement from the american heart association. *Circulation.* (2020) 142:e506–32. doi: 10.1161/CIR.0000000000000912

27. Gartlehner G, Patel SV, Feltner C, Weber RP, Long R, Mullican K, et al. Hormone therapy for the primary prevention of chronic conditions in postmenopausal women: evidence report and systematic review for the US preventive services task force. *JAMA.* (2017) 318:2234–49. doi: 10.1001/jama.2017.16952

28. Bakker EA, Lee DC, Hopman MTE, Oymans EJ, Watson PM, Thompson PD, et al. Dose-response association between moderate to vigorous physical activity and incident morbidity and mortality for individuals with a different cardiovascular health status: a cohort study among 142,493 adults from the Netherlands. *PLoS Med.* (2021) 18:e1003845. doi: 10.1371/journal.pmed.1003845

29. Global BMIMC, Di Angelantonio E, Bhupathiraju Sh N, Wormser D, Gao P, Kaptoge S, et al. Body-mass index and all-cause mortality: individual-participant-data meta-analysis of 239 prospective studies in four continents. *Lancet.* (2016) 388:776–86. doi: 10.1016/S0140-6736(16)30175-1

30. Shi Q, Wang Y, Hao Q, Vandvik PO, Guyatt G, Li J, et al. Pharmacotherapy for adults with overweight and obesity: a systematic review and network meta-analysis of randomised controlled trials. *Lancet.* (2021) 399:259–69. doi: 10.1016/S0140-6736(21)01640-8

31. Hauger AV, Bergland A, Holvik K, Stahle A, Emaus N, Strand BH. Osteoporosis and osteopenia in the distal forearm predict all-cause mortality independent of grip strength: 22-year follow-up in the population-based Tromso Study. *Osteoporos Int.* (2018) 29:2447–56. doi: 10.1007/s00198-018-4653-z

32. Bolland MJ, Grey A, Avenell A. Effects of vitamin D supplementation on musculoskeletal health: a systematic review, meta-analysis, and trial sequential analysis. *Lancet Diabetes Endocrinol.* (2018) 6:847–58. doi: 10.1016/S2213-8587(18)30265-1

33. Compston JE, McClung MR, Leslie WD. Osteoporosis. *Lancet.* (2019) 393:364–76. doi: 10.1016/S0140-6736(18)32112-3

34. Zhang W, Zhang S, Deng Y, Wu S, Ren J, Sun G, et al. Trial of intensive blood-pressure control in older patients with hypertension. *N Engl J Med.* (2021) 385:1268–79. doi: 10.1056/NEJMc2117463

35. Docherty KF, Vaduganathan M, Solomon SD, McMurray JJV. Sacubitril/valsartan: neprilysin inhibition 5 years after PARADIGM-HF. *JACC Heart Fail.* (2020) 8:800–10. doi: 10.1016/j.jchf.2020.06.020

36. American Diabetes A. 9. Pharmacologic approaches to glycemic treatment: standards of medical care in diabetes-2021. *Diabetes Care.* (2021) 44(Suppl 1.): S111–24. doi: 10.2337/dc21-S009

37. Musunuru K. Treating coronary artery disease: beyond statins, ezetimibe, and PCSK9 inhibition. *Annu Rev Med.* (2021) 72:447–58. doi: 10.1146/annurev-med-080819-044918

38. Ray KK, Wright RS, Kallend D, Koenig W, Leiter LA, Raal FJ, et al. Two Phase 3 trials of inclisiran in patients with elevated LDL cholesterol. *N Engl J Med.* (2020) 382:1507–19. doi: 10.1056/NEJMoa1912387

39. O'Sullivan ED, Hughes J, Ferenbach DA. Renal aging: causes and consequences. *J Am Soc Nephrol.* (2017) 28:407–20. doi: 10.1681/ASN.2015121308

40. Mark PB, Sattar N. Implementation, not hesitation, for SGLT2 inhibition as foundational therapy for chronic kidney disease. *Lancet.* (2022) 400:1745–7. doi: 10.1016/S0140-6736(22)02164-X

41. Nuffield Department of Population Health Renal Studies G, Consortium SiM-AC-RT. Impact of diabetes on the effects of sodium glucose co-transporter-2 inhibitors on kidney outcomes: collaborative meta-analysis of large placebo-controlled trials. *Lancet.* (2022) 400:1788–801. doi: 10.1016/S0140-6736(22)02074-8

42. Daehn IS, Duffield JS. The glomerular filtration barrier: a structural target for novel kidney therapies. *Nat Rev Drug Discov.* (2021) 20:770–88. doi: 10.1038/s41573-021-00242-0

43. Hall JE, do Carmo JM, da Silva AA, Wang Z, Hall ME. Obesity, kidney dysfunction and hypertension: mechanistic links. *Nat Rev Nephrol.* (2019) 15:367–85. doi: 10.1038/s41581-019-0145-4

44. Blood Pressure Lowering Treatment Trialists C. Pharmacological blood pressure lowering for primary and secondary prevention of cardiovascular disease across different levels of blood pressure: an individual participant-level data meta-analysis. *Lancet.* (2021) 397:1625–36. doi: 10.1016/S0140-6736(21)00590-0

45. Itoh H, Kaneko H, Okada A, Yano Y, Morita K, Seki H, et al. Fasting plasma glucose and incident colorectal cancer: analysis of a nationwide epidemiological database. *J Clin Endocrinol Metab.* (2021) 106:e4448–e58. doi: 10.1210/clinem/dgab466

46. Teufel F, Seiglie JA, Geldsetzer P, Theilmann M, Marcus ME, Ebert C, et al. Body-mass index and diabetes risk in 57 low-income and middle-income countries: a cross-sectional study of nationally representative, individual-level data in 685 616 adults. *Lancet.* (2021) 398:238–48. doi: 10.1016/S0140-6736(21)00844-8

47. Couser WG, Remuzzi G, Mendis S, Tonelli M. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. *Kidney Int.* (2011) 80:1258–70. doi: 10.1038/ki.2011.368

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 06 January 2023
DOI 10.3389/fnut.2022.1090023

OPEN ACCESS

EDITED BY
Zachary Clayton,
University of Colorado Boulder,
United States

REVIEWED BY
Abigail Casso,
University of Colorado Boulder,
United States
Cameron G. McCarthy,
University of South Carolina,
United States
Camilla Ferreira Wenceslau,
University of South Carolina,
United States

*CORRESPONDENCE
Daniel R. Machin
dmachin@fsu.edu

SPECIALTY SECTION
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

RECEIVED 04 November 2022
ACCEPTED 23 November 2022
PUBLISHED 06 January 2023

CITATION
Zheng X, Li Z, Berg Sen J, Samarah L, Deacon CS, Bernardo J and Machin DR (2023) Western diet augments metabolic and arterial dysfunction in a sex-specific manner in outbred, genetically diverse mice. *Front. Nutr.* 9:1090023. doi: 10.3389/fnut.2022.1090023

Western diet augments metabolic and arterial dysfunction in a sex-specific manner in outbred, genetically diverse mice

Xiangyu Zheng, Zhuoxin Li, Jennifer Berg Sen, Luaye Samarah, Christina S. Deacon, Joseph Bernardo and Daniel R. Machin*

Department of Nutrition and Integrative Physiology, Florida State University, Tallahassee, FL, United States

Western diet (WD), characterized by excess saturated fat and sugar intake, is a major contributor to obesity and metabolic and arterial dysfunction in humans. However, these phenotypes are not consistently observed in traditional inbred, genetically identical mice. Therefore, we sought to determine the effects of WD on visceral adiposity and metabolic/arterial function in UM-HET3 mice, an outbred, genetically diverse strain of mice. Male and female UM-HET3 mice underwent normal chow (NC) or WD for 12 weeks. Body mass and visceral adiposity were higher in WD compared to NC ($P < 0.05$). Female WD mice had greater visceral adiposity than male WD mice ($P < 0.05$). The results of glucose and insulin tolerance tests demonstrated that metabolic function was lower in WD compared to NC mice ($P < 0.05$). Metabolic dysfunction in WD as was driven by male mice, as metabolic function in female WD mice was unchanged ($P > 0.05$). Systolic blood pressure (BP) and aortic stiffness were increased in WD after 2 weeks compared to baseline and continued to increase through week 12 ($P < 0.05$). Systolic BP and aortic stiffness were higher from weeks 2-12 in WD compared to NC ($P < 0.05$). Aortic collagen content was higher in WD compared to NC ($P < 0.05$). Carotid artery endothelium-dependent dilation was lower in WD compared to NC ($P < 0.05$). These data suggest sex-related differences in visceral adiposity and metabolic dysfunction in response to WD. Despite this, arterial dysfunction was similar in male and female WD mice, indicating this model may provide unique translational insight into similar sex-related observations in humans that consume WD.

KEYWORDS

endothelium, fat, sugar, arterial function, blood pressure, aorta, metabolic function

Introduction

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in industrialized societies and advanced age is the major risk factor for the development of CVD (1). In industrialized societies, advanced age is accompanied by visceral obesity and metabolic dysfunction (e.g., insulin resistance), as well as arterial dysfunction (i.e., increased systolic blood pressure [BP], stiffening of the large elastic arteries, and impaired endothelium-dependent dilation [EDD]) (2, 3). While visceral obesity, metabolic dysfunction, and arterial dysfunction are each independent risk factors for CVD (3, 4), CVD risk becomes even greater in individuals with combined risk factors (i.e., metabolic syndrome) (5). This becomes increasingly important in an aged society, as advancing age also increases the prevalence of metabolic syndrome (6).

Lifestyle factors, such as diet, are the most important modifiable risk factors for metabolic syndrome and subsequent CVD (7). Thus, consumption of a Western diet (WD) that consists of excess saturated fat and sugar intake (7), is reported to be a primary driver for metabolic syndrome and subsequent CVD risk in individuals living in the industrialized societies (8). This is evident as pre-industrialized societies shift toward industrialization, which increases the incidence of type-2 diabetes and CVD (9, 10). To determine the mechanisms by which a WD might elevate CVD risk, preclinical rodent models have been used to induce obesity, metabolic dysfunction, and arterial dysfunction. However, the vast majority of these studies use inbred, genetically identical mice, such as the C57BL/6 strain that have strain specific phenotypes that may limit translation to humans. For example, in response to WD, C57BL/6 mice display sex-differences in body mass gain (11–13) and/or a lack of elevation in systolic BP (14–19), which conflicts with observations in humans (20–22).

In recent years, outbred, genetically diverse mice, such as the UM-HET3 strain, have become popular in translational aging research, as they are hypothesized to better phenocopy the genetic diversity in humans (23), while displaying lower phenotypic variation than genetically identical inbred mice (24). However, very few studies have used outbred rodent models to study the effect of WD consumption. In outbred Sprague Dawley rats, studies show a large disparity in body mass gain to diet induced obesity (25–27), indicating a lack of consistency of this rat model. To the best of our knowledge, there is only one study that has used an outbred mouse model of genetic diversity with WD and showed that WD consumption induced a wide range of diabetes-related phenotypes (11). On the other side, the National Institute on Aging (NIA) Interventions Testing Program (ITP) and Study of Longitudinal Aging in Mice (SLAM) are longitudinal studies that target aging in UM-HET3 mice to establish normative aging values and identify potential therapeutics that extend lifespan (28–30). While the ITP and SLAM studies have strong translational importance, it is important to note that mice in these studies consume a standard normal chow (NC) diet. Thus, the translational application of these findings to industrialized societies in which many individuals consume a WD is unclear. To the best of our knowledge, there are no short- or long-term studies that have examined adiposity, metabolic function, and arterial function in UM-HET3 mice in response to a WD. Prior to performing a longitudinal study to assess the effects of WD on aging in UM-HET3 mice, it is imperative to first determine if the short-term adaptations to WD in young UM-HET3 mice phenocopy adaptations in humans to similar conditions. Therefore, the purpose of this study was to determine the effects of 12 weeks of WD consumption on adiposity, metabolic function, and arterial function in young UM-HET3 mice.

Materials and methods

Ethical approval

All animal procedures conform to the *Guide to the Care and Use of Laboratory Animals: Eighth Edition* (31) and were approved by the Florida State University Animal Care and Use Committee.

Animals

Male and female UM-HET3 mice used in this study were first generation offspring of CByB6F1/J male and C3D2F1/J female parents. CByB6F1/J and C3D2F1/J parents were obtained from The Jackson Laboratory, as described previously (32). All mice were housed in standard mouse cages under a 12:12 light:dark cycle in a temperature-controlled environment. At 3 months old, mice were randomized into NC (LabDiet No. 5001; protein: 28.5%, carbohydrate: 58.0%, fat 13.5% by kcal; 1.1% NaCl) or WD (LabDiet No. 5TJN; protein: 15.8%, carbohydrate: 45.1%, fat 39.1% by kcal; 0.4% NaCl) groups for 12 weeks (33). The fat content in WD used in this study is comparable to typical WD used in most preclinical studies (34). Food and water were supplied *ad libitum* in group-housed cages. Body mass and blood glucose were measured in the non-fasting condition on the day of euthanasia. Mice were anesthetized with isoflurane (3%) in room air at 100 ml/min flow rate and euthanized via cardiac puncture.

Blood collection and analyses

Blood was collected in the non-fasting condition via cardiac puncture and immediately centrifuged at 4°C for 20 min at

3,000 g. Plasma was aliquoted and stored at −80°C freezer. Plasma insulin was quantified using mouse insulin ELISA kit (Cat# 9008; Crystal Chem, Elk Grove Village, IL, USA).

Metabolic testing

Metabolic function was determined using glucose- and insulin-tolerance tests (GTT, ITT) at baseline, after 6 weeks and 12 weeks of dietary intervention. Briefly, mice were fasted for 4 h in the morning. Baseline blood glucose was measured using a glucometer (Clarity BG1000; Clarity Diagnostics, Boca Raton, FL, USA) in blood collected via a tail cut. Following baseline measurements, mice were injected intraperitoneally with glucose (2 g/kg body mass) or insulin (1 U/kg body mass). Blood glucose was measured at 15, 30, 45, 60, 90, and 120 min after injection. HOMA-IR, a measurement of insulin sensitivity (35), was calculated using the equation: glucose × insulin ÷ 405.

Systolic blood pressure

Arterial systolic BP was determined *in vivo* in conscious mice using the tail-cuff method (MC4000 BP analysis system; Hatteras Instrument, Cary, NC, USA), as described previously (36), at baseline and after 2, 4, 6, 8, 10, and 12 weeks of dietary intervention. This method has been validated vs. arterial catheter BP (37). Briefly, mice underwent a 5 consecutive days of tail cuff BP measurement that was conducted in a quiet and warm (∼23°C) environment at the same time of the day (38). During each trial, mice were restrained on a heated platform (40°C). Each trial consisted of 5 preliminary measurements that were followed by 10 experimental measurements. Measurements with aberrant movement/behavior or poor signal were excluded and remaining values were used to calculate mean values for each mouse.

Aortic stiffness

Aortic stiffness was assessed by aortic pulse wave velocity (PWV) measurement *in vivo*, as described previously (39), at baseline and after 2, 4, 6, 8, 10, and 12 weeks of dietary intervention. Briefly, mice were anesthetized with isoflurane (3%) in room air at 100 ml/min flow rate and placed in the supine position on a heated platform (37°C). Blood velocity waveforms at the transverse aortic arch and at the abdominal aorta were obtained simultaneously with 2, 20-MHz Doppler probes (Indus Instruments, Webster, TX, USA) and recorded using PowerLab 16/35 with LabChart 8 software (AD Instruments Inc., Colorado Springs, CO, USA) at a sampling rate of 100 k/sec. After blood velocity waveforms were collected, a precise measurement of the traveled distance between the Doppler probes was recorded using a scientific caliper. The transit time between Doppler sites was determined using the foot-to-foot method with LabChart Lightning 1.8 software (AD Instruments Inc., Colorado Springs, CO, USA). Aortic PWV was calculated as the traveled distance divided by the transit time.

Ex vivo arterial function

To assess EDD, carotid arteries were excised, cleared of surrounding tissue, cannulated in the stage of a pressure myograph (DMT Inc., Hinnerup, Denmark), and perfused with physiological salt solution that contained 145.0 mM NaCl, 4.7 mM KCl, 2.0 mM $CaCl_2$, 1.17 mM $MgSO_4$, 1.2 mM NaH_2PO_4, 5.0 mM glucose, 2.0 mM pyruvate, 0.02 mM EDTA, 3.0 mM MOPS buffer, and 0.5% BSA (pH 7.4 at 37°C). Carotid arteries were pressurized to an intraluminal pressure of 68 cm H_2O. Arteries were submaximally pre-constricted with 2 μM phenylephrine and EDD was measured in response to the cumulative addition of acetylcholine (1×10^{-10} to 1×10^{-4} M) in the absence or presence of the nitric oxide (NO) synthase inhibitor, L-NAME (0.1 mM, 30 min), as described previously (40). Endothelium-independent dilation (EID) was assessed in response to the cumulative addition of sodium nitroprusside (1×10^{-10} to 1×10^{-4} M). Following *ex vivo* measurements, carotid arteries were incubated in Ca^{2+}-free physiological salt solution for 1 hour to determine their maximal diameter. Luminal diameters were measured by Vasotracker software 2 minutes after each addition of acetylcholine or sodium nitrorpsuside (41). All *ex vivo* arterial function data are presented as percent of maximal dilation after pre-constriction with phenylephrine. Arteries failing to achieve ≥ 15% pre-constriction were excluded.

Aortic histology

After sacrifice, a 2-3 mm aortic ring with perivascular tissue intact was excised from the thoracic aorta and embedded in Optimal Cutting Temperature medium, as described previously (39). Aortic rings were sliced into 8-micron sections. Each slide contained 2 to 3 aortic sections, which were averaged. For measures of medial cross-sectional area (CSA) the lumen border and the outer medial border were traced in ImageJ and internal areas were measured. These areas were used to calculate medial CSA and were calculated as the outer medial border area minus the lumen area. Collagen was quantified by Masson's

trichrome staining (MilliporeSigma, Burlington, MA, USA) as percentage of the selected area, as described previously (42). Blue channel images from an RGB stack were used for densitometric quantification of collagen content with ImageJ. Elastin was quantified by Verhoff-Van Gieson staining (Abcam, Cambridge, UK), as described previously (39). An 8-bit grayscale was used for densitometric quantification of elastin content with ImageJ. Collagen and elastin content were normalized to NC.

Statistical analysis

A 4-way mixed model ANOVA was used to evaluate the effect of Sex, Group, Week, Time/Concentration on GTT/ITT and *ex vivo* arterial function responses. A 3-way mixed model ANOVA was used to evaluate the effect of Sex, Group, and Week on systolic BP, aortic PWV, GTT/ITT AUC, and *ex vivo* arterial function responses at each drug concentration. To analyze the remaining data, a 2-way ANOVA or 2-way mixed model ANOVA was used to evaluate the effect of Sex, Group, or Time/Concentration on all the variables. Sidak *post-hoc* test was used to further identify values that were significantly different. Bivariate correlations were determined between selected variables. Statistical significance was set at $P < 0.05$ for all analyses. Statistic tests were performed with SPSS software version 26.0 (IBM, Armonk, NY, USA). Data are presented as mean ± SEM.

Results

Dietary intake

Dietary intake is presented in Table 1. There were Sex-related differences in energy intake ($P < 0.05$) but no differences in energy intake were present between groups ($P > 0.05$). There were also Group- and Sex-related differences in carbohydrate, fat, and protein intake ($P < 0.05$). No interaction effects were observed ($P > 0.05$).

Animal characteristics

We observed a significant main effect of Group and Time, as well as a significant interaction effect of Group X Week on body mass and change in body mass (Figure 1; $P < 0.05$ for all). At baseline (i.e., week 0), body mass was similar between NC and WD (Figure 1A; $P > 0.05$). Over the 12-week dietary intervention, body mass was increased from baseline in WD mice at week 2 and continued to increase through week 12 ($P < 0.05$). With the exception of week 3, body mass was greater in WD compared to NC mice in weeks 2-12 ($P < 0.05$). Body mass also increased in NC mice at week 6 and weeks 10-12 compared to baseline ($P < 0.05$). While we did observe a significant main effect of Sex ($P < 0.05$), there was no interaction effect of Sex with body mass (Figure 1B; $P > 0.05$) or change in body mass (Figure 1C; $P > 0.05$). However, the change in body mass over the 12-week intervention was ∼3-fold greater in WD compared to NC mice (Figure 1C; $P < 0.05$). Visceral adipose tissue mass expressed as an absolute mass, as well as normalized to body mass or tibia length were higher in WD compared to NC mice (Table 2; $P < 0.05$). We also observed sex-related differences in visceral adiposity with female WD mice having greater absolute and relative visceral adiposity mass compared to male WD mice ($P < 0.05$).

Metabolic function

We observed a significant main effect of Group and Sex, as well as a significant interaction effect of Group X Sex with non-fasted blood glucose, plasma insulin, and HOMA-IR (Figure 1; $P < 0.05$ for all). Non-fasted blood glucose, plasma insulin, and HOMA-IR were also higher in WD compared to NC mice (Figures 1D-F; $P < 0.05$). These group differences were driven by male WD mice, as they had higher blood glucose, plasma insulin, and HOMA-IR compared to female WD mice ($P < 0.05$). There were no sex-related differences in these variables between male and female NC mice ($P > 0.05$).

We observed a significant main effect of Group, Week, and Time, as well as a significant interaction effects of Group X

TABLE 1 Dietary intake.

	Normal chow		Western diet				
	Male	Female	Male	Female	Group	Sex	Interaction
Sample size, n	6	6	6	6	–	—	—
Energy intake, kcal/d	17.2 ± 0.4	15.9 ± 0.5	17.1 ± 1.2	14.7 ± 0.1	0.329	0.010	0.392
Carbohydrate intake, kcal/d	10 ± 0.2	9.2 ± 0.3	7.7 ± 0.5	6.6 ± 0	<0.001	0.008	0.643
Fat intake, kcal/d	2.3 ± 0.1	2.1 ± 0.1	6.7 ± 0.5	5.7 ± 0	<0.001	0.022	0.105
Protein intake, kcal/d	4.9 ± 0.1	4.5 ± 0.1	2.7 ± 0.2	2.3 ± 0	<0.001	0.008	0.846

Values are means ± SEM. Data were analyzed using 2-way ANOVA. Sidak *post-hoc* test was used to identify group differences.

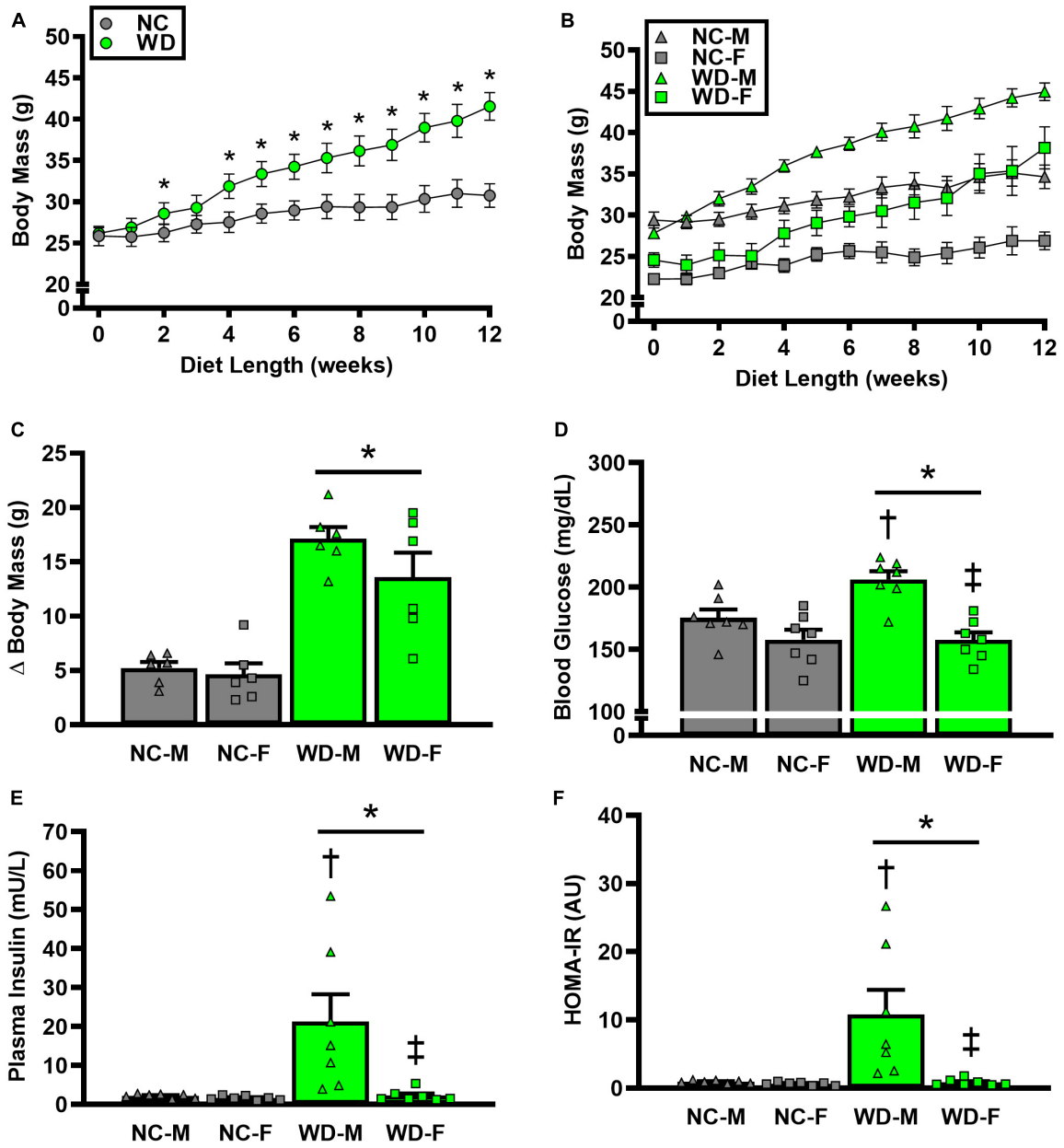


FIGURE 1
Comparisons in normal chow (NC) and Western (WD) diet-treated male (M) and female (F) mice. Data were analyzed using 2-way and 3-way mixed model ANOVA and 2-way ANOVA. Sidak *post-hoc* test was used to identify differences in body mass between groups **(A)** and sexes **(B)**, as well as differences in Δ body mass **(C)**, non-fasting blood glucose **(D)**, non-fasting plasma insulin **(E)**, and HOMA-IR **(F)** between groups/sexes. $^{*}P < 0.05$ vs. NC. $^{\dagger}P$, 0.05 vs. NC within sex. $^{\ddagger}P < 0.05$ vs. male within group. Data are individual values and means ± SEM.

Week and Group X Week X Time with GTT response (Figure 2; $P < 0.05$). At baseline, glucose tolerance was similar between groups (Figure 2A; $P > 0.05$). However, glucose tolerance was lower in WD compared to NC mice at weeks 6 and 12 (Figures 2C,E; $P < 0.05$). We also observed a significant main effect of Sex, as well as significant interaction effects of Sex X Group, Sex X Week, Sex X Group X Week X Time for GTT response ($P < 0.05$). At baseline (Figure 2B), week 6 (Figure 2D), and week 12 (Figure 2F) timepoints, glucose tolerance was lower in males compared to females ($P < 0.05$). Although female NC and WD mice had similar glucose tolerance at all timepoints ($P > 0.05$), only at baseline was glucose tolerance similar between male NC and WD mice ($P > 0.05$). However, at weeks 6 and 12, glucose tolerance was lower in male WD compared to male NC mice ($P < 0.05$).

We observed a significant main effect of Group, Week, and Time, as well as a significant interaction effects of Group X Week and Group X Week X Time with ITT response (Figure 3; $P < 0.05$). At baseline and week 6, insulin tolerance was similar between groups (Figures 3A,C; $P > 0.05$), although insulin tolerance was lower in WD compared to NC mice at week 12 (Figure 3E; $P < 0.05$). We also observed a significant main effect of Sex, as well as significant interaction effects of Sex X Group, Sex X Week, and Sex X Group X Week X Time with ITT response ($P < 0.05$). At baseline (Figure 3B), week 6 (Figure 3D), and week 12 (Figure 3F) timepoints, insulin tolerance was lower in males compared to females ($P < 0.05$). Although female NC and WD mice had similar insulin tolerance at all timepoints ($P > 0.05$), only at baseline was insulin tolerance similar between male NC and WD mice ($P > 0.05$). However, at weeks 6 and 12, insulin tolerance was lower in male WD compared to male NC mice ($P < 0.05$).

We observed a significant main effect of Group and Time, as well as a significant interaction effect with Group X Time with GTT area under the curve (AUC) (Figure 4; $P < 0.05$).

TABLE 2 Animal characteristics.

	Normal chow		Western diet				
	Male	Female	Male	Female	Group	Sex	Interaction
Sample size, n	6	6	6	6	–	–	—
Age, mo	6.7 ± 0.3	6.9 ± 0.0	6.5 ± 0.2	6.9 ± 0.0	0.674	<0.001	0.104
Body mass, g	34.7 ± 1.5	26.7 ± 1.1	45.2 ± 2.2	38.3 ± 2.8	<0.001	0.002	0.802
Tibia length, mm	18.6 ± 0.2	20.0 ± 0.5	19.1 ± 0.3	19.3 ± 0.3	0.876	0.048	0.145
Heart, mg	169 ± 9	139 ± 5	188 ± 12	139 ± 6	0.295	<0.001	0.253
Heart/Body mass, mg/g	4.9 ± 0.2	5.2 ± 0.2	4.2 ± 0.3	3.7 ± 0.3	0.001	0.828	0.163
Heart/Tibia length, mg/mm	9.1 ± 0.5	7.0 ± 0.2	9.8 ± 0.5	7.2 ± 0.3	0.258	<0.001	0.495
Liver, mg	1739 ± 80	1400 ± 68	2233 ± 188	1509 ± 121	0.024	<0.001	0.135
Liver/Body mass, mg/g	50.4 ± 2.2	52.5 ± 2.1	49.1 ± 2.4	40.1 ± 3.3*†	0.014	0.191	0.042
Liver/Tibia length, mg/mm	93.4 ± 3.8	70.2 ± 2.9	116.6 ± 9.0	77.8 ± 5.3	0.014	<0.001	0.189
Spleen, mg	78 ± 8	90 ± 3	86 ± 8	94 ± 7	0.365	0.157	0.742
Spleen/Body mass, mg/g	2.3 ± 0.3	3.4 ± 0.2	1.9 ± 0.1	2.5 ± 0.2	0.003	<0.001	0.181
Spleen/Tibia length, mg/mm	4.2 ± 0.4	4.5 ± 0.2	4.5 ± 0.4	4.8 ± 0.3	0.321	0.311	0.972
Visceral adipose tissue, mg	431 ± 78	255 ± 108	901 ± 101*	1403 ± 251*†	<0.001	0.295	0.036
Visceral adipose tissue/Body mass, mg/g	12.1 ± 1.8	8.8 ± 3.5	19.8 ± 1.6	35.3 ± 5.0*†	<0.001	0.075	0.009
Visceral adipose tissue/Tibia length, mg/mm	23.0 ± 4.0	12.1 ± 4.9	47.1 ± 5.0*	73.0 ± 13.7*†	<0.001	0.356	0.031
Quadricep, mg	240 ± 6	194 ± 18	216 ± 15	211 ± 6	0.782	0.049	0.104
Quadricep/Body mass, mg/g	7.0 ± 0.4	7.4 ± 0.9	4.8 ± 0.4	5.6 ± 0.4	0.002	0.298	0.701
Quadricep/Tibia length, mg/mm	12.9 ± 0.5	9.8 ± 1.0	11.3 ± 0.9	10.9 ± 0.4	0.749	0.028	0.083
Gastrocnemius, mg	164 ± 4	135 ± 9	166 ± 12	115 ± 7	0.269	<0.001	0.195
Gastrocnemius/Body mass, mg/g	4.8 ± 0.2	5.1 ± 0.4	3.7 ± 0.3	3.1 ± 0.3	0.000	0.677	0.163
Gastrocnemius/Tibia length, mg/mm	8.8 ± 0.3	6.8 ± 0.5	8.6 ± 0.5	5.9 ± 0.4	0.239	<0.001	0.423
Soleus, mg	11 ± 1	8 ± 1	11 ± 1	10 ± 2	0.680	0.085	0.583
Soleus/Body mass, mg/g	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.025	0.844	0.715
Soleus/Tibia length, mg/mm	0.6 ± 0.0	0.4 ± 0.0	0.6 ± 0.1	0.5 ± 0.1	0.735	0.025	0.389
Plantaris, mg	22 ± 1	21 ± 1	24 ± 1	18 ± 2	0.664	0.014	0.065
Plantaris/Body mass, mg/g	0.6 ± 0.0	0.8 ± 0.1†	0.5 ± 0.0	0.5 ± 0.1*	<0.001	0.331	0.041
Plantaris/Tibia length, mg/mm	1.2 ± 0.0	1.1 ± 0.1	1.3 ± 0.0	0.9 ± 0.1	0.601	0.002	0.110
Kidney, mg	297 ± 13	215 ± 8	293 ± 22	201 ± 11	0.525	<0.001	0.745
Kidney/Body mass, mg/g	8.6 ± 0.5	8.1 ± 0.3	6.5 ± 0.5	5.4 ± 0.5	<0.001	0.081	0.531
Kidney/Tibia length, mg/mm	16 ± 0.7	10.8 ± 0.4	15.2 ± 0.9	10.4 ± 0.4	0.358	<0.001	0.835
Blood glucose, mg/dl	175 ± 7	158 ± 8	206 ± 7*	158 ± 6†	0.036	<0.001	0.033
Plasma insulin, mU/L	2.2 ± 0.2	1.6 ± 0.2	21.2 ± 8.3*	2.2 ± 0.6†	0.014	0.014	0.020
HOMA-IR, U	0.9 ± 0.1	0.6 ± 0.1	10.7 ± 4.3*	0.8 ± 0.2†	0.015	0.013	0.019

Values are means ± SEM. Data were analyzed using 2-way ANOVA. Sidak *post-hoc* test was used to identify group differences.
*$P < 0.05$ vs. normal chow diet within sex.
†$P < 0.05$ vs. male within group.

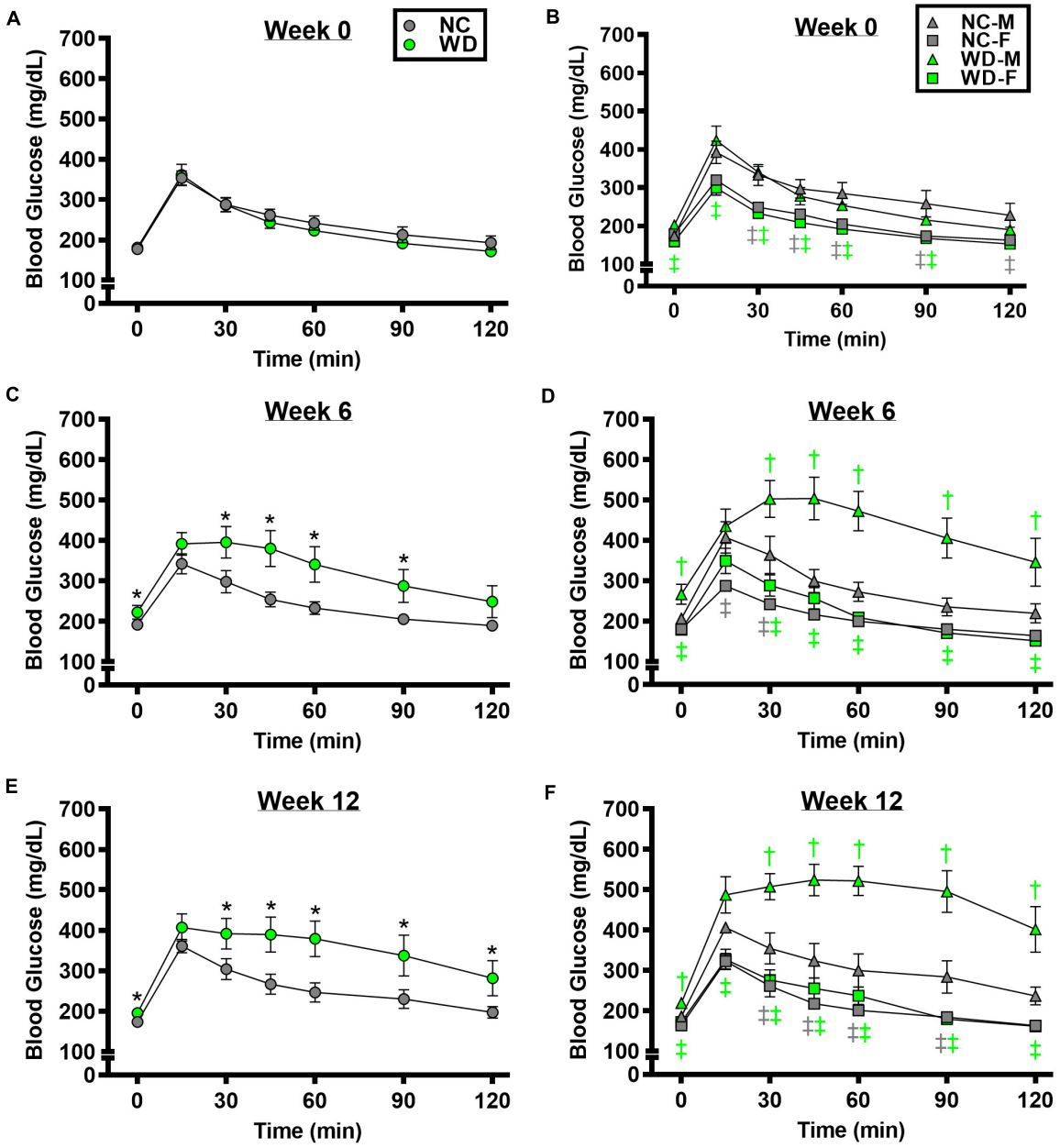


FIGURE 2
Comparisons in normal chow (NC) and Western (WD) diet-treated male (M) and female (F) mice. Data were analyzed using 3-way and 4-way mixed model ANOVA. Sidak *post-hoc* test was used to identify differences in blood glucose in response to glucose tolerance test between groups **(A)** and sexes **(B)** at week 0, week 6 **(C,D)**, and week 12 **(E,F)**. *$P < 0.05$ vs. NC. †$P < 0.05$ vs. NC within sex. ‡$P < 0.05$ vs. male within group. Data are means ± SEM.

At baseline, GTT AUC was similar between NC and WD (Figures 4A; $P < 0.05$). However, GTT AUC was higher in WD compared to NC at weeks 6 and 12 ($P < 0.05$). We also observed a significant main effect of Sex as well as significant interaction effects of Sex X Group, Sex X Time, and Sex X Group X Time with GTT AUC ($P < 0.05$). Within both diet groups, female mice had a lower GTT AUC compared to male mice at all weeks (Figure 4A; $P < 0.05$). Additionally, we observed elevations in GTT AUC in WD male mice compared to NC males at weeks 6 and 12 ($P < 0.05$). We observed a significant main effect of Group and Time, as well as a significant interaction effect with Group X Time with ITT AUC (Figure 4; $P < 0.05$). At baseline and week 6, ITT AUC was similar between NC and WD (Figure 4B; $P < 0.05$). However, ITT AUC was higher in WD compared to NC at week 12 ($P < 0.05$). Also, we observed a significant main effect of Sex as well as significant interaction

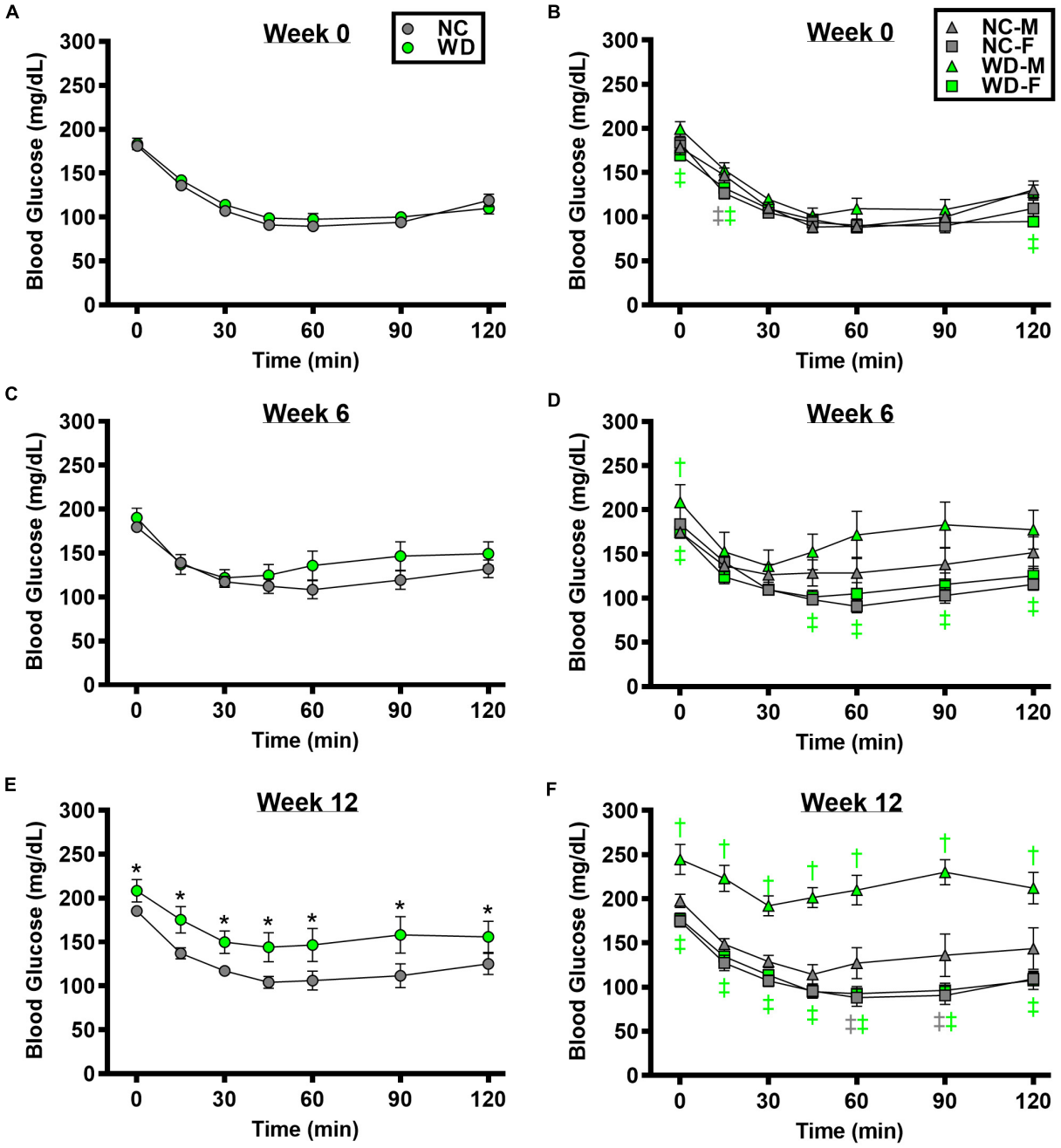


FIGURE 3
Comparisons in normal chow (NC) and Western (WD) diet-treated male (M) and female (F) mice. Data were analyzed using 3-way and 4-way mixed model ANOVA. Sidak *post-hoc* test was used to identify differences in blood glucose in response to insulin tolerance test between groups **(A)** and sexes **(B)** at week 0, week 6 **(C,D)**, and week 12 **(E,F)**. *$P < 0.05$ vs. NC. †$P < 0.05$ vs. NC within sex. ‡$P < 0.05$ vs. male within group. Data are means ± SEM.

effects of Sex X Group, Sex X Time, and Sex X Group X Time with ITT AUC ($P < 0.05$). Within both diet groups, female mice had a lower ITT AUC compared to male mice at most weeks (Figure 4B; $P < 0.05$). Additionally, we observed elevations in ITT AUC in WD male mice compared to NC males at weeks 6 and 12 ($P < 0.05$).

Systolic blood pressure

We observed a significant main effect of Group and Week, as well as a significant interaction effect of Group X Week with systolic BP and change in systolic BP (Figure 5; $P < 0.05$ for all). At baseline, systolic BP was similar between NC and WD

(Figure 5A; $P > 0.05$). Over the 12-week dietary intervention, systolic BP increased from baseline in WD mice at week 2 and continued to increase through week 12 ($P < 0.05$). Systolic BP was greater in WD compared to NC mice in weeks 2-12 ($P < 0.05$). We did not observe any sex-related differences in systolic BP (Figure 5C; $P > 0.05$) or change in systolic BP (Figure 5E; $P > 0.05$). The change in systolic BP over the 12-week intervention was ~3-fold greater in WD compared to NC mice (Figure 5E; $P < 0.05$).

Aortic structure and function

We observed a significant main effect of Group and Week, as well as a significant interaction effect of Group X Week with aortic PWV and change in aortic PWV (Figure 5; $P < 0.05$ for all). At baseline, aortic PWV was similar between NC and WD (Figure 5B; $P > 0.05$). Over the 12-week dietary intervention, aortic PWV increased from baseline in WD mice at week 4 and continued to increase through week 12 ($P < 0.05$). Aortic PWV was greater in WD compared to NC mice in weeks 2-12 ($P < 0.05$). We observed a significant interaction effect of Sex X Group with aortic PWV ($P < 0.05$), indicating female WD mice had greater aortic PWV than male WD mice (Figure 5D; $P < 0.05$). There was no sex-related difference in change in aortic PWV (Figure 5F; $P > 0.05$). The change in aortic PWV over the 12-week intervention was ~7-fold greater in WD compared to NC mice (Figure 5F; $P < 0.05$). There was a strong correlation between aortic stiffness and systolic BP (Figure 6A, $r^2 = 0.34$, $P < 0.05$), as well as a moderate correlation between change in aortic stiffness and systolic BP (Figure 6B, $r^2 = 0.16$, $P < 0.05$). We also observed strong correlations between change in body mass and change in systolic BP (Figure 7A, $r^2 = 0.31$, $P < 0.05$) or aortic stiffness (Figure 7B, $r^2 = 0.51$, $P < 0.05$).

In histological sections of thoracic aortas, there was no difference in lumen diameter (Figure 8A, $P > 0.05$), medial CSA (Figure 8B, $P > 0.05$), or medial wall-to-lumen ratio (Figure 8C, $P > 0.05$) between groups. Collagen content was elevated in WD compared to NC (Figure 8D, $P < 0.05$), but there were no differences in elastin content (Figure 8E, $P > 0.05$). There were no sex-related differences in histological analyses ($P > 0.05$).

Ex vivo arterial function

We observed a significant main effect of Group and Concentration, as well as a significant interaction effect of Group X Concentration with carotid artery vasodilation to acetylcholine (Figure 9; $P < 0.05$ for all). Carotid artery EDD was higher in NC compared to WD mice (Figure 9A; $P < 0.05$). In the presence of L-NAME, EDD was lower in both NC and WD ($P < 0.05$ vs. acetylcholine), but similar between groups ($P > 0.05$). There were no sex-related differences in EDD (Figure 9B; $P > 0.05$). We observed a significant main effect of Concentration with carotid artery vasodilation to sodium nitroprusside (Figure 9C; $P < 0.05$), but there were no group differences in EID ($P > 0.05$). There were no sex-related differences in EID (Figure 9D; $P > 0.05$). We observed no differences in carotid artery maximal luminal diameter (NC-Male: 436 ± 10; NC-Female: 421 ± 10; WD-Male: 444 ± 5; WD-Female: 438 ± 9 μm; $P > 0.05$), wall thickness (NC-Male: 51 ± 3; NC-Female: 51 ± 3; WD-Male: 55 ± 3; WD-Female: 47 ± 4 μm; $P > 0.05$), or phenylephrine-induced preconstriction (NC-Male: 24.5 ± 3.2; NC-Female: 22.0 ± 3.3; WD-Male: 25.7 ± 3.6; WD-Female: 23.6 ± 2.2 % preconstriction; $P > 0.05$). There were strong, inverse correlations between maximal vasodilation to acetylcholine and systolic BP (Figure 10A, $P < 0.05$) or aortic stiffness (Figure 10B, $P < 0.05$). However, no relationship between systolic BP (Figure 10C, $P > 0.05$) or aortic stiffness (Figure 10D, $P > 0.05$) and maximal vasodilation to sodium nitroprusside was present.

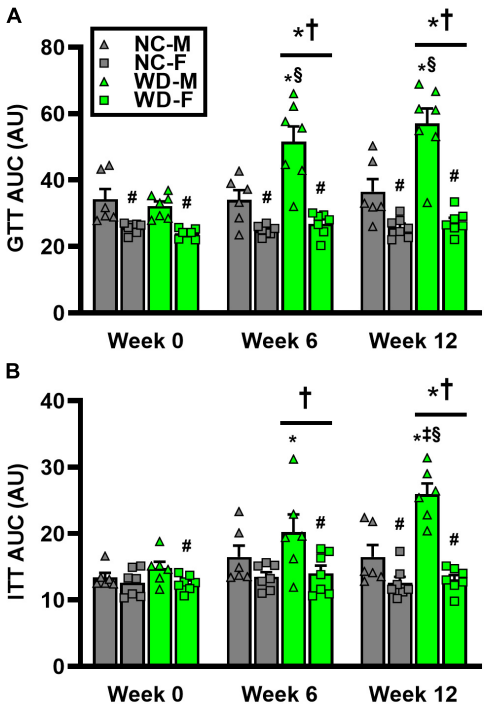


FIGURE 4
Comparisons in normal chow (NC) and Western (WD) diet-treated male (M) and female (F) mice. Data were analyzed using 2-way and 3-way mixed model ANOVA. Sidak *post-hoc* test was used to identify differences in glucose tolerance test (GTT) area under the curve (AUC) between groups/sexes **(A)**, as well as differences in insulin tolerance test (ITT) AUC between groups/sexes **(B)**. $^{*}P < 0.05$ vs. NC. $^{\dagger}P < 0.05$ vs. Week 0. $^{\ddagger}P < 0.05$ vs. Week 6. $^{\S}P < 0.05$ vs. NC within sex. $^{\#}P < 0.05$ vs. male within group. Data are individual values and means ± SEM.

10.3389/fnut.2022.1090023

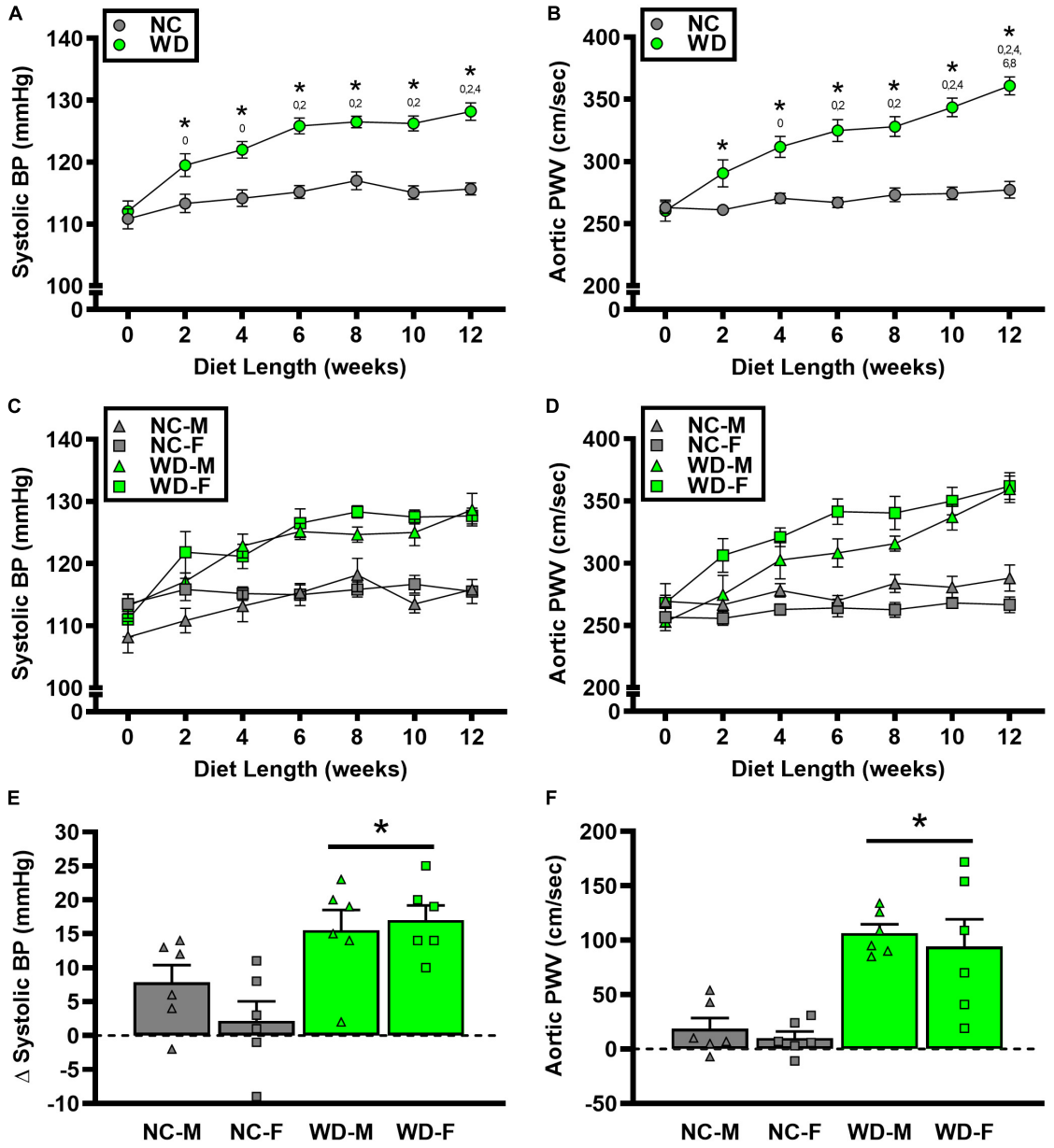


FIGURE 5
Comparisons in normal chow (NC) and Western (WD) diet-treated male (M) and female (F) mice. Data were analyzed using 2-way and 3-way mixed model ANOVA and 2-way ANOVA. Sidak *post-hoc* test was used to identify group differences in systolic blood pressure (BP) **(A)** and aortic pulse wave velocity (PWV) **(B)**, sex-differences in systolic BP **(C)** and aortic PWV **(D)**, as differences in Δ systolic BP **(E)** and Δ aortic PWV **(F)** between groups/sexes. *$P < 0.05$ vs. NC. 0, 2, 4, 6, 8, 10 $P < 0.05$ vs. the corresponding week, respectively. Data are individual values and means ± SEM.

Discussion

In the present study, 12 weeks of WD resulted in increased visceral adiposity, metabolic dysfunction, and arterial dysfunction in outbred, genetically diverse young mice. Interestingly, visceral adiposity was observed in both male and female WD mice. However, metabolic dysfunction (i.e., impaired GTT and ITT responses and elevated HOMA-IR) was only observed in male WD mice. Unlike metabolic dysfunction, arterial dysfunction was present in both male and female WD mice, which was indicated by augmented systolic BP and aortic PWV and impaired EDD. WD-induced arterial dysfunction was also accompanied by structural adaptations within the aorta that resulted in augmented aortic collagen content. Taken together, these findings provide evidence that WD-induced visceral adiposity and arterial dysfunction occur in male and

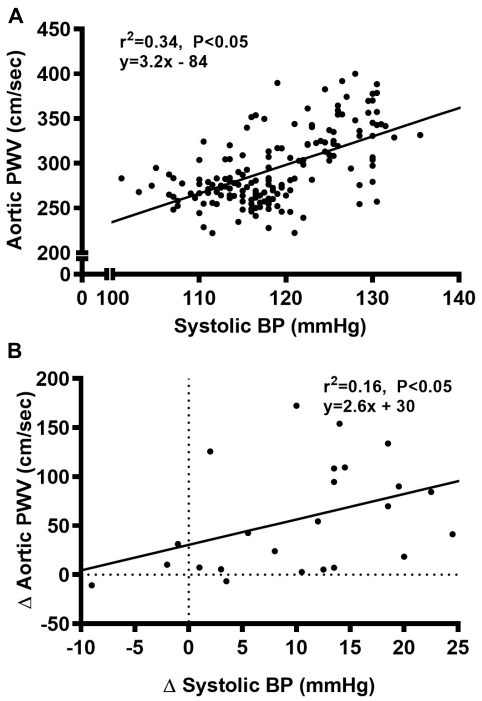


FIGURE 6
Bivariate correlational analysis was used to determine the relationship between aortic pulse wave velocity (PWV) and systolic blood pressure (BP) **(A)**, as well as the relationship between Δ in systolic BP and aortic PWV **(B)** in normal chow and Western diet-treated mice. Data are individual values.

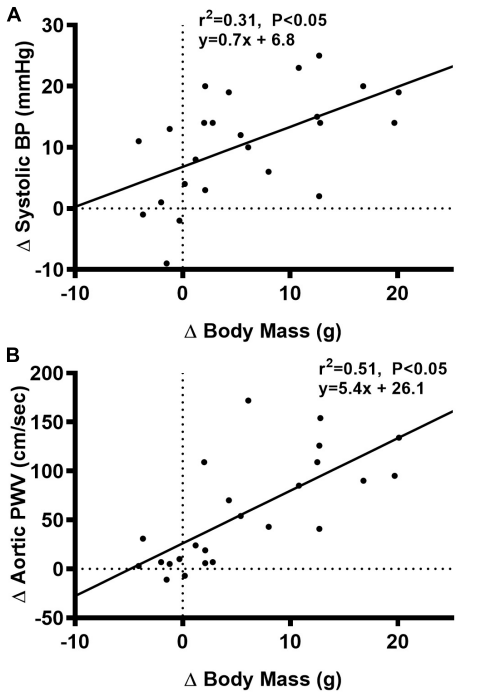


FIGURE 7
Bivariate correlational analysis was used to determine the relationship between Δ in systolic blood pressure (BP) **(A)** or aortic pulse wave velocity (PWV) **(B)** with Δ in body mass in normal chow and Western diet-treated mice. Data are individual values.

female UM-HET3 mice, despite preserved metabolic function in WD-fed female mice.

Sex-related differences in metabolic response to Western diet

Body mass and visceral adiposity were higher in male and female WD mice, compared to their NC counterparts. Although the change in body mass in response to WD was similar between males and females, visceral adiposity was ∼56% heavier in female WD mice compared to male WD mice. We observed no sex-related differences in visceral adiposity between male and female NC mice. Thus, sex-related differences in visceral adiposity between male and female WD mice indicate that WD exerts a greater obesogenic effect on female mice. As a group, metabolic dysfunction was worse in WD compared to NC mice. Interestingly, this was entirely driven by worsened metabolic dysfunction in male WD mice. Indeed, compared to NC mice and female WD mice, male WD mice had a higher non-fasted blood glucose, plasma insulin concentrations, and HOMA-IR. Moreover, male WD mice had worse glucose and insulin tolerance in response to GTTs and ITTs, respectively, compared to NC mice and female WD mice. Indeed, GTT and ITT responses were similar at all timepoints (i.e., 0-, 6-, and 12-weeks) between NC and WD female mice. It has been previously reported that female mice have greater insulin sensitivity in diet-induced obesity models (13, 43), and this has also been shown in women (44, 45). Thus, female mice may have a greater ability to store adipose tissue, which is critical to maintain insulin sensitivity (43, 46), and might explain why metabolic function was preserved in female WD mice. Although this appears to be a beneficial feature in female mice, we still observed similar or worse arterial dysfunction in female WD mice, compared to male WD mice. Thus, further study in this area is warranted.

Western diet augments systolic blood pressure and aortic stiffness

Despite sex-related differences in metabolic function in WD mice, we observed a similar time course of elevation in systolic BP between male and female across the 12-week intervention. Indeed, 2 weeks after beginning WD, systolic BP was increased in male and female mice. Systolic BP continued to rise throughout the 12-week dietary intervention. These data are in contrast to other studies that have shown a minimal effect of WD on systolic BP in mice (14–19). However, it is important

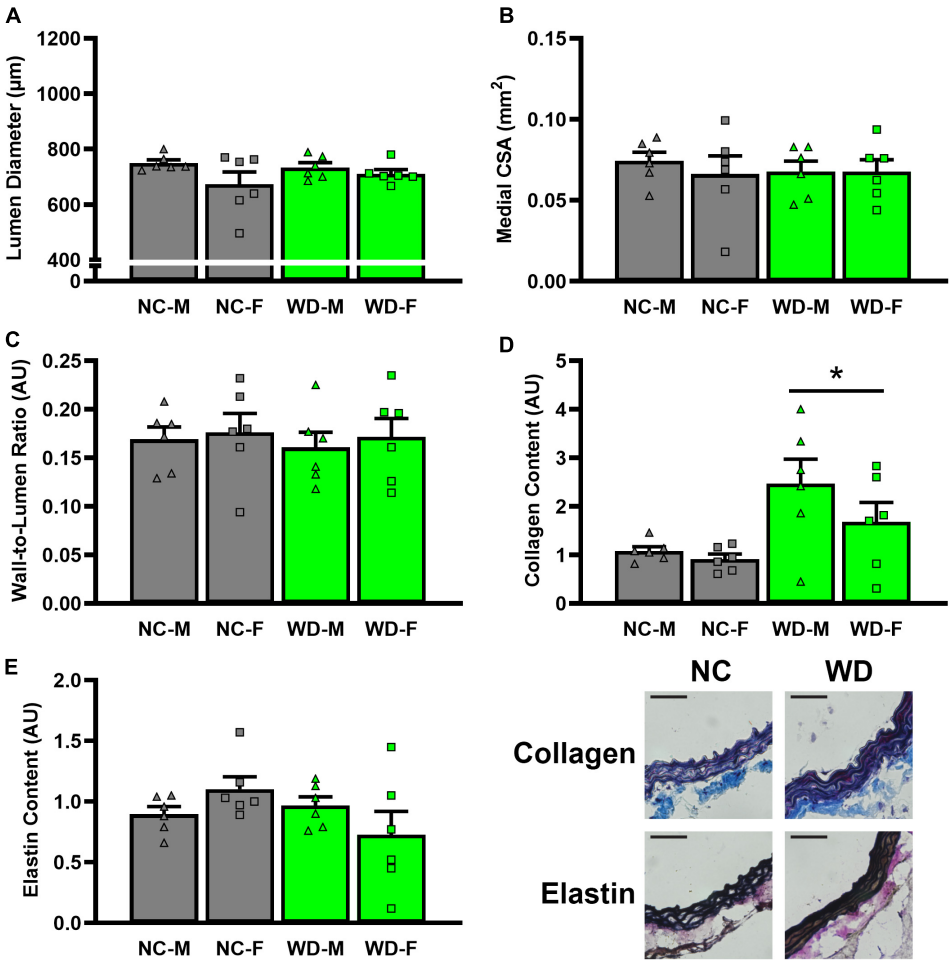


FIGURE 8
Comparisons in normal chow (NC) and Western (WD) diet-treated mice. Data were analyzed using 3-way ANOVA. Sidak *post-hoc* test was used to identify groups/sex differences in aortic lumen diameter **(A)**, medial cross-sectional area (CSA) **(B)**, wall-to-lumen area **(C)**, collagen **(D)**, and elastin **(E)** content. Figures are accompanied by representative images of collagen and elastin staining. Black scale bars are equal to 500 μm. *$P < 0.05$ vs. NC. Data are individual values and means ± SEM.

to note that these studies used genetically identical, inbred mouse strains, such as C57BL/6. Moreover, WD consumption in human is associated with elevated systolic BP (47–49). Also, there is direct evidence demonstrating experimental weight gain over a short period of time in humans increases systolic BP (20–22). Thus, the lack of a hypertensive effect of WD in inbred mice may be a strain-specific phenotype that may be avoided by using outbred, genetically diverse mice, which also increases the translatability of the current findings to humans. Thus, our findings in the present study that UM-HET3 mice more closely resemble systolic BP responses to WD and its association to weight gain in humans should be considered as a translational strength of this study and this mouse model.

Elevated systolic BP in WD mice was accompanied by augmented aortic stiffness. Although increases in systolic BP do not commonly occur this early after beginning WD in inbred strains, augmented aortic stiffening has been shown in response to WD (50–53). To the best of our knowledge, the present study is the first to examine systolic BP and aortic stiffening in response to WD in UM-HET3 mice. Early in the intervention, systolic BP and aortic stiffness tended to increase at a similar rate across the 12-week intervention. However, in the latter weeks of the intervention aortic stiffness appeared to continue increases at the same rate, while increases in systolic BP tended to slow down.

Precise determination of whether elevations in systolic BP augment aortic stiffness or vice versa with WD is beyond the scope of this study. However, some mechanistic insight may be achieved by examining the time course of changes in these measurements. Changes in systolic BP and aortic stiffness were also accompanied by significant increases in body mass. While aortic stiffening has been shown to precede elevations in systolic BP in WD-fed C57BL/6 mice (54), these changes were shown to be dependent on adiposity, as reductions in

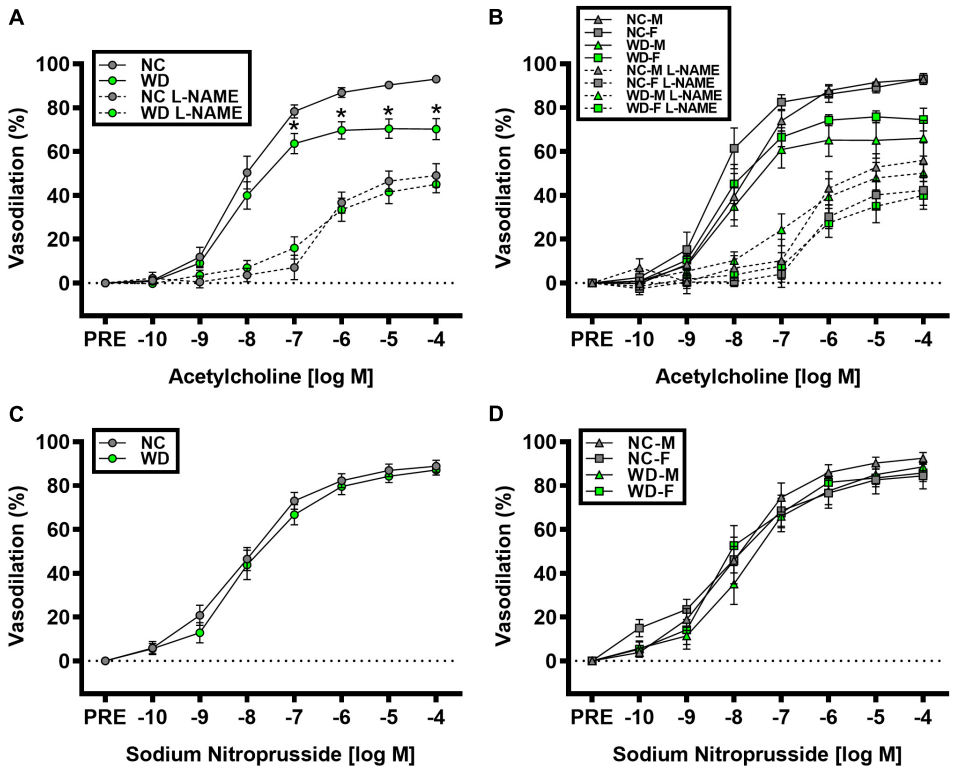


FIGURE 9
Comparisons in normal chow (NC) and Western (WD) diet-treated male (M) and female (F) mice. Data were analyzed using 2-way and 3-way mixed model ANOVA. Sidak *post-hoc* test was used to identify differences in carotid artery vasodilation to acetylcholine in the presence and absence of L-NAME between groups **(A)** and sexes **(B)**, as well as to identify differences in carotid artery vasodilation to sodium nitroprusside between groups **(C)** and sexes **(D)**. *$P < 0.05$ vs. NC. Data are means ± SEM.

adiposity lead to a decrease in systolic BP and aortic stiffness. We observed a strong relationship between the body mass gain and changes in systolic BP. In addition to augmented systolic BP and aortic stiffness, aortic collagen content was also augmented in WD mice. Increased arterial collagen content is a hallmark of arterial aging and is thought to be due to age-related changes to aortic structure and function (55). Thus, it is possible that the increase in collagen content represents arterial remodeling in response to WD consumption, and this remodeling is responsible for the continued increase in aortic stiffness and systolic BP throughout the intervention (56, 57). The direct mechanism responsible for augmented aortic stiffness cannot be determined from this study. However, modest weight gain has been associated with increased arterial stiffness in nonobese men but not nonobese women (58), although total and visceral adiposity have been shown to have a greater association with arterial stiffness in obese women than obese men (59). To the best of our knowledge, only one study measured systolic BP and aortic stiffness across the lifespan of WD-fed C57BL/6 mice and showed continually increased aortic stiffness and no change in systolic BP (16). Although it is unknown whether systolic BP and aortic stiffness would continue to rise past week 12 in these WD-fed UM-HET3 mice, it is possible that systolic BP and aortic stiffness in female WD mice would start to elevate with a faster rate compared to male WD mice as metabolic function starts to deteriorate with aging in female WD mice (60). Thus, future studies are warranted in this area.

Western diet may impair endothelium-dependent dilation by decreasing NO-mediated vasodilation

Carotid artery EDD was impaired in WD mice, demonstrated by blunted vasodilation to acetylcholine in WD compared to NC mice. Reductions in EDD appeared to be due to lower NO-mediated vasodilation in WD mice, indicating that EDD was reduced via decreases in NO bioavailability. We did not observe any blunted vasodilation to sodium nitroprusside. Thus, impaired EDD does not appear to be caused by dysfunction in vascular smooth muscle, as there was no impairment in EID. Moreover, the increase in systolic BP and aortic stiffness is linked with impaired EDD,

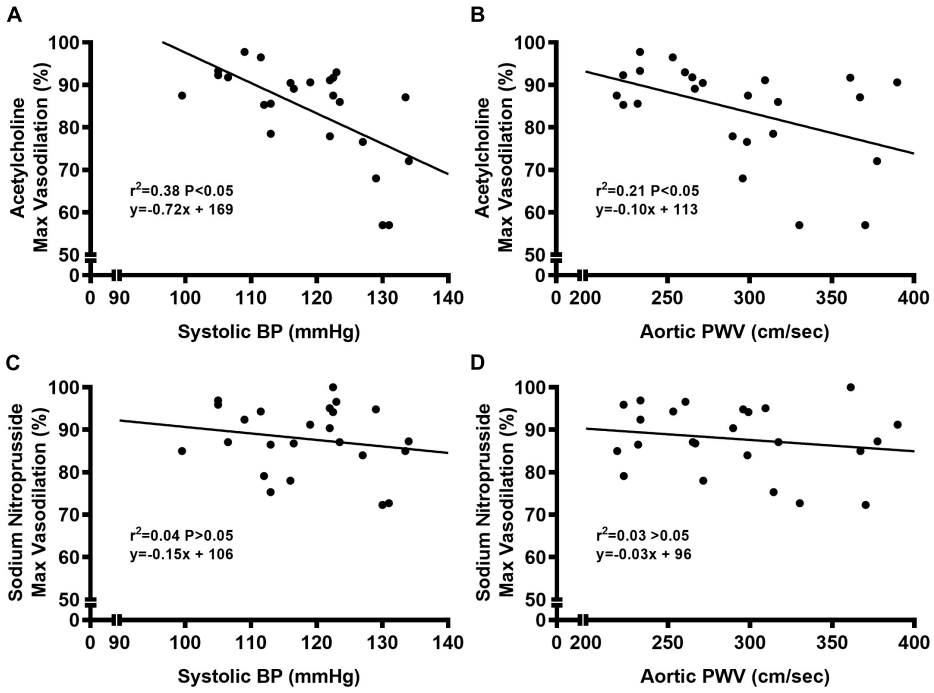


FIGURE 10
Bivariate correlational analysis was used to determine relations between maximal vasodilation to acetylcholine with systolic blood pressure (BP) **(A)** or aortic pulse wave velocity (PWV) **(B)**, as well as relations between maximal vasodilation to sodium nitroprusside with systolic blood pressure (BP) **(C)** or aortic pulse wave velocity (PWV) **(D)** in normal chow and Western diet-treated mice. Data are individual values.

but not EID (61, 62). Indeed, we observed strong inverse relationships between systolic BP or aortic stiffness and maximal vasodilation to acetylcholine in carotid artery. This relationship was not present between systolic BP or aortic stiffness and maximal vasodilation to sodium nitroprusside in carotid artery. Thus, it seems that the mechanism by which WD consumption induces elevation in systolic BP and aortic stiffening also plays a role in impaired EDD in carotid artery.

Limitations

This study is not without limitations. We did not collect subcutaneous adipose tissue, therefore, was not able to determine whether sex-difference exits in subcutaneous adipose tissue mass as it has been shown that obese men typically have greater visceral adipose tissue and obese women have greater subcutaneous adipose tissue (63). We did not observe any differences in aortic lumen diameter, media CSA, or wall-to-lumen in WD mice. It should be noted that these measurements were derived from histological sections that were cut from unpressurized aortic rings. However, we also observed no differences in lumen diameter or wall thickness in pressurized carotid arteries that we used during *ex vivo* arterial function. While these data in pressurized carotid arteries support our histological findings in the aorta, further examination of the aorta structural characteristics when pressurized is warranted.

Conclusion

In the present study, we observed a rapid increase in body mass in outbred, genetically diverse, male and female UM-HET3 mice fed WD. Interestingly, we observed WD resulted in metabolic dysfunction in male mice only, demonstrating a sex-specific manner by which WD impairs metabolic function in these mice. Although female WD mice did not develop metabolic dysfunction, they had greater visceral adiposity compared to male WD mice. Systolic BP and aortic stiffness also had a rapid increase in response to WD, which continued to increase through the end of the 12-week dietary intervention. Elevated systolic BP and aortic stiffness were strongly related to EDD, which was also impaired at the end of the intervention in male and female WD-fed mice. Although the precise physiological mechanism for these changes in response to WD is unclear, these data provide preliminary support for the use of UM-HET3 mice, an outbred, genetically diverse strain, as a mouse model for translational research on this topic. Future studies are warranted to elucidate the mechanism of WD on arterial aging and metabolic function in this mouse model, as they may provide important translational insight into

sex-specific WD-induced metabolic and arterial dysfunction in industrialized human populations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This animal study was reviewed and approved by Florida State University Animal Care and Use Committee.

Author contributions

XZ, ZL, JBS, LS, CD, JB, and DM performed the experiments. DM prepared the figures. XZ and DM drafted the manuscript. All authors have analyzed the data, conception, design of research, interpreted results, edited, revised manuscript, and approved the final version of manuscript.

Funding

This work was supported by a grant from the National Institutes of Health (R00 AT010017).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Heidenreich P, Trogdon J, Khavjou O, Butler J, Dracup K, Ezekowitz M, et al. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American heart association. *Circulation.* (2011) 123:933–44. doi: 10.1161/CIR.0b013e31820a55f5

2. Barzilai N, Huffman D, Muzumdar R, Bartke A. The critical role of metabolic pathways in aging. *Diabetes.* (2012) 61:1315–22. doi: 10.2337/db11-1300

3. Lakatta E, Levy D. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part I: aging arteries: a "set up" for vascular disease. *Circulation.* (2003) 107:139–46. doi: 10.1161/01.CIR.0000048892.83521.58

4. Adeva-Andany M, Martinez-Rodriguez J, Gonzalez-Lucan M, Fernandez-Fernandez C, Castro-Quintela E. Insulin resistance is a cardiovascular risk factor in humans. *Diabetes Metab Syndr.* (2019) 13:1449–55. doi: 10.1016/j.dsx.2019.02.023

5. Dominguez L, Barbagallo M. The biology of the metabolic syndrome and aging. *Curr Opin Clin Nutr Metab Care.* (2016) 19:5–11. doi: 10.1097/MCO.0000000000000243

6. Moore J, Chaudhary N, Akinyemiju T. Metabolic syndrome prevalence by race/ethnicity and sex in the United States, national health and nutrition examination survey, 1988-2012. *Prev Chronic Dis.* (2017) 14:E24. doi: 10.5888/pcd14.160287

7. Kopp W. How western diet and lifestyle drive the pandemic of obesity and civilization diseases. *Diabetes Metab Syndr Obes.* (2019) 12:2221–36. doi: 10.2147/DMSO.S216791

8. Oikonomou E, Psaltopoulou T, Georgiopoulos G, Siasos G, Kokkou E, Antonopoulos A, et al. Western dietary pattern is associated with severe coronary artery disease. *Angiology.* (2018) 69:339–46. doi: 10.1177/0003319717721603

9. Galloway J. Cardiovascular health among American Indians and Alaska Natives: successes, challenges, and potentials. *Am J Prev Med.* (2005) 29:11–7. doi: 10.1016/j.amepre.2005.07.023

10. West K. Diabetes in American Indians and other native populations of the new world. *Diabetes.* (1974) 23:841–55. doi: 10.2337/diab.23.10.841

11. Keller M, Gatti D, Schueler K, Rabaglia M, Stapleton D, Simecek P, et al. Genetic drivers of pancreatic islet function. *Genetics.* (2018) 209:335–56. doi: 10.1534/genetics.118.300864

12. Yang Y, Smith D Jr., Keating K, Allison D, Nagy T. Variations in body weight, food intake and body composition after long-term high-fat diet feeding in C57BL/6J mice. *Obesity.* (2014) 22:2147–55. doi: 10.1002/oby.20811

13. Casimiro I, Stull N, Tersey S, Mirmira R. Phenotypic sexual dimorphism in response to dietary fat manipulation in C57BL/6J mice. *J Diabetes Complications.* (2021) 35:107795. doi: 10.1016/j.jdiacomp.2020.107795

14. DeMarco V, Habibi J, Jia G, Aroor A, Ramirez-Perez F, Martinez-Lemus L, et al. Low-dose mineralocorticoid receptor blockade prevents western diet-induced arterial stiffening in female mice. *Hypertension.* (2015) 66:99–107. doi: 10.1161/HYPERTENSIONAHA.115.05674

15. Brunt V, Greenberg N, Sapinsley Z, Casso A, Richey J, VanDongen N, et al. Suppression of trimethylamine N-oxide with DMB mitigates vascular dysfunction, exercise intolerance, and frailty associated with a Western-style diet in mice. *J Appl Physiol.* (1985) 133:798–813. doi: 10.1152/japplphysiol.00350.2022

16. Gioscia-Ryan R, Clayton Z, Zigler M, Richey J, Cuevas L, Rossman M, et al. Lifelong voluntary aerobic exercise prevents age- and Western diet- induced vascular dysfunction, mitochondrial oxidative stress and inflammation in mice. *J Physiol.* (2021) 599:911–25. doi: 10.1113/JP280607

17. Ramirez-Perez F, Cabral-Amador F, Whaley-Connell A, Aroor A, Morales-Quinones M, Woodford M, et al. Cystamine reduces vascular stiffness in Western diet-fed female mice. *Am J Physiol Heart Circ Physiol.* (2022) 322:H167–80. doi: 10.1152/ajpheart.00431.2021

18. Heinonen I, Rinne P, Ruohonen S, Ruohonen S, Ahotupa M, Savontaus E. The effects of equal caloric high fat and western diet on metabolic syndrome, oxidative stress and vascular endothelial function in mice. *Acta Physiol.* (2014) 211:515–27. doi: 10.1111/apha.12253

19. Chaar L, Coelho A, Silva N, Festuccia W, Antunes V. High-fat diet-induced hypertension and autonomic imbalance are associated with an upregulation of CART in the dorsomedial hypothalamus of mice. *Physiol Rep.* (2016) 4:e12811. doi: 10.14814/phy2.12811

20. Covassin N, Sert-Kuniyoshi F, Singh P, Romero-Corral A, Davison D, Lopez-Jimenez F, et al. Experimental weight gain increases ambulatory blood pressure in healthy subjects: implications of visceral fat accumulation. *Mayo Clin Proc.* (2018) 93:618–26. doi: 10.1016/j.mayocp.2017.12.012

21. Gentile C, Orr J, Davy B, Davy K. Cardiorespiratory fitness influences the blood pressure response to experimental weight gain. *Obesity.* (2007) 15:3005–12. doi: 10.1038/oby.2007.358

22. Yang G, Xiang Y, Zheng W, Xu W, Zhang X, Li H, et al. Body weight and weight change in relation to blood pressure in normotensive men. *J Hum Hypertens.* (2007) 21:45–52. doi: 10.1038/sj.jhh.1002099

23. Koks S, Dogan S, Tuna B, Gonzalez-Navarro H, Potter P, Vandenbroucke R. Mouse models of ageing and their relevance to disease. *Mech Ageing Dev.* (2016) 160:41–53. doi: 10.1016/j.mad.2016.10.001

24. Tuttle A, Philip V, Chesler E, Mogil J. Comparing phenotypic variation between inbred and outbred mice. *Nat Methods.* (2018) 15:994–6. doi: 10.1038/s41592-018-0224-7

25. Archer Z, Rayner D, Rozman J, Klingenspor M, Mercer J. Normal distribution of body weight gain in male Sprague-Dawley rats fed a high-energy diet. *Obes Res.* (2003) 11:1376–83. doi: 10.1038/oby.2003.186

26. Levin B, Keesey R. Defense of differing body weight set points in diet-induced obese and resistant rats. *Am J Physiol.* (1998) 274:R412–9. doi: 10.1152/ajpregu.1998.274.2.R412

27. Mercer J, Archer Z. Diet-induced obesity in the Sprague-Dawley rat: dietary manipulations and their effect on hypothalamic neuropeptide energy balance systems. *Biochem Soc Trans.* (2005) 33:1068–72. doi: 10.1042/BST0331068

28. Palliyaguru D, Vieira Ligo Teixeira C, Duregon E, di Germanio C, Alfaras I, Mitchell S, et al. Study of longitudinal aging in mice: presentation of experimental techniques. *J Gerontol A Biol Sci Med Sci.* (2021) 76:552–60. doi: 10.1093/gerona/glaa285

29. Nadon N, Strong R, Miller R, Harrison D. NIA interventions testing program: investigating putative aging intervention agents in a genetically heterogeneous mouse model. *EBioMedicine.* (2017) 21:3–4. doi: 10.1016/j.ebiom.2016.11.038

30. Miller R, Harrison D, Astle C, Fernandez E, Flurkey K, Han M, et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell.* (2014) 13:468–77. doi: 10.1111/acel.12194

31. 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*. Washington, DC: National Academies Press (2011). p. 220.

32. Miller R, Chrisp C. Lifelong treatment with oral DHEA sulfate does not preserve immune function, prevent disease, or improve survival in genetically heterogeneous mice. *J Am Geriatr Soc.* (1999) 47:960–6. doi: 10.1111/j.1532-5415.1999.tb01291.x

33. Zheng X, Deacon C, King A, Machin D. Microcirculatory and glycocalyx properties are lowered by high salt diet but augmented by western diet in genetically heterogeneous mice. *Am J Physiol Heart Circ Physiol.* (2022) 322:H328–35. doi: 10.1152/ajpheart.00656.2021

34. Hintze K, Benninghoff A, Cho C, Ward R. Modeling the western diet for preclinical investigations. *Adv Nutr.* (2018) 9:263–71. doi: 10.1093/advances/nmy002

35. Sarafidis P, Lasaridis A, Nilsson P, Pikilidou M, Stafilas P, Kanaki A, et al. Validity and reproducibility of HOMA-IR, 1/HOMA-IR, QUICKI and McAuley's indices in patients with hypertension and type II diabetes. *J Hum Hypertens.* (2007) 21:709–16. doi: 10.1038/sj.jhh.1002201

36. Trott D, Thabet S, Kirabo A, Saleh M, Itani H, Norlander A, et al. Oligoclonal CD8+ T cells play a critical role in the development of hypertension. *Hypertension.* (2014) 64:1108–15. doi: 10.1161/HYPERTENSIONAHA.114.04147

37. Feng M, Whitesall S, Zhang Y, Beibel M, D'Alecy L, DiPetrillo K. Validation of volume-pressure recording tail-cuff blood pressure measurements. *Am J Hypertens.* (2008) 21:1288–91. doi: 10.1038/ajh.2008.301

38. Bastianini S, Silvani A, Berteotti C, Lo Martire V, Zoccoli G. Mice show circadian rhythms of blood pressure during each wake-sleep state. *Chronobiol Int.* (2012) 29:82–6. doi: 10.3109/07420528.2011.635231

39. Machin D, Auduong Y, Gogulamudi V, Liu Y, Islam M, Lesniewski L, et al. Lifelong SIRT-1 overexpression attenuates large artery stiffening with advancing age. *Aging.* (2020) 12:11314–24. doi: 10.18632/aging.103322

40. Trott D, Machin D, Phuong T, Adeyemo A, Bloom S, Bramwell R, et al. T cells mediate cell non-autonomous arterial ageing in mice. *J Physiol.* (2021) 599:3973–91. doi: 10.1113/JP281698

41. Wilson C, Lawton P, Lee M, Saunter C, Girkin J, McCarron J. VasoTracker: an open access pressure myography platform. *FASEB J.* (2019) 33:525.6. doi: 10.1096/fasebj.2019.33.1_supplement.525.6

42. Walker A, Breevoort S, Durrant J, Liu Y, Machin D, Dobson P, et al. The pro-atherogenic response to disturbed blood flow is increased by a western diet, but not by old age. *Sci Rep.* (2019) 9:2925. doi: 10.1038/s41598-019-39466-x

43. Medrikova D, Jilkova Z, Bardova K, Janovska P, Rossmeisl M, Kopecky J. Sex differences during the course of diet-induced obesity in mice: adipose tissue expandability and glycemic control. *Int J Obes.* (2012) 36:262–72. doi: 10.1038/ijo.2011.87

44. Broussard J, Perreault L, Macias E, Newsom S, Harrison K, Bui H, et al. Sex differences in insulin sensitivity are related to muscle tissue acylcarnitine but not subcellular lipid distribution. *Obesity.* (2021) 29:550–61. doi: 10.1002/oby.23106

45. Tramunt B, Smati S, Grandgeorge N, Lenfant F, Arnal J, Montagner A, et al. Sex differences in metabolic regulation and diabetes susceptibility. *Diabetologia.* (2020) 63:453–61. doi: 10.1007/s00125-019-05040-3

46. Kim J, van de Wall E, Laplante M, Azzara A, Trujillo M, Hofmann S, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. *J Clin Invest.* (2007) 117:2621–37. doi: 10.1172/JCI31021

47. Hojhabrimanesh A, Akhlaghi M, Rahmani E, Amanat S, Atefi M, Najafi M, et al. Western dietary pattern is associated with higher blood pressure in Iranian adolescents. *Eur J Nutr.* (2017) 56:399–408. doi: 10.1007/s00394-015-1090-z

48. Ruan Y, Huang Y, Zhang Q, Qin S, Du X, Sun Y. Association between dietary patterns and hypertension among Han and multi-ethnic population in southwest China. *BMC Public Health.* (2018) 18:1106. doi: 10.1186/s12889-018-6003-7

49. Monge A, Lajous M, Ortiz-Panozo E, Rodriguez B, Gongora J, Lopez-Ridaura R. Western and Modern Mexican dietary patterns are directly associated with incident hypertension in Mexican women: a prospective follow-up study. *Nutr J.* (2018) 17:21. doi: 10.1186/s12937-018-0332-3

50. Manrique-Acevedo C, Ramirez-Perez F, Padilla J, Vieira-Potter V, Aroor A, Barron B, et al. Absence of endothelial ERalpha results in arterial remodeling and decreased stiffness in western diet-fed male mice. *Endocrinology.* (2017) 158:1875–85. doi: 10.1210/en.2016-1831

51. Manrique C, Lastra G, Ramirez-Perez F, Haertling D, DeMarco V, Aroor A, et al. Endothelial estrogen receptor-alpha does not protect against vascular stiffness induced by western diet in female mice. *Endocrinology.* (2016) 157:1590–600. doi: 10.1210/en.2015-1681

52. Lesniewski L, Zigler M, Durrant J, Nowlan M, Folian B, Donato A, et al. Aging compounds western diet-associated large artery endothelial dysfunction in mice: prevention by voluntary aerobic exercise. *Exp Gerontol.* (2013) 48:1218–25. doi: 10.1016/j.exger.2013.08.001

53. Foote C, Castorena-Gonzalez J, Ramirez-Perez F, Jia G, Hill M, Reyes-Aldasoro C, et al. Arterial stiffening in western diet-fed mice is associated with increased vascular elastin, transforming growth factor-beta, and plasma neuraminidase. *Front Physiol.* (2016) 7:285. doi: 10.3389/fphys.2016.00285

54. Weisbrod R, Shiang T, Al Sayah L, Fry J, Bajpai S, Reinhart-King C, et al. Arterial stiffening precedes systolic hypertension in diet-induced obesity. *Hypertension.* (2013) 62:1105–10.

55. Pierce G, Coutinho T, DuBose L, Donato A. Is it good to have a stiff aorta with aging? Causes and consequences. *Physiology.* (2022) 37:154–73. doi: 10.1152/physiol.00035.2021

56. Stakos D, Tziakas D, Chalikias G, Mitrousi K, Tsigalou C, Boudoulas H. Associations between collagen synthesis and degradation and aortic function in arterial hypertension. *Am J Hypertens.* (2010) 23:488–94. doi: 10.1038/ajh.2010.2

57. Humphrey J. Mechanisms of arterial remodeling in hypertension: coupled roles of wall shear and intramural stress. *Hypertension.* (2008) 52:195–200. doi: 10.1161/HYPERTENSIONAHA.107.103440

58. Orr J, Gentile C, Davy B, Davy K. Large artery stiffening with weight gain in humans: role of visceral fat accumulation. *Hypertension.* (2008) 51:1519–24. doi: 10.1161/HYPERTENSIONAHA.108.112946

59. Scuteri A, Orru M, Morrell C, Tarasov K, Schlessinger D, Uda M, et al. Associations of large artery structure and function with adiposity: effects of age, gender, and hypertension. The SardiNIA Study. *Atherosclerosis.* (2012) 221:189–97. doi: 10.1016/j.atherosclerosis.2011.11.045

60. Hasegawa Y, Chen S, Sheng L, Jena P, Kalanetra K, Mills D, et al. Long-term effects of western diet consumption in male and female mice. *Sci Rep.* (2020) 10:14686. doi: 10.1038/s41598-020-71592-9

61. Panza J, Garcia C, Kilcoyne C, Quyyumi A, Cannon R III. Impaired endothelium-dependent vasodilation in patients with essential hypertension. Evidence that nitric oxide abnormality is not localized to a single signal transduction pathway. *Circulation.* (1995) 91:1732–8. doi: 10.1161/01.CIR.91.6.1732

62. Bellien J, Favre J, Iacob M, Gao J, Thuillez C, Richard V, et al. Arterial stiffness is regulated by nitric oxide and endothelium-derived hyperpolarizing factor during changes in blood flow in humans. *Hypertension.* (2010) 55:674–80. doi: 10.1161/HYPERTENSIONAHA.109.142190

63. Geer E, Shen W. Gender differences in insulin resistance, body composition, and energy balance. *Gend Med.* (2009) 6(Suppl. 1):60–75. doi: 10.1016/j.genm.2009.02.002

frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 14 March 2023
DOI 10.3389/fnut.2023.1101764

OPEN ACCESS

EDITED BY
Devin Wahl,
Colorado State University,
United States

REVIEWED BY
Zachary Clayton,
University of Colorado Boulder,
United States
Sophia Mahoney,
University of Colorado Boulder,
United States

*CORRESPONDENCE
Xinmin Wei
✉ wxinmindct@outlook.com

SPECIALTY SECTION
This article was submitted to Nutrition and Metabolism, a section of the journal Frontiers in Nutrition

RECEIVED 29 November 2022
ACCEPTED 23 February 2023
PUBLISHED 14 March 2023

CITATION
Wei X (2023) Dietary magnesium and calcium intake is associated with lower risk of hearing loss in older adults: A cross-sectional study of NHANES.
Front. Nutr. 10:1101764.
doi: 10.3389/fnut.2023.1101764

Dietary magnesium and calcium intake is associated with lower risk of hearing loss in older adults: A cross-sectional study of NHANES

Xinmin Wei*

Department of Otolaryngology, Affiliated Nanjing Jiangbei Hospital of Nantong University, Nanjing, China

Aim: Dietary intake as a modifiable factor has been reported to be associated with hearing loss (HL). The relationship between magnesium (Mg) and calcium (Ca) as common dietary nutrients and HL in the elderly has rarely been reported. This study aimed to assess the association between Mg and Ca intake and HL in older adults.

Method: This cross-sectional study included participants aged ≥70years from the National Health and Nutrition Examination Survey (NHANES) 2005–2006, 2009–2010, and 2017–2018. Outcomes were low-frequency [pure-tone averages (PTAs) at 500, 1000, and 2000Hz >25dB] and speech-frequency (PTAs at 500, 1000, 2000, and 4,000Hz >25dB) HL. Multivariate logistic analysis was utilized to explore the association between dietary Mg and Ca intake and their combined intake (Ca/Mg, Ca*Mg) and HL, and was described as odds ratio (OR) and 95% confidence interval (CI).

Results: A total of 1,858 participants were included, of which 1,052 (55.95%) had low-frequency HL and 1,349 (72.62%) had speech-frequency HL. Dietary Ca intakes [OR=0.86, 95%CI: (0.74–0.99)] and Mg intakes [OR=0.81, 95%CI: (0.68–0.95)] and Ca * Mg [OR=0.12, 95%CI: (0.02–0.87)] were associated with lower odds of low-frequency HL after adjusting for confounders. Similar, dietary Ca intakes [OR=0.85, 95%CI: (0.77–0.95)] and Mg intakes [OR=0.78, 95%CI: (0.68–0.90)] and Ca * Mg [OR=0.23, 95%CI: (0.05–0.78)] were related to lower odds of speech-frequency HL. For different levels of Mg and Ca intake, the combined intake of Ca (≥1,044mg) and Mg (≥330mg) was related to lower odds of low-frequency HL [OR=0.02, 95%CI: (0.00–0.27)] and speech-frequency HL [OR=0.44, 95%CI: (0.21–0.89)].

Conclusion: Dietary intakes of Mg and Ca were associated with lower odds of HL and are a promising intervention to be further explored in older adults with HL.

KEYWORDS

hearing loss, magnesium, calcium, dietary intakes, NHANES

Introduction

Approximately 360 million people suffer from permanent hearing loss (HL) worldwide, accounting for 5.3% of the global population (1). The prevalence of HL is rapidly rising due to an aging population, increasingly noisy environments, and increased use of hearing devices (2). Several studies have shown that more than half of people over the age of 70 suffer from age-related HL (3, 4). HL affects elderly people's daily life and quality of life, negatively affects

their mental health (5, 6), and the health costs consumed by the prevention and treatment of HL also put pressure on socioeconomic development (7, 8). Identifying modifiable factors contributing to HL and good health management of HL are important to reduce the burden of HL in the elderly.

Age-related HL experienced by older adults usually starts with high-frequency HL and gradually affects the middle and low frequencies (9). Škerková et al. showed that hearing thresholds increase significantly with age, and people over 50 years of age can only perceive sounds at 11.2 kHz (10). Moreover, once HL has progressed into the 2–4 kHz range, speech understanding in any situation is compromised (9). It is necessary to explore the relevant factors affecting the low- and middle-frequency Hl in the elderly. Studies have shown that risk factors for HL include age, smoking, and noise exposure (11). Recently, the relationship between dietary intake and HL has received attention, and good nutritional status can help prevent or repair initial HL (12). Choi et al. found that dietary magnesium (Mg) intakes may contribute to lower hearing thresholds (13). Jung et al. reported that higher dietary Mg intake was associated with a lower incidence of HL (12). This may be due to the fact that Mg can reduce HL caused by noise-induced vasoconstriction and free radical formation (14, 15). Furthermore, calcium dysregulation is a well-recognized cause of noise HL (16), and there is an antagonistic effect between Ca and Mg (17). Ca levels affect bone density, decreased bone density is common in older adults. Curhan et al. found that osteoporosis caused by decreased bone density may increase the risk of HL (18). However, the association between dietary Mg and Ca intakes and HL at different frequency HL in the elderly remain unclear.

Therefore, the purpose of this study was to examine the association between dietary Ca and Mg intakes and the risk of HL and their interaction effects. From the perspective of improving eating habits, explore and propose health management countermeasures, to lay a theoretical foundation for hearing loss health management services.

Methods

Study population

Data for this cross-sectional study were extracted from the National Health and Nutrition Examination Survey (NHANES) 2005–2006, 2009–2010, and 2017–2018 (19). NHANES was conducted by the National Centers for Health Statistics (NCHS), the Centers for Disease Control and Prevention (CDC) to assess health and nutritional information for a representative sample of the U.S. civilian, noninstitutionalized U.S. population (20). The NHANES survey uses complex, multistage, probability sampling methods based on broad population distributions. Each survey cycle in the NHANES database focused on the hearing status of participants in a specific age group (21). For example, the 2007–2008 survey focused on the hearing status of adolescents aged 12–19 years, the 2009–2010 survey focused on the hearing status of adolescents aged 12–19 years and seniors aged 70 years and older, and the 2011–2012 survey focused on the hearing status of adults aged 20–69 years. After the screening, only three survey cycles of 2005–2006, 2009–2010, and 2017–2018 focused on the hearing status of participants aged 70 and above.

A total of 3,935 eligible participants aged ≥70 who received audiometric examination was in NHANES 2005–2006, 2009–2010, and 2017–2018. Participants were excluded for nonresponse or unavailable response at the audiometric examination ($n = 7{,}243$). Moreover, participants with missing information on dietary and zero total energy intake ($n = 205$) were excluded. Finally, 1,858 eligible participants were included in the analysis. NHANES is a publicly available dataset and was approved by the NCHS Ethics Review Board, and all participants provided written informed consent.

Outcome variable

Audiometry examination was performed in a dedicated sound-isolating room in the Mobile Examination Center by trained examiners on participants. The outcome variables of this study were low-frequency HL and speech-frequency HL. Low-frequency HL was defined as the pure-tone averages (PTAs) at 500, 1000, and 2000 Hz >25 dB HL in either ear and speech-frequency HL was defined as PTAs at 500, 1,000, 2,000, and 4,000 Hz >25 dB HL in either ear (22). For patients with low-frequency HL, the control group was all participants except those with low-frequency HL. For patients with speech-frequency HL, the control group was all participants except those with speech-frequency HL. Among patients with both low-frequency and speech-frequency HL, the control group was participants with only low-frequency or speech-frequency HL, and participants with neither low-frequency nor speech-frequency HL.

Intake of calcium and magnesium

Dietary Ca, Mg, vitamin C, vitamin E, and dietary supplement intakes were estimated by 24-h dietary recall interviews. Diet recall interviews require respondents to report all food and beverages (other than regular drinking water) consumed in the 24 h prior to the interview, the quantity of food reported, and a detailed description of the food (23). Food consumption data were converted to United States Department of Agriculture (USDA) standard reference codes, and food intake was linked to the USDA's Food and Nutrient Database for Dietary Studies (FNDDS) (23, 24).

Covariates

Demographic variables included age (70–80/ ≥80 years), sex (male/ female), race (Mexican American/ other Hispanic/ non-Hispanic White/ non-Hispanic Black/ other race), marital status (married/ widowed/ divorced or separated/never married), body mass index (BMI), education level (less than high school/high school/more than high school), physical activity, and ratio of family income to poverty (PIR). PIR was categorized as ≤130, >130 to 350%, and ≥ 350% by the federal poverty level (FPL) (25). The FPL reflects income relative to household size and was used as an indicator of socioeconomic status (26). BMI was calculated as weight (kg)/height $(m)^2$. Hypertension, diabetes mellitus and cerebrovascular disease (CVD) were defined as self-reported physician diagnoses. Smoking status was defined as smoking at least 100 cigarettes in life. Loud noise exposure in past 24-h was assessed by the question of "Outside of a job, have you ever been exposed to steady loud noise or music for 5 or more hours a week? This is noise so loud that you have to raise your

voice to be heard. Examples are noise from power tools, lawn mowers, farm machinery, cars, trucks, motorcycles, or loud music." Hear protection was determined by the survey item, "How often do you wear hearing protection devices (ear plugs, ear muffs) when exposed to loud sounds or noise? (Include both job- and off-work exposures)," and it was divided into four groups: most of the time, sometimes, rarely/seldom and never. Loud noise exposure at work was participants ever had a job where exposed to loud noise for 5 or more hours a week. Physical activity was expressed as the metabolic equivalent task (MET) and calculated as follows: physical activity (met·min/week) = recommended MET × exercise time for corresponding activities (min/day) × the number of exercise days per week (day) (27). Ototoxic drug use was identified based on participants' self-reported use of the following drugs: aminoglycosides, macrolides, antineoplastic drugs, loop diuretics, salicylates, and antimalarials (28).

Statistical analysis

The study population was divided into two groups according to whether HL, characteristics were performed for comparison between groups. Continuous data were expressed as mean and standard error (S.E.), and the weighted t-test was used for comparison between groups. Categorical variables were described as the number and percentage [*n* (%)], and comparisons between groups used the weighted χ^2 test. Missing values were filled using multiple imputations. Weighted logistic multivariate analysis was utilized to explore the association between dietary Ca intakes, dietary Mg intakes, Ca/Mg and Ca*Mg and HL. Model 1 was the crude model. Model 2 adjusted for age, sex, race/ethnicity, marital status, education level, and PIR. Model 3 adjusted for age, sex, race/ethnicity, marital status, education level, PIR, loud noise exposure in the past 24 h, loud noise exposure at work, total energy intake, vitamin C intake, vitamin E intake, physical activity, ototoxic medication use, and dietary supplement. Sensitivity analysis was performed to compare whether the results were different before and after data imputation. Moreover, Ca and Mg levels were categorized into quartiles to explore the relationship between different levels of Ca and Mg intake and HL. Odds ratio (OR) and 95% confidence interval (CI) were used to assess the association. All statistical analyzes were performed using R v. 4.20 (R Foundation for Statistical Computing, Vienna, Austria) and SAS v. 9.4 (SAS Institute, Cary, North Carolina) software. A *p*-value <0.05 (two-sided) was considered statistically significant.

Results

Characteristics of the study population

A total of 1,858 participants were included in the study. Characteristics of included participants were shown in Table 1. Of these participants, 1,052 (55.95%) had low-frequency HL, 1,349 (72.62%) had speech-frequency HL, and 1,046 (55.68%) had both low-and speech-frequency HL. There were 1,220 (65.32%) participants aged 70–80 years, 950 (51.37%) male participants, and 1,268 (68.10%) non-Hispanic white participants. The mean (S.E.) BMI was 28.52 (0.13) kg/m^2. The mean (S.E.) Ca and Mg intakes were 825.84 (10.20) mg and 268.69 (3.23) mg, respectively. Dietary supplements were used by 1,503 (80.11%) participants. There were 228 (12.15%) participants who had loud noise exposure in the past 24 h and 651 (34.95%) participants who had loud noise exposure at work.

Comparison between the HL group and the non-HL group

Comparing the differences between low-frequency HL and non-low-frequency HL groups, the results showed that the two groups were significant in age ($p < 0.001$), race/ethnicity ($p < 0.001$), marital status ($p < 0.001$), education level ($p < 0.001$), PIR ($p = 0.047$), loud noise exposure in the past 24 h ($p < 0.001$), hear protection when noise exposure ($p < 0.001$), total energy intake ($p = 0.014$), and ototoxic medication use ($p = 0.043$; Table 1). In the speech-frequency HL and non-speech-frequency HL groups, there were significant differences in age ($p < 0.001$), race/ethnicity ($p < 0.001$), sex ($p < 0.001$), marital status ($p = 0.022$), educational level ($p < 0.001$), loud noise exposure in the past 24 h ($p < 0.001$), loud noise exposure at work ($p = 0.004$), hear protection when noise exposure ($p < 0.001$), dietary supplements ($p = 0.034$), and ototoxic medication use ($p = 0.018$).

Association between dietary ca and mg intake and HL

Table 2 reports the effects of dietary Ca and Mg intake and their combination (Ca/Mg and Ca * Mg) on HL. Dietary Ca intakes [OR = 0.86, 95%CI: (0.75–0.99)] and Mg intakes [OR = 0.77, 95%CI: (0.67–0.90)] and Ca * Mg [OR = 0.22, 95%CI: (0.05–0.86)] were associated with lower odds of low-frequency HL. After adjusting for age, sex, race/ethnicity, marital status, education level, PIR, loud noise exposure in the past 24 h, loud noise exposure at work, total energy intake, vitamin C intake, vitamin E intake, physical activity, ototoxic medication, and dietary supplements (model 3), dietary Ca intakes [OR = 0.86, 95%CI: (0.74–0.99)] and Mg intakes [OR = 0.81, 95%CI: (0.68–0.95)] and Ca * Mg [OR = 0.12, 95%CI: (0.02–0.87)] were still associated with lower odds of low-frequency HL. In the analysis of speech-frequency HL, dietary Ca intakes [OR = 0.85, 95%CI: (0.77–0.95)] and Mg intakes [OR = 0.78, 95%CI: (0.68–0.90)] and Ca * Mg [OR = 0.23, 95%CI: (0.05–0.78)] were related to lower odds of speech-frequency HL after adjusting for all confounders. Among participants with both low- and speech-frequency HL, dietary Ca intakes [OR = 0.85, 95%CI: (0.77–0.95)] and Mg intakes [OR = 0.78, 95%CI: (0.68–0.90)] and Ca * Mg [OR = 0.23, 95%CI: (0.05–0.78)] were also related to lower odds of low- and speech-frequency HL after adjusting for all confounders. In the sensitivity analysis (Supplementary Table S1), there was no significant difference in the results before and after data imputation.

Association between different levels of ca and mg intake and HL

Compared with Ca intake <545 mg, only participants with Ca intake ≥1,044 mg [OR = 0.61, 95%CI: (0.46–0.81)] were related to lower odds of low-frequency HL after adjusting for all confounders.

TABLE 1 Comparison of hearing loss (HL) group and non-HL group.

Variables	Total (*n*=1858)	Low-frequency HL		*P*	Speech-frequency HL		*P*
		No (*n*=806)	Yes (*n*=1,052)		No (*n*=509)	Yes (*n*=1,349)	
Age, *n* (%)				<0.001			<0.001
70–80 years	1,220 (65.32)	650 (80.67)	570 (54.46)		436 (86.30)	784 (58.36)	
≥80 years	638 (34.68)	156 (19.33)	482 (45.54)		73 (13.70)	565 (41.64)	
Race/ethnicity, *n* (%)				<0.001			<0.001
Mexican American	148 (7.78)	63 (7.45)	85 (7.69)		45 (8.65)	103 (7.18)	
Other Hispanic	72 (4.04)	41 (5.01)	31 (2.95)		25 (4.67)	47 (3.55)	
Non-Hispanic White	1,268 (68.10)	506 (63.67)	762 (73.80)		288 (57.62)	980 (73.76)	
Non-Hispanic Black	278 (14.92)	155 (18.82)	123 (11.30)		118 (22.94)	160 (11.47)	
Other race	92 (5.15)	41 (5.06)	51 (4.27)		33 (6.13)	59 (4.04)	
Sex, *n* (%)				0.059			<0.001
Male	908 (48.63)	378 (46.08)	530 (50.18)		195 (37.88)	713 (52.33)	
Female	950 (51.37)	428 (53.92)	522 (49.82)		314 (62.12)	636 (47.67)	
Marital status, *n* (%)				<0.001			0.022
Married	1,001 (53.65)	452 (56.01)	549 (53.51)		270 (53.15)	731 (55.16)	
Widowed	572 (30.67)	205 (25.42)	367 (33.34)		139 (27.24)	433 (30.84)	
Divorced/separated	220 (12.06)	118 (14.67)	102 (9.81)		78 (15.39)	142 (10.65)	
Never married	65 (3.62)	31 (3.90)	34 (3.33)		22 (4.22)	43 (3.34)	
BMI, kg/m^2, Mean (S.E)	28.52 (0.13)	28.80 (0.22)	28.37 (0.15)	0.090	28.63 (0.32)	28.54 (0.14)	0.809
Education level, *n* (%)				<0.001			<0.001
Less than high school	540 (29.13)	201 (24.07)	339 (31.65)		113 (21.57)	427 (30.86)	
High school	491 (26.49)	208 (25.71)	281 (26.63)		125 (24.53)	364 (26.87)	
More than high school	827 (44.38)	397 (50.21)	432 (41.72)		271 (53.89)	558 (42.28)	
PIR, *n* (%)				0.047			0.229
<130	432 (23.05)	204 (24.23)	312 (28.33)		129 (23.88)	387 (27.52)	
130–350%	906 (49.08)	373 (47.49)	499 (48.27)		237 (47.92)	635 (47.93)	
≥350%	520 (27.87)	229 (28.28)	241 (23.39)		143 (28.20)	327 (24.55)	
Smoking, *n* (%)				0.727			0.533
Yes	968 (51.89)	428 (52.53)	540 (51.61)		260 (50.83)	708 (52.46)	
No	890 (48.11)	378 (47.47)	512 (48.39)		249 (49.17)	641 (47.54)	
Physical activity, MET·min, Mean (S.E)	1486.64 (111.58)	1701.22 (209.16)	1328.44 (139.84)	0.159	1542.94 (166.27)	1473.71 (139.16)	0.734
Diabetes mellitus, *n* (%)				0.832			0.981
No	1,414 (75.74)	609 (75.75)	805 (76.18)		387 (75.95)	1,027 (76.00)	
Yes	444 (24.26)	197 (24.25)	247 (23.82)		122 (24.05)	322 (24.00)	
Hypertension, *n* (%)				0.432			0.625
No	452 (24.26)	199 (25.14)	254 (23.60)		126 (25.04)	327 (23.99)	
Yes	1,406 (75.74)	607 (74.86)	798 (76.40)		383 (74.96)	1,022 (76.01)	
CVD, *n* (%)				0.146			0.444
No	442 (23.72)	201 (25.27)	242 (22.19)		126 (24.87)	317 (23.05)	
Yes	1,416 (76.28)	605 (74.73)	810 (77.81)		383 (75.13)	1,032 (76.95)	
Loud noise exposure in past 24-h, *n* (%)				<0.001			<0.001
Yes	228 (12.15)	81 (10.31)	147 (13.97)		40 (7.94)	188 (14.02)	

(Continued)

TABLE 1 (Continued)

Variables	Total (*n*=1858)	Low-frequency HL		*P*	Speech-frequency HL		*P*
		No (*n*=806)	Yes (*n*=1,052)		No (*n*=509)	Yes (*n*=1,349)	
No	1,630 (87.85)	725 (89.69)	905 (86.03)		469 (92.06)	1,161 (85.98)	
Loud noise exposure at work, *n* (%)				0.052			0.004
Yes	651 (34.95)	254 (31.74)	400 (37.67)		139 (27.75)	515 (37.82)	
No	1,207 (65.05)	552 (68.26)	652 (62.33)		370 (72.25)	834 (62.18)	
Hear protection, *n* (%)				<0.001			<0.001
Most of the time	344 (18.58)	124 (15.42)	184 (17.55)		75 (14.39)	233 (17.45)	
Sometimes	141 (7.55)	58 (7.31)	69 (6.30)		33 (6.58)	94 (6.81)	
Rarely/seldom	1,130 (60.44)	400 (48.75)	603 (57.48)		249 (48.49)	754 (55.58)	
Never	243 (13.42)	224 (28.51)	196 (18.67)		152 (30.55)	268 (20.16)	
Calcium intake, mg, Mean (S.E)	825.84 (10.20)	846.84 (17.04)	817.59 (11.87)	0.152	840.98 (20.15)	826.52 (10.38)	0.477
Magnesium intake, mg, Mean (S.E)	268.69 (3.23)	279.39 (4.77)	261.62 (4.01)	0.004	280.11 (6.45)	265.43 (3.29)	0.029
Vitamin C intake, mg, Mean (S.E)	85.46 (2.21)	86.03 (2.98)	86.35 (2.38)	0.907	86.03 (3.84)	86.28 (2.20)	0.941
Vitamin E intake, mg, Mean (S.E)	7.88 (0.16)	8.16 (0.26)	7.63 (0.23)	0.159	7.92 (0.29)	7.84 (0.19)	0.812
Total energy, kcal, Mean (S.E)	1742.34 (18.22)	1789.74 (26.35)	1712.71 (23.11)	0.014	1761.09 (27.05)	1741.20 (20.71)	0.456
Dietary supplements, *n* (%)				0.088			0.034
Yes	1,503 (80.11)	463 (59.75)	557 (54.87)		300 (61.46)	720 (55.34)	
No	355 (19.89)	343 (40.25)	495 (45.13)		209 (38.54)	629 (44.66)	
Ototoxic medication use, *n* (%)				0.043			0.018
No	1,573 (84.35)	696 (86.19)	877 (82.91)		450 (88.28)	1,123 (82.87)	
Yes	285 (15.65)	110 (13.81)	175 (17.09)		59 (11.72)	226 (17.13)	

PIR: ratio of family income to poverty; BMI: body mass index; CVD: cerebrovascular disease.

Compared with Mg intake <190 mg, only participants with Mg intake ≥330 mg [OR = 0.67, 95%CI: (0.46–0.99)] were related to lower odds of low-frequency HL after adjusting for all confounders (Supplement Table S2). Similar, only participants with Ca intake ≥1,044 mg [OR = 0.59, 95%CI: (0.38–0.90)] or Mg intake ≥330 mg [OR = 0.64, 95%CI: (0.41–0.99)] were associated with lower odds of speech-frequency HL after adjusting for all confounders. Table 3 shows the effect of combined intake of different levels of Ca and Mg on HL. Combined intake of Ca (545-756 mg) and Mg (≥330 mg) [OR = 0.14, 95%CI: (0.02–0.74)] or Ca (≥1,044 mg) and Mg (≥330 mg) [OR = 0.02, 95%CI: (0.00–0.27)] was related to lower odds of low-frequency HL. Combined intake of Ca (545-756 mg) and Mg (≥330 mg) [OR = 0.34, 95%CI: (0.12–1.00)] or Ca (≥1,044 mg) and Mg (252-330 mg) [OR = 0.37, 95%CI: (0.17–0.84)] or Ca (≥1,044 mg) and Mg (≥330 mg) [OR = 0.44, 95%CI: (0.21–0.89)] was associated with lower odds of speech-frequency HL. Similar results were observed in participants with both low- and speech-frequency HL.

Discussion

HL is one of the chronic non-communicable diseases that can affect people's quality of life. HL not only has a negative impact on daily life and mental health, but its prevention and treatment costs also bring pressure to social and economic development. The present study found that dietary Mg and Ca intake and their combined intake were inversely associated with the odds of low- and speech-frequency HL. Among different levels of Mg and Ca intake, Mg levels ≥330 mg combined with Ca levels ≥1,044 mg were related to lower odds of low- and speech-frequency HL.

HL at different frequencies can be divided into low-frequency, speech-frequency, and high-frequency HL (29). High-frequency audiometry is mainly used for early detection of hearing loss. Age-related HL experienced by older adults usually starts with high-frequency HL and gradually affects the middle and low frequencies and audiometry in the 2–4 kHz range is important for the assessment of speech comprehension (9). Therefore, the current study focused on

TABLE 2 Association of dietary calcium (Ca) and magnesium (Mg) intakes with hearing loss (HL).

Outcomes	Model 1		Model 2		Model 3	
	OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
Low-frequency HL						
Ca	0.86 (0.75–0.99)	0.032	0.85 (0.74–0.97)	0.020	0.86 (0.74–0.99)	0.035
Mg	0.77 (0.67–0.90)	<0.001	0.80 (0.69–0.94)	0.008	0.81 (0.68–0.95)	0.011
Ca/Mg	1.09 (0.93–1.28)	0.258	1.03 (0.89–1.19)	0.710	1.02 (0.88–1.18)	0.809
Ca * Mg	0.22 (0.05–0.86)	0.035	0.25 (0.04–0.93)	0.044	0.12 (0.02–0.87)	0.041
Speech-frequency HL						
Ca	0.89 (0.77–1.03)	0.120	0.91 (0.84–0.99)	0.032	0.85 (0.77–0.95)	0.004
Mg	0.81 (0.69–0.94)	0.007	0.85 (0.77–0.95)	0.003	0.78 (0.68–0.90)	0.001
Ca/Mg	1.14 (0.99–1.32)	0.073	1.06 (0.95–1.19)	0.265	1.04 (0.93–1.16)	0.470
Ca * Mg	0.11 (0.02–0.76)	0.030	0.11 (0.03–0.75)	0.036	0.23 (0.05–0.78)	0.041
Low- and speech-frequency HL						
Ca	0.89 (0.81–0.97)	0.010	0.83 (0.73–0.95)	0.006	0.83 (0.72–0.95)	0.010
Mg	0.82 (0.75–0.91)	<0.001	0.82 (0.72–0.95)	0.008	0.83 (0.76–0.91)	<0.001
Ca/Mg	1.06 (0.97–1.17)	0.206	1.00 (0.85–1.19)	0.973	0.96 (0.88–1.05)	0.358
Ca * Mg	0.20 (0.05–0.89)	0.040	0.26 (0.06–0.95)	0.048	0.19 (0.04–0.79)	0.027

OR: odds ratio; CI: confidence interval. Model 1: the crude model. Model 2: adjusted for age, sex, race/ethnicity, marital status, education level, and PIR. Model 3: adjusted for age, sex, race/ethnicity, marital status, education level, PIR, loud noise exposure in the past 24 h, loud noise exposure at work, total energy intake, vitamin C intake, vitamin E intake, physical activity, ototoxic medication, and dietary supplement.

low- and speech-frequency HL in older adults aged ≥70 years. Our results showed that the prevalence of high-frequency hearing loss in participants aged ≥70 years was 95.07%, which was why we chose low- and speech-frequency HL as the outcome of our study. Many studies have reported the association between dietary Mg intake and HL. The cross-sectional study of Choi et al. showed that dietary Mg intake was associated with lower risks of HL (13), which was consistent with our results. Spankovich et al. suggested that higher Mg intake was significantly associated with better pure tone thresholds in the populations of Sydney Australia (30). Several studies have pointed out that oxidative stress was related to HL (14). The relationship between antioxidants and HL has been confirmed in many animal studies, the formation of free radicals in the inner ear is a key factor in HL, and Mg can reduce the vasoconstriction caused by the formation of free radicals, thereby protecting hearing (14, 15). These studies provide a scientific basis to support our epidemiological findings. Weyh et al. reported the role of minerals such as Mg in immune system function and regulation of inflammation (31). Therefore, dietary Mg intake is a promising intervention for elderly patients with HL and further studies are needed.

Currently, few studies were studied on the association between dietary Ca intakes and HL. The present study found an inversely associations between dietary Ca intake and low- and speech-frequency HL. Moreover, the product of dietary Ca and dietary Mg was found to be associated with lower odds of low-frequency and speech-frequency HL after adjusting for all confounders (including vitamin C, vitamin E, physical activity). To the best of our knowledge, this study was the first to analyze the association between combined intake of Ca and Mg and HL. The present study may provide epidemiological evidence for the relationship between Ca and Mg intake and HL in older adults. Dietary Ca and Mg intake is a promising intervention to be further explored in older adults with HL. However, the specific mechanism by which Mg and Ca affect HL is still unknown, and the mechanisms that can be speculated are as follows. Mg is a Ca antagonist, and Mg antagonizes Ca when absorbed in the small intestine, and chronic low levels of Ca may be related to underlying Mg deficiency (32). Mg could block the excessive release of Ca in hair cells and the cochlear vasculature, limits cellular energy consumption and induces arteriole vasodilation (33). Through this mechanism, Mg could inhibit ischemia caused by hearing damage (33). Hypomagnesemia disrupted Ca homeostasis, thereby enhancing the pro-inflammatory effects of Mg deficiency (34). Inflammatory effects are generally associated with free radicals and thus have an effect on HL (14, 35).

Several limitations of this study should be considered. First, we only used three surveys of 2005–2006, 2009–2010, and 2017–2018, which may lead to small sample size and reduced statistical power due to the different ages of participants who participated in the audiometry examination in different years in the NHANES database. Second, the cross-sectional study design of this study could not establish a causal relationship between dietary Ca and Mg intake and HL. Third, the dietary data was from a dietary recall survey, and there may be participants with cognitive impairments among the surveyed elderly, which may cause a certain recall bias. Prospective large-scale studies are needed to further explore the relationship between dietary Ca and Ma and HL.

Conclusion

This study found that dietary Mg and Ca intake and their combination were inversely associated with low- and

TABLE 3 Association between combined intake of different levels of calcium (Ca) and magnesium (Mg) and hearing loss (HL).

Outcomes	Levels	Model 2		Model 3	
		OR (95%CI)	*P*	OR (95%CI)	*P*
Low-frequency HL	Ca (<545 mg) and Mg (<190 mg)	Ref		Ref	
	Ca (545-756 mg) and Mg (190-252 mg)	1.37 (0.89–2.10)	0.149	0.66 (0.24–1.77)	0.405
	Ca (545-756 mg) and Mg (252-330 mg)	0.92 (0.47–1.81)	0.815	1.23 (0.37–4.13)	0.734
	Ca (545-756 mg) and Mg (≥330 mg)	0.47 (0.20–1.10)	0.082	0.14 (0.02–0.74)	0.021
	Ca (756-1,044 mg) and Mg (190-252 mg)	0.92 (0.54–1.58)	0.772	1.44 (0.48–4.30)	0.510
	Ca (756-1,044 mg) and Mg (252-330 mg)	0.76 (0.48–1.21)	0.245	3.37 (0.99–11.47)	0.052
	Ca (756-1,044 mg) and Mg (≥330 mg)	0.69 (0.41–1.15)	0.157	0.61 (0.11–3.33)	0.571
	Ca (≥1,044 mg) and Mg (190-252 mg)	1.51 (0.54–4.17)	0.432	0.07 (0.00–1.36)	0.080
	Ca (≥1,044 mg) and Mg (252-330 mg)	0.59 (0.35–0.99)	0.049	0.08 (0.00–1.44)	0.087
	Ca (≥1,044 mg) and Mg (≥330 mg)	0.61 (0.40–0.93)	0.020	0.02 (0.00–0.27)	0.004
Speech-frequency HL	Ca (<545 mg) and Mg (<190 mg)	Ref		Ref	
	Ca (545-756 mg) and Mg (190-252 mg)	1.52 (0.68–3.40)	0.303	1.31 (0.58–2.94)	0.515
	Ca (545-756 mg) and Mg (252-330 mg)	0.71 (0.28–1.79)	0.466	0.67 (0.25–1.81)	0.429
	Ca (545-756 mg) and Mg (≥330 mg)	0.36 (0.13–0.98)	0.045	0.34 (0.12–1.00)	0.050
	Ca (756-1,044 mg) and Mg (190-252 mg)	0.58 (0.27–1.23)	0.156	0.62 (0.27–1.43)	0.262
	Ca (756-1,044 mg) and Mg (252-330 mg)	1.15 (0.52–2.55)	0.739	1.10 (0.50–2.43)	0.809
	Ca (756-1,044 mg) and Mg (≥330 mg)	0.58 (0.22–1.54)	0.285	0.52 (0.16–1.66)	0.270
	Ca (≥1,044 mg) and Mg (190-252 mg)	1.47 (0.40–5.34)	0.561	1.37 (0.38–4.90)	0.601
	Ca (≥1,044 mg) and Mg (252-330 mg)	0.41 (0.19–0.88)	0.023	0.37 (0.17–0.84)	0.018
	Ca (≥1,044 mg) and Mg (≥330 mg)	0.47 (0.28–0.80)	0.005	0.44 (0.21–0.89)	0.023
Low- and speech-frequency HL	Ca (<545 mg) and Mg (<190 mg)	Ref		Ref	
	Ca (545-756 mg) and Mg (190-252 mg)	0.68 (0.27–1.74)	0.425	0.59 (0.23–1.51)	0.272
	Ca (545-756 mg) and Mg (252-330 mg)	1.33 (0.42–4.19)	0.623	1.21 (0.37–3.95)	0.753
	Ca (545-756 mg) and Mg (≥330 mg)	0.18 (0.03–1.02)	0.053	0.16 (0.03–0.87)	0.034
	Ca (756-1,044 mg) and Mg (190-252 mg)	1.64 (0.55–4.85)	0.374	1.57 (0.53–4.69)	0.418
	Ca (756-1,044 mg) and Mg (252-330 mg)	3.88 (1.25–12.11)	0.019	3.63 (1.09–12.05)	0.035
	Ca (756-1,044 mg) and Mg (≥330 mg)	0.92 (0.17–4.92)	0.926	0.72 (0.13–3.93)	0.707
	Ca (≥1,044 mg) and Mg (190-252 mg)	0.05 (0.00–0.92)	0.438	0.06 (0.00–0.92)	0.044
	Ca (≥1,044 mg) and Mg (252-330 mg)	0.08 (0.01–1.24)	0.070	0.08 (0.01–1.22)	0.069
	Ca (≥1,044 mg) and Mg (≥330 mg)	0.02 (0.00–0.31)	0.005	0.02 (0.00–0.26)	0.003

OR: odds ratio; CI: confidence interval; Ref, reference. Model 2: adjusted for age, sex, race/ethnicity, marital status, educational level, and PIR. Model 3: adjusted for age, sex, race/ethnicity, marital status, educational level, PIR, loud noise exposure in the past 24 h, loud noise exposure at work, total energy intake, vitamin C intake, vitamin E intake, physical activity, ototoxic medication, and dietary supplements.

speech-frequency HL. Identification of modifiable factors affecting elderly patients with HL plays an important role in patient management and prevention. Dietary Mg and Ca intake is a promising intervention to be further explored in older adults with HL.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here NHANES, https://wwwn.cdc.gov/nchs/nhanes/.

Ethics statement

Ethical approval was not provided for this study on human participants because NHANES is a publicly available data set. The patients/participants provided their written informed consent to participate in this study.

Author contributions

XW designed the study, wrote the manuscript, collected, analyzed and interpreted the data, and critically reviewed, edited and approved the manuscript.

Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2023.1101764/full#supplementary-material

References

1. Murphy WJ, Eichwald J, Meinke DK, Chadha S, Iskander J. CDC grand rounds: promoting hearing health across the lifespan. *Morb Mortal Wkly Rep*. (2018) 67:243–6. doi: 10.15585/mmwr.mm6708a2

2. Olusanya BO, Davis AC, Hoffman HJ. Hearing loss: rising prevalence and impact. *Bull World Health Organ*. (2019) 97:646–646A. doi: 10.2471/blt.19.224683

3. Wu PZ, O'Malley JT, de Gruttola V, Liberman MC. Primary neural degeneration in noise-exposed human Cochleas: correlations with outer hair cell loss and word-discrimination scores. *J Neurosci*. (2021) 41:4439–47. doi: 10.1523/JNEUROSCI.3238-20.2021

4. Yamoah EN, Li M, Shah A, Elliott KL, Cheah K, Xu PX, et al. Using Sox2 to alleviate the hallmarks of age-related hearing loss. *Ageing Res Rev*. (2020) 59:101042. doi: 10.1016/j.arr.2020.101042

5. Bonfiglio V, Umegaki H, Kuzuya M. A study on the relationship between cognitive performance, hearing impairment, and frailty in older adults. *Dement Geriatr Cogn Disord*. (2020) 49:156–62. doi: 10.1159/000507214

6. GBD 2019 Ageing Collaborators. Global, regional, and national burden of diseases and injuries for adults 70 years and older: systematic analysis for the global burden of disease 2019 study. *BMJ*. (2022) 376:e068208. doi: 10.1136/bmj-2021-068208

7. Guan L, Liu Q, Chen D, Chen C, Wang Z. Hearing loss, depression, and medical service utilization among older adults: evidence from China. *Public Health*. (2022) 205:122–9. doi: 10.1016/j.puhe.2022.01.025

8. Tordrup D, Smith R, Kamenov K, Bertram MY, Green N, Chadha S. Global return on investment and cost-effectiveness of WHO's HEAR interventions for hearing loss: a modelling study. *Lancet Glob Health*. (2022) 10:e52–62. doi: 10.1016/S2214-109X(21)00447-2

9. Gates GA, Mills JH. Presbycusis. *Lancet*. (2005) 366:1111–20. doi: 10.1016/S0140-6736(05)67423-5

10. Škerková M, Kovalová M, Mrázková E. High-frequency audiometry for early detection of hearing loss: A narrative review. *Int J Environ Res Public Health*. (2021) 18:4702. doi: 10.3390/ijerph18094702

11. Nieman CL, Oh ES. Hearing loss. *Ann Intern Med*. (2020) 173:ITC81–ITC96. doi: 10.7326/AITC202012010

12. Jung SY, Kim SH, Yeo SG. Association of nutritional factors with hearing loss. *Nutrients*. (2019) 11:307. doi: 10.3390/nu11020307

13. Choi Y-H, Miller JM, Tucker KL, Hu H, Park SK. Antioxidant vitamins and magnesium and the risk of hearing loss in the US general population. *Am J Clin Nutr*. (2014) 99:148–55. doi: 10.3945/ajcn.113.068437

14. Le Prell C, Yamashita D, Minami S, Yamasoba T, Miller J. Mechanisms of noise-induced hearing loss indicate multiple methods of prevention. *Hear Res*. (2007) 226:22–43. doi: 10.1016/j.heares.2006.10.006

15. Scheibe F, Haupt H, Vlastos GA. Preventive magnesium supplement reduces ischemia-induced hearing loss and blood viscosity in the Guinea pig. *Eur Arch Otorhinolaryngol*. (2000) 257:355–61. doi: 10.1007/s004050000252

16. Shen H, Zhang B, Shin JH, Lei D, Du Y, Gao X, et al. Prophylactic and therapeutic functions of T-type calcium blockers against noise-induced hearing loss. *Hear Res*. (2007) 226:52–60. doi: 10.1016/j.heares.2006.12.011

17. Messler HH, Koch W, Münzenberg KJ. Analogous effects of organic calcium antagonists and magnesium on the epiphyseal growth plate. *Clin Orthop Relat Res*. (1990) 258:135–41. doi: 10.1097/00003086-199009000-00016

18. Curhan SG, Stankovic K, Halpin C, Wang M, Eavey RD, Paik JM, et al. Osteoporosis, bisphosphonate use, and risk of moderate or worse hearing loss in women. *J Am Geriatr Soc*. (2021) 69:3103–13. doi: 10.1111/jgs.17275

19. CDC (Centers for Disease Control and Preventio). *National Health and Nutrition Examination Survey, 2005–2006 Data Documentation, Codebook, and Frequencies*. (2005). Available at: https://wwwn.cdc.gov/Nchs/Nhanes/2005-2006/DEMO_D.htm (Accessed October 16, 2022).

20. Chen T-C, Clark J, Riddles MK, Mohadjer LK, Fakhouri TH. National Health and nutrition examination survey, 2015–2018: Sample design and estimation procedures. *Vital Health Stat 2*. (2020) 184:1–35.

21. CDC (Centers for Disease Control and Preventio). *National Health and Nutrition Examination Survey 2009–2010 Data Audiometry*. (2009). Available at: https://wwwn.cdc.gov/Nchs/Nhanes/2009-2010/AUX_F.htm (Accessed October 16, 2022).

22. Szeto B, Valentini C, Lalwani A. Low vitamin D status is associated with hearing loss in the elderly: a cross-sectional study. *Am J Clin Nutr*. (2021) 113:456–66. doi: 10.1093/ajcn/nqaa310

23. CDC (Centers for Disease Control and Preventio). *National Health and Nutrition Examination Survey, 2017–2018, Dietary Interview*. (2017). Available at: https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/DR1IFF_J.htm#Component_Description (Accessed October 16, 2022).

24. Raper N, Perloff B, Ingwersen L, Steinfeldt L, Anand J. An overview of USDA's dietary intake data system. *J Food Compos Anal*. (2004) 17:545–55. doi: 10.1016/j.jfca.2004.02.013

25. Ogden CL, Fakhouri TH, Carroll MD, Hales CM, Fryar CD, Li X, et al. Prevalence of obesity among adults, by household income and education - United States, 2011-2014. *MMWR Morb Mortal Wkly Rep*. (2017) 66:1369–73. doi: 10.15585/mmwr.mm6650a1

26. ASPE. *Prior HHS Poverty Guidelines and Federal Register References*. (2022). Available at: https://aspe.hhs.gov/prior-hhs-poverty-guidelines-and-federal-register-references (Accessed February 16, 2023).

27. Mendes MA, da Silva I, Ramires V, Reichert F, Martins R, Ferreira R, et al. Metabolic equivalent of task (METs) thresholds as an indicator of physical activity intensity. *PLoS One*. (2018) 13:e0200701. doi: 10.1371/journal.pone.0200701

28. Guo J, Chai R, Li H, Sun S. Protection of hair cells from ototoxic drug-induced hearing loss. *Adv Exp Med Biol*. (2019) 1130:17–36. doi: 10.1007/978-981-13-6123-4_2

29. Kiely KM, Gopinath B, Mitchell P, Luszcz M, Anstey KJ. Cognitive, health, and sociodemographic predictors of longitudinal decline in hearing acuity among older adults. *J Gerontol A Biol Sci Med Sci*. (2012) 67:997–1003. doi: 10.1093/gerona/gls066

30. Spankovich C, Hood L, Silver H, Lambert W, Flood V, Mitchell P. Associations between diet and both high and low pure tone averages and transient evoked otoacoustic emissions in an older adult population-based study. *J Am Acad Audiol*. (2011) 22:49–58. doi: 10.3766/jaaa.22.1.6

31. Weyh C, Krüger K, Peeling P, Castell L. The role of minerals in the optimal functioning of the immune system. *Nutrients*. (2022) 14:644. doi: 10.3390/nu14030644

32. Dennehy C, Tsourounis C. A review of select vitamins and minerals used by postmenopausal women. *Maturitas*. (2010) 66:370–80. doi: 10.1016/j.maturitas.2010.06.003

33. Vink R, Nechifor M. *Magnesium in the Central Nervous System*. Adelaide, AU: University of Adelaide Press (2011).

34. Malpuech-Brugère C, Rock E, Astier C, Nowacki W, Mazur A, Rayssiguier Y. Exacerbated immune stress response during experimental magnesium deficiency results from abnormal cell calcium homeostasis. *Life Sci*. (1998) 63:1815–22. doi: 10.1016/S0024-3205(98)00455-X

35. Gopinath B, Flood V, McMahon C, Burlutsky G, Spankovich C, Hood LJ, et al. Dietary antioxidant intake is associated with the prevalence but not incidence of age-related hearing loss. *J Nutr Health Aging*. (2011) 15:896–900. doi: 10.1007/s12603-011-0119-0

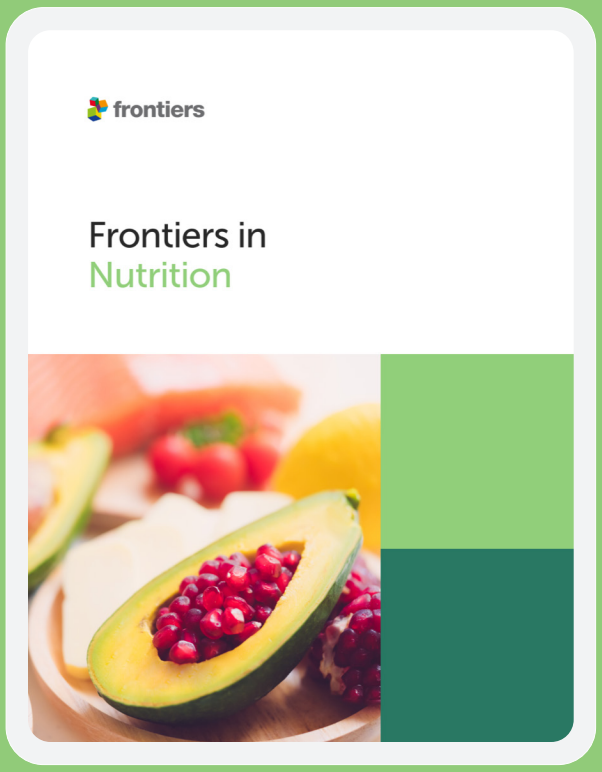
Frontiers in Nutrition

Explores what and how we eat in the context of health, sustainability and 21st century food science

A multidisciplinary journal that integrates research on dietary behavior, agronomy and 21st century food science with a focus on human health.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact