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Advances in obesity prevention, treatment and management: Lifecycle and complex system approaches

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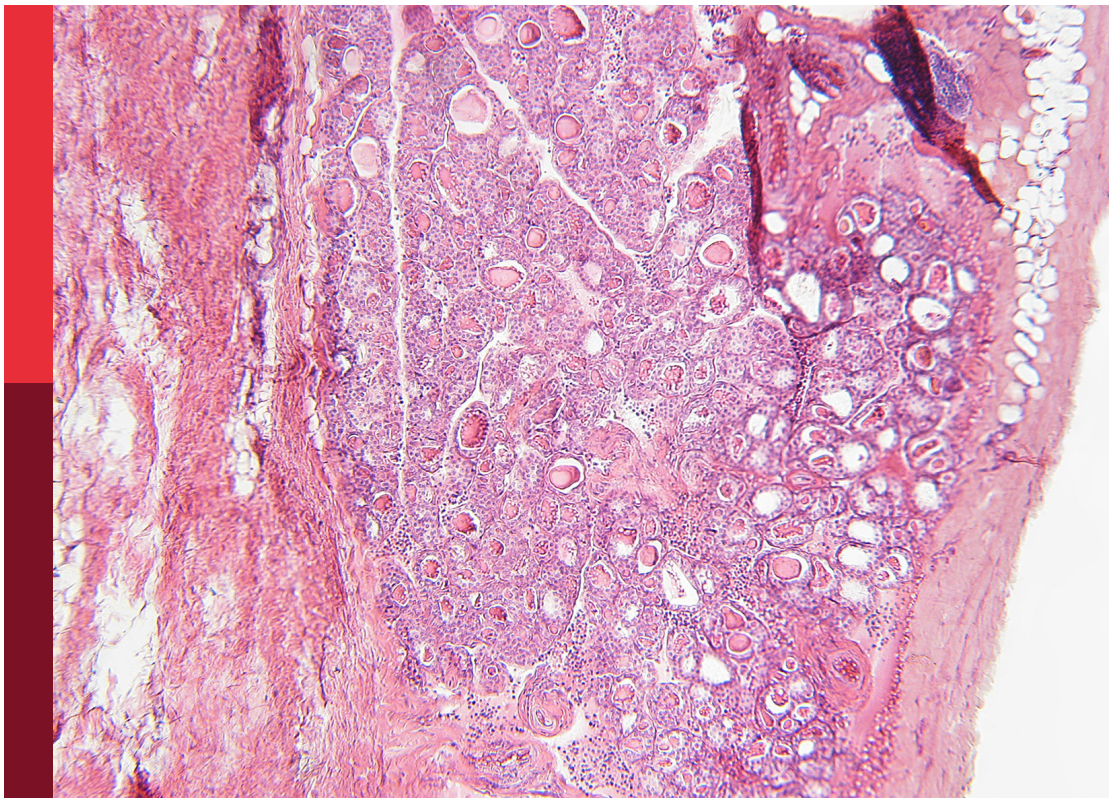

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Advances in obesity prevention, treatment and management: Lifecycle and complex system approaches

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Modeling obesity in complex food systems: Systematic review

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Obesity-related data derived from multiple complex systems spanning media, social, economic, food activity, health records, and infrastructure (sensors, smartphones, etc.) can assist us in understanding the relationship between obesity drivers for more efficient prevention and treatment. Reviewed literature shows a growing adaptation of the machine-learning model in recent years dealing with mechanisms and interventions in social influence, nutritional diet, eating behavior, physical activity, built environment, obesity prevalence prediction, distribution, and healthcare cost-related outcomes of obesity. Most models are designed to reflect through time and space at the individual level in a population, which indicates the need for a macro-level generalized population model. The model should consider all interconnected multi-system drivers to address obesity prevalence and intervention. This paper reviews existing computational models and datasets used to compute obesity outcomes to design a conceptual framework for establishing a macro-level generalized obesity model.

KEYWORDS

obesity, complex system, simulation model, machine learning, statistical methods, computational models, system dynamics

1 Introduction

Over the past two decades, there has been a significant rise in the prevalence of obesity, which has gradually turned into a global epidemic. Obesity is a global public health and economic issue that harms people's physical and mental health, reduces their quality of life and life expectancy, and significantly increases the cost of healthcare systems (1). There are multiple causes and effects that contribute to obesity. Obesity is a multidimensional, systemic issue that affects a variety of domains, including social interactions, infrastructure, environment, and biology (2). Due to obesity's global scope, heterogeneous drivers interacting in non-linear ways, and the lack of a single solution for variation in outcomes, a complex system is needed (3). A complex system model that can explain inter-connections and correlations of drivers and can examine non-linear

dynamics, time-delay effects, multiple interactions, and feedback is required. Multiple authors used different system dynamic (SD) techniques and methods to model, predict, classify, and explain the prevalence of obesity and driver's interconnection. Most models are designed with a specific question and focus on the limited number of links specific to a region or county. A holistic model explaining the indirect drivers of obesity in complex food system is missing. The multi-level sub-systems interconnections of obesity drivers must be investigated to derive general model, potentially able to clarify complex and indirect interconnections; Modeling multi-level model can become confusing and complex that results are no longer transparent, making validation impossible. To model a comprehensive general model, the multi-level sub-systems links of obesity should be explored, which can become so unwieldy and complex that results are no longer transparent, and validation becomes nearly impossible. So, currently, there is a need for a global level model, able to addresses the driver of interconnections and multi-level sub-systems of the obesity system (2). Vandenbroeck et al. (2) developed the qualitative obesity system map to understand the complex systemic structure of obesity using the causal loop model. The obesity system map can be used as a reference framework to design a conceptual, quantitative general obesity model. The obesity system map includes a variety of drivers, some of which have quantified causal relationships represented as mechanistic equations in the literature and others for which there is no quantitative data, such as environmental and social drivers. By using machine learning models (4), one can approximate the missing causative relationship and fill the gap to build a general complex obesity system model. The obesity system model has implications for future research aimed at early detection of obesity by hospitals and health professionals as well as to assist policymakers in testing interventions to analyze hotspots and where to intervene.

This review a) provides an overview of computational obesity models present in the scientific literature, including machine learning, agent-based, system dynamics, and simulation models that can explain the interconnections and non-linear dynamics between obesity drivers and b) analyzes the studies which have datasets and were reproducible c) and determines different modeling techniques strengths and limitations. Such an analysis should determine the potential of different models in defining and tracing the non-linear effects of key drivers of obesity. The review is aimed at the development of a conceptual framework for a global-level model by using and combining strengths of different modeling techniques.

The study begins with a bibliographic search and selection of literature for review, explaining the search terms and literature selection criteria for reviewing different models. We then examine the various modeling techniques of complex obesity systems and analyze their purpose, outcome, and limitations using the selected studies. The selected models are reviewed and discussed further, explaining the global level of a proposed framework and future development and application potential.

2 Methods

A systematic literature review (SLR) is an accessible and well-organized technique to define relevant research questions, keywords, and search phrases and study (5). An SLR analyses the research question and provides different methods for analyzing the problem thoroughly and broadly. The summary of the research methodology and SLR procedure is shown in following sub-section.

2.1 Overview

In order to conduct the SLR research and disclose the findings, this study adheres to the PRISMA guidelines (6). The SLR includes following tasks: define the search strategy and keywords and describe the inclusion and exclusion criteria. The review consists of computational models of obesity (6) which were written in English and published between January 1, 2002 and January 1, 2022 and had obesity-related search phrases and computational models.

After removing duplicate results, the potential studies were identified using relevant terms and keywords in electronic databases. Prior to text screening, the titles and abstracts of the records were first checked for inclusion and exclusion criteria for inclusion in the review.

2.2 Electronic searches

We searched scientific databases PubMed/Medicine (US National Library of Medicine) and Google Scholar (Google) for potential articles. We used a "backward and forward" search to determine the study references. To "go on" and find the articles cited in particular reviews, the google search engine was employed. The chosen studies serve as a starting point for finding articles that were relevant for our investigation. The logical search strategy based on Medical Subheading terms from PubMed: MeSH terms "obesity", "overweight", "obese", "model", "simulation model", "system dynamics", "agent-based model", "ABM", "machine learning" using query [(obesity [MeSH Terms] OR obese[MeSH Terms] or overweight[MeSH Terms]) AND (model[MeSH Terms] OR simulation model [MeSH Terms] OR system dynamic [MeSH Terms] OR agent-based model[MeSH Terms] or ABM[MeSH Terms] OR machine

learning [MeSH Terms])]. The following were the chosen keywords for databases and Google scholar engine:

- ("Obesity" OR "Overweight" OR "Obese" OR "Adiposity") AND ("Simulation model" OR "Simulation")
- ("Obesity" OR "Overweight" OR "Obese" OR "Adiposity") AND ("Agent based model" OR "ABM" OR "Agent-based model")
- ("Obesity" OR "Overweight" OR "Obese" OR "Adiposity") AND ("Machine learning" OR "Prediction")
- ("Obesity" OR "Overweight" OR "Obese" OR "Adiposity") AND ("System dynamics" OR "Model" OR "Computational model") AND ("model")

The search strategy included the databases, limiting research to 20 years (from May 2012 to May 2022). The studies were selected using a systematic and logical search strategy based on Medical Subheading terms from PubMed, IEEE, and query for Google Scholar engine as explained in Figure 1. After eliminating ten duplicates, the total articles examined on 6, Jan 2022 for the year (2002–2022) generated 136 hits.

2.3 Inclusion and exclusion criteria

To assure the relevance of the chosen articles to the study purpose of the research, inclusion comprises the characteristics that qualify the studies for inclusion, and exclusion includes the characteristics that disqualify the studies for inclusion. First, the article's abstract and title was carefully reviewed to evaluate its suitability for the current SLR. After that, each study was examined to determine if it met the exclusion or inclusion criteria.

For this review, only articles about obesity and obesity computational models published in English from January 2002 and January 2022 were taken into consideration. The explicit inclusion and exclusion criteria used in the study summarized in Table 1.

2.4 Study selection process

The primary goal of the selection procedure is to find relevant articles. Using the keywords provided above, an electronic search resulted in 353 articles. After deleting 10 duplicate articles with Mendeley's reference management tool (Elsevier, UK), the results were narrowed down to 343 articles. We eliminated 185 studies from consideration by carefully reading abstracts, title and conclusions of studies and applying inclusion and exclusion criteria.

Exclusion criteria were used to assess the relevance of the remaining 136 articles to the research objectives. The manual and electronic search yielded 41 different computational models. Genetic factors and infant studies were not considered.

3 Modeling techniques for obesity

For simulating the complexity of obesity, there are a variety of methodologies available. Because of the depth and scale of the obesity epidemic, the model should be able to capture multi-level analysis when modeling the obesity complex system (7–9). Modeling at a single level does not allow to identify links and feedback loops between different levels (individual, population, national, and global levels) and its degree of influence on obesity outcomes. Second, the model should be able to capture individual heterogeneity as well as variation in adaptation over time. Finally, the model explains the problem and its mechanism to slow or reverse the epidemic (10). Given these requirements, computational and simulation models taken from the literature on obesity research and complexity science offers a set of useful tools.

TABLE 1 Inclusion and exclusion criteria.

Criteria	Principle
Inclusion	Studies published between January 2002- January 2022
	Studies written in English
	Studies that are complete and models have been validated on datasets
	Studies related to computational and machine learning models of obesity
	Studies explaining the relation of obesity drivers, prediction, trends, and prevalence of obesity
Exclusion	Duplicate studies
	Studies conducted in languages other than English
	Studies in which models were not validated against datasets
	Studies that did not explain the relation between obesity drivers using model
	Studies related to infants and genetic concepts
	Studies based on simulation model software

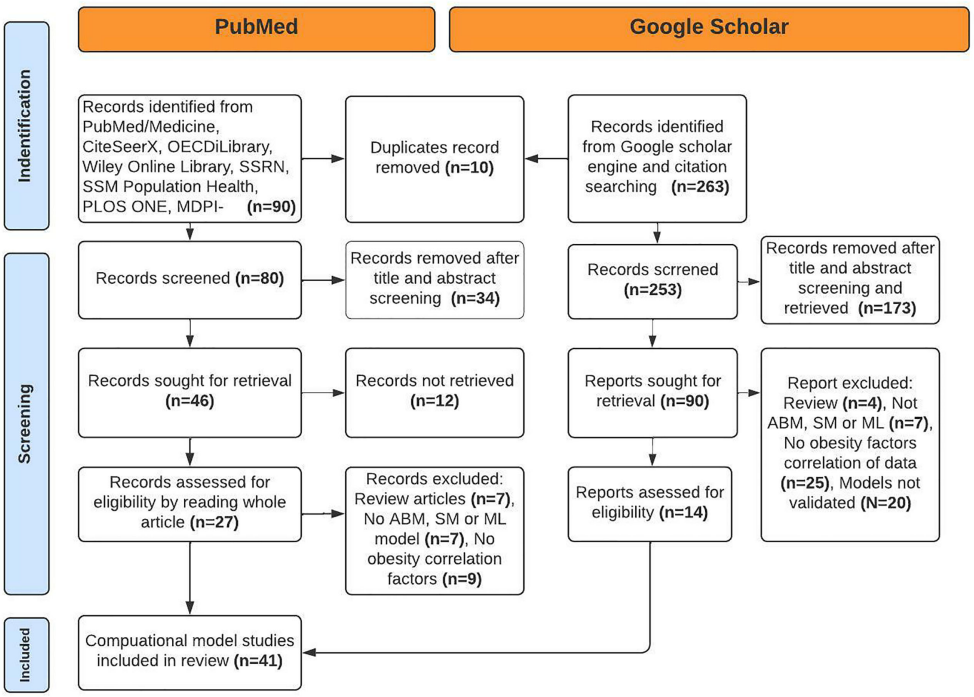

FIGURE 1
PRISMA diagram of identification and selection of studies for the review.

3.1 System dynamics

System dynamics (SD) (11, 12) is a modeling technique in which a system is modeled using key drivers, flows and feedback loops to investigate the dynamics of a system under a set of scenarios, assumptions and datasets. The simulated computer model helps us determine under what conditions and how a part of the system might fail and identify the gaps in current knowledge. A simulation model has the following properties: a) stochastic and deterministic behavior, b) static and dynamic time series, and c) discrete and continuous data. Many simulation models based on food, physical activity, social environment, and economic trends have been implemented in the literature, as shown in Table S1.

3.1.1 Mechanistic models of obesity

Hodgkin and Huxley (13) proposed the mechanistic modeling paradigm. It typically involves generating simplified mathematical equations of the causal mechanism by establishing a causal relationship between inputs and outputs based on observations of the phenomenon of interest (14). The mechanistic model can be implemented at both individual and population levels. Frerichs implemented the micro-level mechanistic model to assess the sensitivity of childhood obesity and social transmission rates. Fallah Fini et al. (15, 16) explored micro-level dynamics of Body Mass Index (BMI) distribution and prevalence to quantify the energy gap responsible for obesity in gender and racial population using mechanistic models and statistics.

3.1.2 Markov simulation models of obesity

Markov models are stochastic models that can capture the distribution of attributes and model their dynamics (17, 18). Markov simulation model can recognize patterns, make predictions and learn the statistics of sequential data. Basu (19) forecasted BMI using Medical Expenditure Panel Survey (MEPS) data and a micro-simulated probabilistic population-level model. The model was validated using the National Health and Nutrition Examination Survey (NHANES) to project that obesity growth will continue in children aged 6-9, while overall obesity prevalence will remain constant. Individual-level Markov models explain how BMI trends differ across cohorts by taking demographics (age, race, height, BMI), socioeconomic and dietary composition, and individuals at household parameters. Ball et al. (20) created an individual-level dynamic discrete-time Markov micro-simulation model for estimating lifetime healthcare costs for healthy, obese, and smoker cohorts. The economic burden and productivity loss brought on by obesity were predicted by Lightwood et al. (21) using Discrete-time Markov cohort macro model.

3.1.3 Statistical simulation models of obesity

Statistical modeling uses mathematical models and assumptions to study complex systems with arbitrary event flows at the inputs and distributions of time intervals of events. Sassi et al. (22) used the Log-Linear model to show BMI trends and the prevalence of obesity and overweight to access associated social gradients. In order to investigate the dynamics of obesity by body mass index (BMI), nutritional stage dynamics and trends in obesity by gender and socioeconomic status at the national, regional and sector level, Meisel et al. (23) developed a statistical model. Vreeman et al. (24) implemented a mathematical simulation model to understand better how food advertising and television contribute to obesity and determine the conditions under which changes in price and income affect body weight. Schroeter et al. (25) developed a micro-simulation economic model to explore eating behavior, food environment, and obesity interventions. The scalable mobility model based on the maximum utility and mobility framework can simulate individual-level weight change to show population-level weight change (26). Chen et al. (27) used a population-level model to see how population weight status and socioeconomic dispersion in longitudinal data projected the potential impact of socioeconomic interventions on obesity prevalence.

3.1.4 Dynamic microsimulation models of obesity

Dynamic microsimulation models have a bottom-up approach and individual-level focus property. The microsimulation model assumes no assumptions about agents' interaction. Using metabolic micro-simulated model one can achieve health goal by adjusting energy intake and physical activity (28). Using data from NHANES and British and Foreign School Society (BFSS) surveys, the network-based simulation model simulates how behavioural and social influences contribute to the spread of obesity and forecasts the efficacy of weight-loss interventions (29). To estimate how long will it take to reach the government's goal of reducing the prevalence of obesity, Abidin et al. (30) simulated changes in children's eating behavior using sub-models of food and energy intake, energy expenditure, and body composition.

3.2 Agent-based models of obesity

The complex dynamics are modeled in the agent-based model (ABM) by replicating the agent's interactions and actions in the system's environment in software code. The agents are positioned in a spatial environment with predetermined rules and initial conditions. At the individual systemic levels, interactions and decisions determine the outcomes. Complex systems can benefit from the computer simulation since it shows macro-level trends and patterns utilizing individual level outcomes and a bottom-up methodology (31). Different agent-based model studies are present in the literature on obesity. The studies were categorized according to characteristics, use cases, and variables involved, as shown in Table S2, with individual advantages and limitations. An ABM approach would enable the simulation of various systems at various scales while taking unique diversity into account (32).

3.2.1 Mechanistic models

Different agent-based mechanistic models are present in the literature to understand the food decision-making process, food environment relation, and activity environment's effects on obesity. Food costs, diet composition and food budget all factors that influence food decisions. Food inequalities can persist at the national level as a result of residential segregation, social networks, group preferences, and complex networks of social influence (33, 34). Food prices and store locations can influence low-income households' diet quality, and food store spatial segregation promotes disparities in diet quality across income levels (35). The agent-based theoretical framework presented by Burke et al. (36) investigates the effects of decreasing food prices on weight gain, human metabolism, and social interaction. Rational addiction and variation in the self-control framework quantitatively predict the disproportionate growth in weight distribution using the discrete-time mechanistic model. Agents in the model a) compare their weight to the group's averagely desired weight b) interactively and incrementally change their diets until they are less expensive than agent's food budget (37). The relationship between the food reward environment and eating behavior and obesity can be explained by food reward hypothesis (38). To better comprehend how families, make restaurant selections based on socioeconomic, demographic, environmental, and nutritional characteristics, Li et al. (39) applied agent-based Huff model. to understand better how families, choose restaurants based on. Body weight can be affected by physical activity, location accessibility, leisure-time physical activity (LTPA), and obesity (40). The impact of social networks on adolescent body size, BMI, screen time, and sports participation are not well demonstrated (41).

Agent-based policy intervention model offers distinct insights into the dynamics of different obesity combat policy interventions. Zhang et al. (42) investigated the effects of societal norms, food price policies, and regulations influence on people's eating behavior. Sugar-sweetened beverage warning labels and taxes can lower sugar-sweetened beverage consumption and body mass among youth (33, 43). Environmental and nutritional characteristics and proximity to food outlets (44) affect dietary habits. The food environment influences aggregated consumption habits and, food outlets open or closed, household income (45). The proximity of a walking destination encourages low-income neighbors to do physical activity, and the social context influences energy balance and

obesity (46, 47). Increasing the community's availability of neighborhood healthy food outlets, improving physical activity infrastructure, and higher school quality policies can help reduce body mass index disparities (48). Li et al. (49) looked at how mass media and nutrition education campaigns influence dietary habits and food intake. Household income, neighborhood income, school quality, food availability (neighborhood food environment), and exercise opportunity are the critical variables of complex obesity systemic structure. The key outcomes include educational attainment, socioeconomic status, social influence, physical activity, body mass index, cardiovascular health, and morbidity (50).

3.3 Machine learning models of obesity

By utilizing sensors, smartphone apps, electronic medical health records, and digital data, machine learning (ML) uses computer algorithms to automatically learn from experience and categorize risks and outcomes associated with obesity. As shown in Table S3, machine learning offers a novel approach to examining multivariate data and predicting the complex inter-relationships likely to cause obesity risks.

The machine learning algorithms provides a distinct picture of the current state of machine learning algorithms and data analysis. The learning algorithms can be used to characterize, adapt, learn, predict, and analyze data, thereby enhance our understanding of obesity and our ability for precise prediction. Nowadays, there is a massive amount of big data in the literature; gadgets, surveys, and data points alone have no value. Machine learning can decipher enormous amount of contradictory information and acquire new knowledge. Researchers used machine learning techniques like regression, Random Forest, Decision Tree, Convolution Neural Networks (CNN), and SVN to find connections between many causes of obesity. The inability of such models to explain the causal connection between the drivers is a major drawback for obesity modeling. To function with deep neural networks requires big data; the model will exaggerate biased outcomes due to its reliance on survey data.

Obesity levels can be detected using obesity-causing parameters, caloric intake, energy expenditure, physical activity, dietary and genetic disorders, socioeconomic factors, and anxiety or depression. Using computational intelligence methods, supervised (Decision Tree and SVN) for comparative analysis and classification, and unsupervised techniques like K-Means for clustering and validation of models (51). Using low-dimensional (Decision Tree, Random Forest, etc.) and high-dimensional ML approaches (SVN, CNN, etc.), statistical, and data visualization methodologies, we can identify potential risks associated with obesity models using different learning models. Regression analysis and data visualization approaches were used with publicly accessible health datasets from Kaggle, UCI, and Physio Net (52, 53) to comprehend how identified risk factors relate to weight change (54). Using food sales data, a population/country level model can be used to estimate the prevalence of obesity and identify the food categories that are most important for obesity prediction (55). Gender, age, and race/ethnicity were only marginally significant predictors of weight status, while physical activity was the most important factor (56). Different machine learning regression techniques can estimate the relative predictive relevance of BMI demographics and psychological, behavioral, and cognitive traits. Adolescent Brain Cognitive Development (ABCD) study (57) explained the role of fixed and potentially modifiable variables of the obesity system map and BMI datasets.

According to the study, social problems and screen time correlated significantly linked with BMI and modifiable therapy targets (58). Country-level demographics, socioeconomic, environmental, and healthcare factors explain the heterogeneity in country-level obesity prevalence better than conventional epidemiological techniques, which consider only small number of preselected variables. The basis of interpretation can be greatly explained by machine learning models (59). Conditional Random Forests were used to find diverse set of social, physical activity, and food characteristics that make up the obesogenic environment. Geographically co-occurring risk variables can be accessed using machine learning approach that is data-driven yet non-parametric (60). Using deep learning Convolution neural networks with Google Static Maps API images, one can analyze the link between features and obesity incidence based on built environment information. These algorithms extract relevant environment features, and regression can be used for quantifying the association (61).

4 Discussion

Computational models enable system-level thinking, modeling techniques, and tools to be used to study obesity as an integrated system and to explicitly model the complex system's dynamics as well as non-linear and circular causality. Several reviews and studies available in the literature discuss the possible techniques for developing an obesity system model. According to Hammod et al. (62), complex systems and system modeling methodologies offer a promising field for researching complex dynamics of obesity. Levy et al. (63) summarize existing simulation models of obesity and the strengths and weaknesses of these models to suggest future research directions. Xue et al. (64) study the applications of system modeling in obesity. To address all essential aspects of obesity and highlight the most significant gaps and overlaps, Morshed et al. (65) summarize system dynamics and agent-based models. The machine learning, ABM, and SM models can predict childhood and adolescent obesity to assess obesity as a worldwide epidemic (2, 4, 65, 66). DeGregory et al. (66) review provides a unique overview of data

analysis and machine learning methods explicitly applied to obesity. Vandenbroeck et al. (2) provide the casual loop system dynamic that explains all the possible drivers of obesity and their interconnections but is qualitative in nature with no real datasets for validation. In this review, we made an attempt to identify the majority of recent computational models that function on real-world data sets and provide quantified output to quantify the energy surplus that causes obesity.

Our review of the literature generated several key findings. First, during the past 20 years, obesity research mostly relied on SM and ABM. Most of the models implemented in SM and ABM were designed to answer specific question. Machine learning models can emerge as a valuable tool to deal with high-dimensional data (66–68), due to their high predictive power, ability to model complex, non-linear relationships between variables, and capacity. The application of computational models can cover multiple domains of obesity ranging from human metabolism to behavior, environment, activity, and social influence, allowing for a general view instead of focusing on individual pieces of the system. The SM based Markov simulation (16, 19–21, 62, 69, 70) statistical, network-based (29) and mechanistic (22, 23, 26) simulation models have been used in the literature to estimate the healthcare cost due to obesity outcomes; to predict the BMI distribution; and to stimulate metabolism, eating behavior, environment relationship, to quantify the energy balance responsible for obesity. An agent-based model (ABM) provides a platform to model interactions between agents to simulate social behavior, network structure, and interventions.

Second, computational models were implemented at the individual-level and population level. The individual-level models were used to understand the spatial-temporal dynamics of the epidemic. In contrast, the population level models were mechanistic models that relate individual-level responses to population density and structure, to study population dynamics. Seven of the 41 studies used empirical data to reproduce real-world scenarios at the country level, while the others were carried at the individual-level.

Third, most of the included studies used data from government sources such as National Health and Nutritional Examination Survey (NHANES 1971-2010) for studies based in the United States (US), the health survey for England (HSE), Longitudinal Cost and Medicare Current Beneficiary Survey (MCBS 1992-2001), Medical Expenditure Panel Survey (2001-2005) datasets, World Bank Data and country-specific health, and nutritional statistics.

This review demonstrated that analyzed methodologies have limits as shown in Table 2, and can be addressed by

TABLE 2 Advantages and limitations of simulation modeling techniques of a complex system.

Model	Advantages	Limitation
Markov simulation model	Markov models can capture attribute distributions and model their dynamics	Presume typically low-dimensional data space, which make them less versatile
	Straightforward to implement in standard software packages	Unable to deal with complex state transition such as path dependence and individual learning
Mechanistic model	Logic principles combined with deductive reasoning allow extrapolation to predict behavior that is not present in data	Data that span multi-space and time scales cannot be handled
	Establish a causal relationship between inputs and outputs	Can handle only small datasets
		No consideration of patterns and non-predictive in nature
		It is challenging to incorporate information from multiple spaces and times
Statistical model	Can understand any pattern in data	Applicable to quantitative data and results might be misleading
	Comprehensive statistical analysis is less subject to bias	Cannot apply to homogeneous data
	Results inform better decision making	Deals with groups and aggregates only
Microsimulation model	Using computer simulation, macro-level trends and patterns can be generated, making them suitable for complex system modeling	Microsimulation assumes no interaction between components
	Individual-level focus property allows substantial diversity and heterogeneity among agents	It is challenging to measure social influence and transmission
Agent-based model	Using computer simulation, the bottom-up approach can generate macro-level trends and patterns	Not beneficial when dealing with homogeneous data as ABM focuses on the individual difference and how this difference contributes to system patterns
	Allow substantial diversity and heterogeneity among agents	Computationally expensive
	Capable of incorporating spatial contexts and feedback dynamics	The model must be built at the proper degree of description and with the appropriate level of details to serve its purpose
	Can examine non-equilibrium dynamics and focus on mechanisms	The general-purpose model cannot work
Machine learning model	Inductive capability- from past data, one can identify patterns in the data	Require large datasets
	Can tackle multiple spaces and time scales	Can only predict based on patterns in the provided data

symbiotically combining their strengths and techniques into a viable solution for bridging the gaps. We can implement the quantified causal system that can explain the interconnection and causation between the drivers and can explain the indirect drivers (emergence properties) linked to the physical phenomenon of obesity.

4.1 Limitations of the studies

System modeling techniques have certain limitations as shown in Table 2. Firstly, while building a detailed and comprehensive obesity system map framework, the model can become so sophisticated that the results will no longer be transparent, and validation will become almost impossible. To overcome this limitation, the whole complex system can be divided into sub-systems (2). Obesity interventions vary across populations and regions and are sensitive to assumptions and settings. As a result, SD has limitations in guiding global future intervention development.

Second, the models depended on survey data, prone to bias due to self-reported datasets. Only a few model results are reproducible and documented in selected studies (26, 35, 43, 46, 54, 55, 71), the remaining studies were either slightly or moderately reproducible. Available models were created with a single or a few questions in mind and occasionally could not incorporate demographic data directly. They cannot scale to other variables and sub-populations, as described in system dynamics limitations and machine learning techniques Tables S1–S3. The global system model simulated counterfactual comparison, and the lack of empirical data and uncertainty of assumptions remains a significant challenge.

4.2 Matching existing models to the theoretical framework of current study

One of the most significant challenges in developing and implementing the obesity model is the complexity of the systems and the scarcity of datasets for all the drivers). One feasible option for modeling obesity's complex structure is to use a modular approach. When studying multi-level feedback and interactions, a modular approach allows for separate analysis of each system and level, allowing easy integration. Each sub-system (for example, physical activity patterns, food intake and production, social and individual psychology, environment, and physiology) would have its module, with mechanisms theory and impact paths incorporated (3). We can use both machine learning and the SM technique to investigate the system dynamics of obesity. SM allows for the integration of data from wide range of disciplines, including statistics, epidemiology, biology, nutrition, and so on. Data-driven machine learning approaches can investigate the driver's relationship and trends (72). Mechanistic model equations (7) can interpolate food production and consumption drivers (purchasing power, nutritional value, alcohol intake, portion size) to energy balance. Some drivers in the model's psychology sub-system, such as social tolerance of fatness and food literacy, are not quantitative, and data-driven machine learning models (73) can be used to approximate the relationship between the drivers.

These models are proposed to be combined to form a framework (Figure 2) to model the quantitative obesity systemic map (74). To implement the framework, the complex obesity system model needs to be divided into sub-systems of Food production and consumption, Psychology, Physical and Environment activity. Then, collected data from the available databases and literature would be used to define the level of importance of drivers related to obesity sub-systems along with the mechanistic mathematical equations that explain the causal relations between the drivers. Using the available datasets from NHANES and MPES etc. and mechanistic equations, new datasets can be generated and can be a part of a knowledge-based system.

The machine-learning models will be included in the framework as the certain driver's causal relation connections are missing. Machine learning techniques can fill the gap. Mechanistic models supply the causality that machine learning methods lack, and machine learning models supply inductive capability and handle multi-space and time scale data to approximate the relationships between drivers using past observations.

The knowledge-based system consists of discrete and continuous data of obesity can be used to train and analyze the machine learning model. The ensemble mechanistic machine learning model allow to quantify the correlation between the drivers and predict the obesity emergence dynamics and the driver's role in the prediction which can be possible using the application of Rule based explanations of machine learning and knowledge graphs (75). Machine learning can overcome the scalability limitation of mechanistic model. The predicted values will act as a behavioral description of the model, which can then be used to identify hotspots to intervene and predict future scenarios and assist policymakers in testing policies in the complex food system.

Conclusions

Obesity is a multi-dimensional system problem. To design full capacity model to enhance obesity research and intervention, theoretical and practical issues with current computational models must be taken into consideration. Due to obesity's non-linear dynamics and complex system effect, there is a need for emergence-focused design in complex system simulations to reproduce the multi-level emergence seen in the real world. The analysis of the literature indicated that both population-level data (weight status, socioeconomic position, physical activity, behavior and social network, and so on) and longitudinal data are required

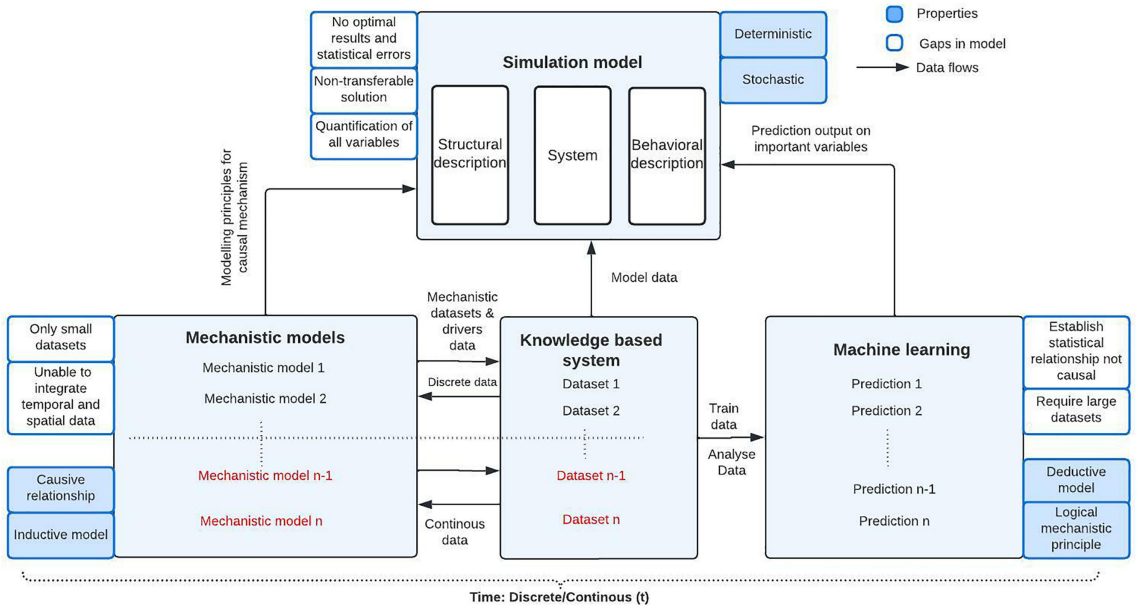

FIGURE 2
Framework of complex system model of obesity (national-global level of application).

to assess the global level effect. Despite the limitations of individual simulation model, agent-based and machine learning models can fill missing gaps and function together symbiotically to model individual-level models. These individual-level models can be aggregated to global levels and estimate individual and population-level obesity dynamics. The complex hybrid system framework based on a synergetic combination of mechanistic, machine learning, and simulation model components may provide an innovative systematic approach to determine the complex interactions between obesity factors and fight the obesity epidemic.

Author contributions

The study was conceptualized and designed by AB, SS, VH and JH. AB performed the literature review and wrote the first draft of the manuscript. JH and SS edited and proofread the manuscript. All authors contributed to the article and read and approved the submitted version.

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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.

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Supplementary material

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A bibliometric and visual analysis of obesity and polycystic ovary syndrome from 2012 to 2022

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Background: Obesity is associated with polycystic ovary syndrome (PCOS). We aimed to elucidate the research status and explore research trends and future directions of research on obesity and PCOS.

Methods: A bibliometric analysis of the published papers in the field of obesity and PCOS between 2012 and 2022 was conducted on the basis of the Web of Science Core Collection database. The collaboration networks, research trends, literature sources, citation analysis, co-citation analysis, and keywords analysis were statistically analyzed and visualized using the VOSviewer software.

Results: We retrieved 2843 records from 681 journals by 12307 authors from 2942 institutes in 99 countries. The number of published papers and citations had a roughly increasing trend annually. The United States and China contributed the majority of the records. Monash University, Shanghai Jiaotong University, Aristotle University of Thessaloniki, Karolinska Institute, University of São Paulo, and Tehran University of Medical Sciences were the biggest nodes in their cluster of the collaboration network map, and Moran LJ, Teede HJ, Joham AE, Escobar-Morreale HF, and Macut D were prolific authors. Research trends and hotspots were identified and visualized in the field of obesity and PCOS. Research hotspots in this field focused on insulin resistance (IR), metabolic syndrome, metformin, and inflammation. Bariatric surgery, mitochondrial dysfunction, binding globulins, and comorbidities may be the frontiers of future research.

Conclusions: We concluded the research status and trends in the field of obesity and PCOS. A better understanding of collaboration patterns, research hotspots, and frontiers may be useful for researchers.

KEYWORDS

obesity, polycystic ovary syndrome, bibliometric analysis, visualization, VOSviewer

1 Introduction

Obesity, defined as abnormal or excessive fat accumulation that may impair health, is a worldwide pandemic disease, especially in women (1, 2). Obesity increases the risk of multiple diseases, such as diabetes mellitus (3), cardiovascular disease (3, 4), and PCOS (5). PCOS, characterized by ovulatory dysfunction, hyperandrogenism, and polycystic ovaries, is one of the most common heterogeneous endocrine conditions and affected 5% to 10% of reproductive-age women, depending on the Rotterdam criteria (6, 7). Approximately 50% of women with PCOS are overweight or obese (8). Although the etiology of PCOS remains unclear, IR and hyperandrogenemia (HA) play a major role in the pathogenesis of PCOS development and complications (9). However, obesity is strongly associated with IR and HA. Obesity amplifies and worsens the adverse metabolic and reproductive outcomes of PCOS and increases IR and compensatory hyperinsulinemia and generates testosterone while suppressing gonadotropin production (10). Meanwhile, Obesity sensitizes thecal cells to luteinizing hormone stimulation and amplifies HA by upregulating ovarian androgen levels (11). The symptoms of PCOS can be improved with weight loss through lifestyle modification, excise, drug therapy, and bariatric surgery (12). However, the pathophysiologic mechanism and intricate relationship between obesity and PCOS remain uncertain, numerous studies have been conducted to identify the question (13–15).

Based on this, it is necessary for us to learn about the advances and novel trends in this field. Bibliometric analysis, an approach to quantifying and visualizing published documents, can provide an overview of the current research status and establish future research orientations in a specialized field (16). Bibliometric analysis revealed the research characteristics, status, and trends of PCOS in specific times and regions (17, 18). Some studies also have explored the research status, hotspots, and trends of research between PCOS and infertility as well as IR by the method of bibliometrics (19, 20). To the best of our knowledge, no bibliometric analysis has focused on the collaboration patterns and research trends, and hotspots of research on obesity and PCOS. To clarify the current research status and provide references for future research, we performed a bibliometric analysis to quantify and visualize this research field through analysis of the information in published papers.

2 Materials and methods

2.1 Literature source and retrieval

The retrieved documents were based on the Web of Science Core Collection (Science Citation Index Expanded, SCI-E) from 1 January 2012 to 27 July 2022. The detailed retrieval strategy was as follows: TS= ("Polycystic Ovary Syndrome" OR "Polycystic Ovarian Syndrome" OR "Stein-Leventhal Syndrome") AND TS= ("obesity"). The document type was limited to articles and reviews. Only publications in English were included.

2.2 Inclusion and exclusion criteria

Inclusion criteria were peer-reviewed published original articles or reviews about obesity and PCOS. Exclusion criteria were: 1) Early access, meeting abstract, editorial material, and other types; 2) repeated publications.

2.3 Literature analysis methods

All the records were downloaded as plain text and imported to VOSviewer to conduct bibliometric analysis. Corresponding data statistics and analysis were used by Excel 2019 (Microsoft) and GraphPad Prism (version 8.0). VOSviewer (version 1.6.18, Leiden University) was an available software for creating and viewing bibliometric maps and was used for visualizing the collaborations between countries, institutions, authors, keywords, and references (21). Each node represents an individual, the same color represents the same cluster, the size of the circles means the number or frequency of individuals, and the lines between circles express the intensity of cooperation (22).

3 Results

3.1 Search results

Totally 2976 publications were identified from SCI-E. Duplicates, non-article and review publications, and non-English publications were excluded. Finally, 2843 was identified as the number of published records and included in the final bibliometric analysis (Figure 1).

3.2 General trends and annual publications

Papers, published over the past 10 years, roughly conformed to the increasing trend year by year, and the cumulative number of published articles showed an overall upward trend (Figure 2). Most records were original articles and reviews (2843/2927, 97.1%), which can greatly reflect the development trends and changes in the field of obesity and PCOS. Through database searching, the 2843 documents were cited 66178 times, with an average of 23.3 times per paper, and an H-index of 101

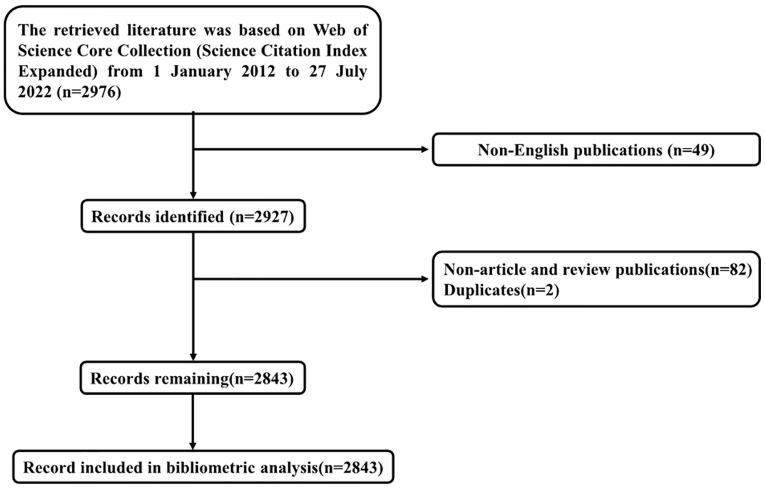

FIGURE 1
Flow chart of document selection and identification.

(Figure 3). It can be seen that the direction of obesity and PCOS has attracted more and more researchers' attention.

3.3 Countries and districts

Ninety-nine countries contributed to publications in the field of obesity and PCOS worldwide. The top 3 countries that published papers were the United States, China, and Turkey, accounting for 47.7% (1356/2843). Australia had the highest average number of citations (38.9), followed by the United Kingdom (38.4), the United States (37.2), Italy (35.6), and Turkey (15.4) (Table 1).

The top 30 most prolific countries had formed 3 stable clusters and created a network map. There was active cooperation between these countries, especially between the United States and China (Figure 4A). Viewed from the dynamics and trends, research about obesity and PCOS mainly focused on the United States, Greece, Australia, and Turkey from 2012 to 2017. The field and direction

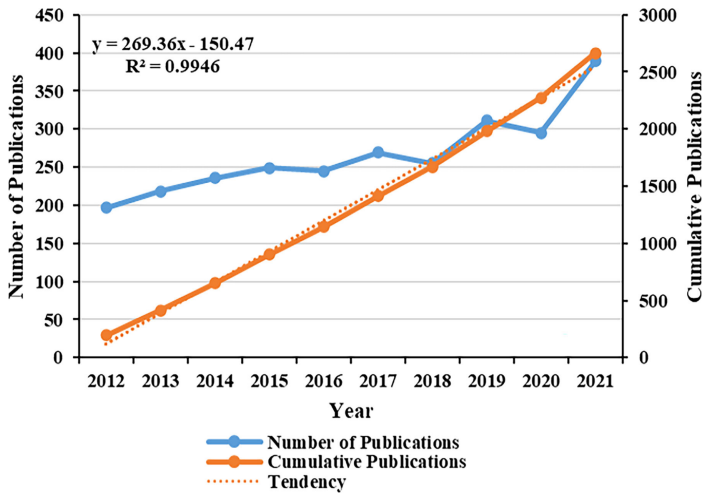

FIGURE 2
Annual and cumulative publications.

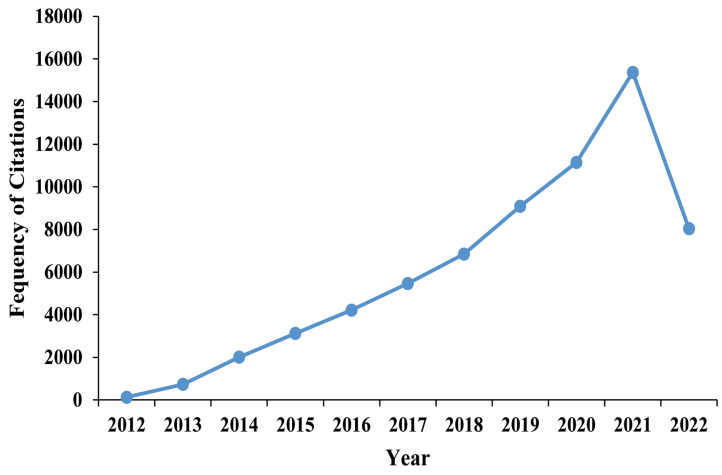

FIGURE 3
Annual frequency of citations.

gradually received greater attention from other countries after 2017 (Figure 4B).

3.4 Universities and institutions

A total of 2942 institutions were involved in these documents. Collaborative network analyses have been conducted between universities or institutions. In a network and overlay visualization of institutions in the top 100, 6 clusters were formed and the biggest node represented that the institution published the most article in their cluster (Figure 5A). Monash University (n=88, 3.1%, Cluster 6), Shanghai Jiaotong University (n=57, 2.0%, Cluster 3), Aristotle University of Thessaloniki (n=41, 1.4%, Cluster 2), Karolinska Institute (n=40, 1.4%, Cluster 4), University of São Paulo (n=38, 1.3%, Cluster 1), and Tehran University of Medical Sciences (n=32, 1.1%, Cluster 5) were the biggest nodes in their cluster, Cluster 1 was the biggest cluster, which contained 40 nodes that represented its related different universities or institutions, while Cluster 6 was the smallest, which included 4 nodes. The other clusters included 20 nodes (Cluster 2), 19 nodes (Cluster 3), and 12 nodes (Cluster 4). Furthermore, the results showed that 3 universities mainly contributed to this field from 2012 to 2017, which included Aristotle University of Thessaloniki, The University of Adelaide, and University of São Paulo. Since then, these studies have gradually gained popularity among other institutes, such as Monash University, and Shanghai Jiaotong University (Figure 5B).

3.5 Authors

Totally 12307 authors contributed to the publications in this domain. The author visualization map of the top 150 productive authors formed 10 clusters (Figure 6). The biggest cluster was

TABLE 1 Analysis of top 10 productive countries.

Rank	Country	Documents, n	Citations, n	Average citations	Total link strength
1	United States	636	23718	37.2	279
2	China	508	6595	12.9	128
3	Turkey	212	3267	15.4	70
4	United Kingdom	198	7621	38.4	214
5	Italy	163	5803	35.6	131
6	Australia	161	6273	38.9	126
7	India	123	1771	14.3	41
8	Poland	122	1549	12.6	35
9	Iran	120	1579	13.1	29
10	Brazil	116	1783	15.3	37

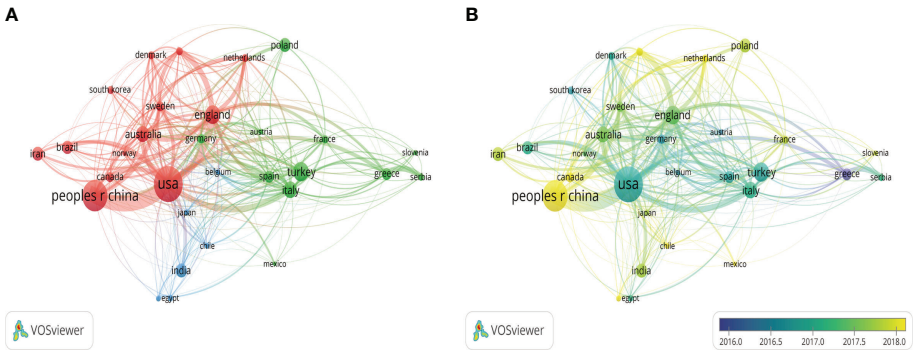

FIGURE 4
Visualization map of countries and districts. **(A)** Network diagram of the top 30 countries/districts. **(B)** Dynamics and trends of the top 30 countries/districts.

Qiao Jie (34/12307), who came from Peking University. Stener-Victorin E (30/12307) had the second biggest cluster and was from Karolinska Institute. The third biggest cluster was Moran LJ (27/12307) who has published the most documents and collaborated with other researchers frequently (Figure 6 and Table 2).

3.6 Journals

This study showed that a total of 681 journals published records about obesity and PCOS. The results showed that the most productive journal was Gynecological Endocrinology (n=156), the most cited journal was The Journal of Clinical Endocrinology & Metabolism (4100 citation times), and the journal with the highest average citation was Endocrine Reviews (n=6, an average citation was 298.1 times). The top productive journals, top cited journals, and journals of top average citations were listed (n=10) in Supplement File 1.

3.7 Citations and co-citations

3.7.1 Top 10 citation publications

Of the 2843 records, the top 10 publications ranked by citation were listed in Table 3. The most cited article was published in Endocrine Reviews by Diamanti-Kandarakis E et al. in 2012 (23) and reported an update on mechanisms and implications of IR and PCOS with 874 citations, which was higher than that of the second paper (525 citations) (24). The third cited paper was on the pathogenesis of PCOS, with 454 citations (10).

3.7.2 Co-citation references

VOSviewer was used to visualize the co-citation network of references, which were divided into 3 clusters, with 93571 references (Figure 7). The larger node represents that the references are cited more frequently, and the line thickness

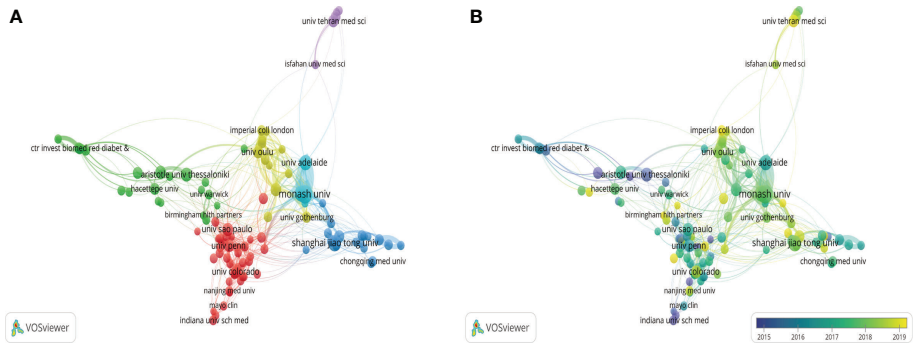

FIGURE 5
Visualization map of universities and institutions. **(A)** Network diagram of the top 100 universities/institutions. **(B)** Dynamics and trends of the top 100 universities/institutions.

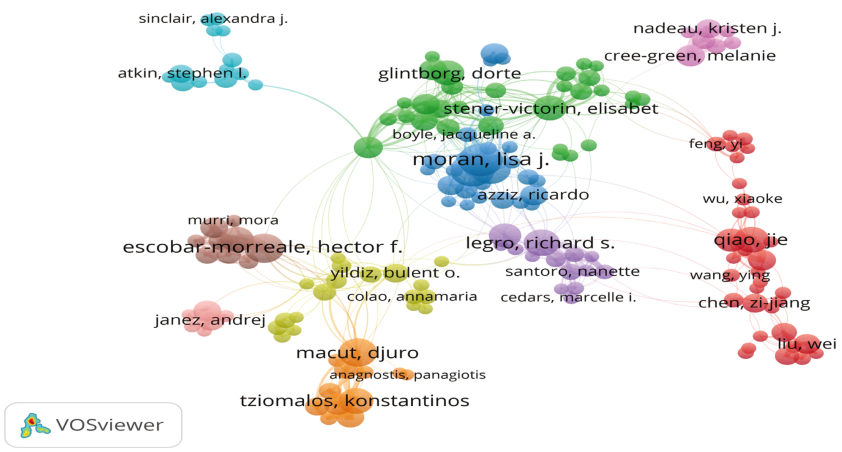

FIGURE 6
Network map of the top 150 authors.

represents the co-citation frequency. The top 10 co-cited references were shown in Table 4. And these co-citation papers were mainly published in Fertility and Sterility, The Journal of Clinical Endocrinology & Metabolism, and Human Reproduction.

3.8 Analysis of keywords

A total of 7462 keywords were extracted from the retrieved records. The network map of the top 100 keywords was grouped into 3 clusters (Figure 8A), and most of the top 10 keywords were clustered in the red cluster. The top 3 keywords were "obesity (n=1627)", "polycystic ovary syndrome (n=1064)", and "insulin-resistance (n=1037)". From Figure 8B and Table 5, we can find that the hot research directions of obesity and PCOS in recent 10 years were insulin resistance, metabolic syndrome, metformin, inflammation, and so on.

3.9 Research frontier analysis

By analyzing the keywords from 2021 to 2022, a keyword table of research frontiers was obtained (Table 6). According to the table, comorbidities of obesity and PCOS, mitochondrial dysfunction, binding globulin, and bariatric surgery may become the research frontier in the future.

4 Discussion

In contrast to traditional literature reviews, bibliometrics analysis can systematically analyze the published literature in a specific field (25). This study summarizes collaboration networks, research trends and hotspots, and future directions in obesity and PCOS based on bibliometric analysis. A total of 2843 records were retrieved and published in 681 journals by 12307 authors from 2942 institutes in 99 countries.

TABLE 2 Analysis of top 10 productive authors.

Rank	Author	Documents, n	Citations, n	Average citations	Total link strength
1	Moran LJ	36	1268	35.2	91
2	Teede HJ	35	1360	38.8	93
3	Joham AE	31	840	27.0	94
4	Escobar-Morreale HF	30	2123	70.7	100
5	Macut D	27	892	33.0	71
6	Legro RS	25	1183	47.3	58
7	Tziomalos K	23	493	21.4	71
8	Qiao J	23	962	41.8	35
9	Luque-Ramirez M	22	962	43.7	79
10	Stener-Victorin E	22	1130	51.3	71

TABLE 3 Analysis of top 10 citations.

Rank	Author	Number of citations	Title	Journal
1	Diamanti-Kandarakis E, 2012	874	Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications	Endocrine Reviews
2	Manikkam M, 2013	525	Plastics derived endocrine disruptors (BPA, DEHP and DBP) induce epigenetic transgenerational inheritance of obesity, reproductive disease and sperm epimutations	PloS One
3	Rosenfield RL, 2016	454	The Pathogenesis of Polycystic Ovary Syndrome (PCOS): the Hypothesis of PCOS as Functional Ovarian Hyperandrogenism Revisited	Endocrine Reviews
4	Escobar-Morreale HF, 2018	449	Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment	Nature Reviews Endocrinology
5	Dumesic DA, 2015	390	Scientific Statement on the Diagnostic Criteria, Epidemiology, Pathophysiology, and Molecular Genetics of Polycystic Ovary Syndrome	Endocrine Reviews
6	Lim SS, 2012	386	Overweight, obesity and central obesity with polycystic ovary syndrome: a systematic review and meta-analysis	Human Reproduction Update
7	Garvey WT, 2016	349	American association of clinical endocrinologists and American college of endocrinology comprehensive clinical practice guidelines for medical care of patients with obesity	Endocrine Practice
8	Yildiz BO, 2012	347	Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria	Human Reproduction
9	Conway G, 2014	335	The polycystic ovary syndrome: a position statement from the European Society of Endocrinology	European Journal of Endocrinology
10	Murri M, 2013	273	Circulating markers of oxidative stress and polycystic ovary syndrome (PCOS): a systematic review and meta-analysis	Human Reproduction Update

Based on our results, the number of published articles showed an increasing trend, especially in 2021, which indicated that this field had been a research hotspot, suggesting that novel researchers can engage in this research field. There is a correlation between academic productivity and socio-economic status for most countries (26). Ilagan-Vega et al. revealed that the research and development expenditures were significantly related to the academic impact of PCOS in Southeast Asia (17). In terms of article production, the United States ranked first, followed by China, Turkey, the United Kingdom, and Italy. Most of the top 5 countries with the most articles published were developed countries. The citation rate is an important index to measure the academic influence of a paper, which reflects the acceptance and recognition of a country's scientific research achievements by other countries or institutions (27). The United States (n=23718) and the United Kingdom (n=7621) were the top 2 countries with the

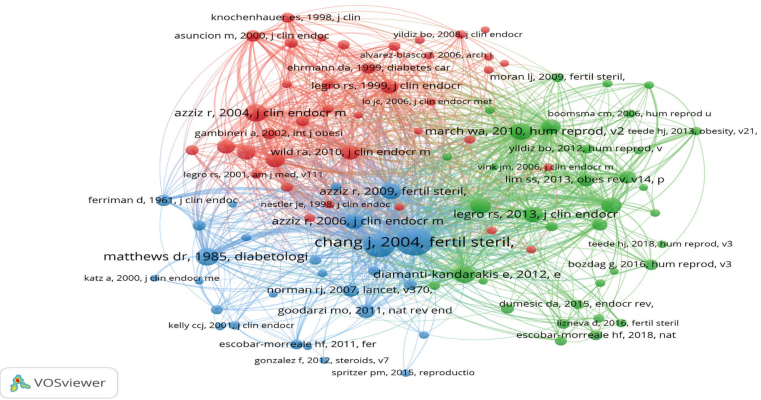

FIGURE 7
Network map of co-citation references.

TABLE 4 Analysis of top 10 co-citations.

Rank	Author	Number of citations	Title	Journal
1	Chang J, 2004	679	Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome	Fertility and Sterility
2	Fauser BCJM, 2004	517	Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS)	Human Reproduction
3	Matthews DR, 1985	302	Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man	Diabetologia
4	Azziz R, 2004	286	The prevalence and features of the polycystic ovary syndrome in an unselected population	The Journal of Clinical Endocrinology & Metabolism
5	March WA, 2010	278	The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria	Human Reproduction
6	Diamanti-Kandarakis E, 2012	264	Insulin resistance and the polycystic ovary syndrome revisited: an update on mechanisms and implications	Endocrine Reviews
7	Azziz R, 2009	253	The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report	Fertility and Sterility
8	Azziz R, 2006	229	Positions statement: criteria for defining polycystic ovary syndrome as a predominantly hyperandrogenic syndrome: an Androgen Excess Society guideline	The Journal of Clinical Endocrinology & Metabolism
9	Fauser BCJM, 2012	225	Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group	Fertility and Sterility
10	Legro RS 2013	219	Diagnosis and treatment of polycystic ovary syndrome: an Endocrine Society clinical practice guideline	The Journal of Clinical Endocrinology & Metabolism

highest total citation frequency, but the average citation frequency in Australia was higher than these two countries, which indicated that Australia had greater influence in this field. It has been reported that international collaboration is a powerful factor in promoting academic productivity in a given field (28). In our analysis, different countries and regions had different degrees of cooperation, the United States, which published the most, showed the strongest links with China, followed by the United Kingdom. At the same time, these countries were also among the top 5 most prolific countries. Regional imbalances were observed in different districts and countries, and it was essential to strengthen collaboration and communication among developed and developing countries worldwide.

Meanwhile, the analysis of institutions and authors was similar to the distribution of countries and districts. Among 2942 institutions, Monash University and The University of Adelaide (Australia), Shanghai Jiao Tong University (China), Karolinska Institute, and Oulu University (European) made great contributions to this field and maintained a steady collaboration with other teams. In the last decade, Moran LJ, published the most articles, mainly focused on lifestyle intervention and metabolomics research in this direction (29–31). Qiao Jie, from Peking University, was in the biggest cluster and mainly focused on the pathogenesis and theoretical research in this area, such as immunity (32), gut microbiota (33), and single-cell sequencing (34).

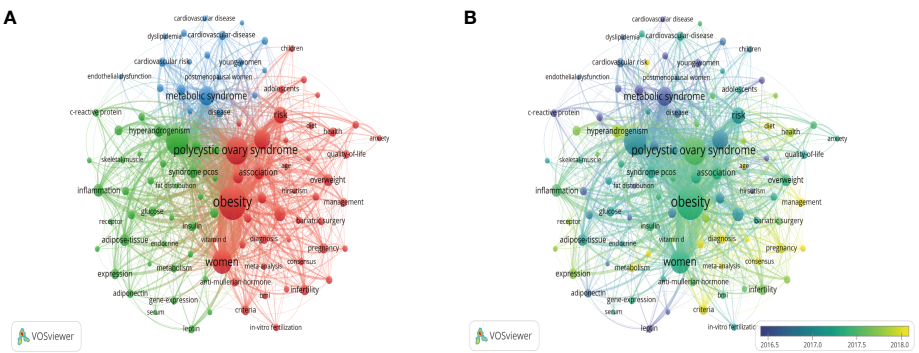

FIGURE 8
Visualization map of keywords. **(A)** Network diagram of the top 100 keywords. **(B)** Dynamics and trends of the top 100 keywords.

TABLE 5 Occurrences and total link strength of top 10 keywords.

Rank	Keyword	Occurrences	Total link strength
1	Obesity	1627	9528
2	Polycystic ovary syndrome	1064	6459
3	Insulin-resistance	1037	6298
4	Women	821	4864
5	Prevalence	533	3486
6	Metabolic syndrome	525	3397
7	Risk	376	2308
8	Body-mass index	282	1558
9	Association	257	1550
10	Metformin	230	1430

Obesity and PCOS often interacted with each other and caused a significant risk to women's health (5). Therefore, it received the attention and recognition of many journal editors. According to our results, hundreds of journals have published articles on this topic. Of 681 journals, the top 10 journals contributed the most. The most published journal was Gynecological Endocrinology, and the most cited journal was The Journal of Clinical Endocrinology & Metabolism, while Endocrine Reviews with the highest average paper citation. In addition, the top 3 most average cited journals in the included records were published in top-ranked journals, which indicated that these journals were widely recognized and deserved the attention of researchers in the field. These top-cited records, published in Endocrine Reviews, PloS One, Human Reproduction Update, and European Journal of Endocrinology, together had been cited more than 1853 times in the past 10 years. The common themes in this field's top 10 cited research articles were the pathogenesis, epidemiology, diagnosis, and treatment of PCOS and its relationship to obesity. Co-citation analysis of references can tell researchers which references had made important contributions in this field. In co-citation analysis, "insulin resistance", "mechanisms", "implications", and "criteria" were the keywords of the top co-citation references, which deserved reference for new researchers (23, 35, 36).

High-frequency keywords are usually used to identify research hotspots in a research field. Shi et al. revealed that the research trends of PCOS were gradually shifting from treatment to mechanistic exploration (18). According to the results of the keywords network map, we found that the research about obesity and PCOS mainly focused on insulin resistance, metabolic syndrome, drug therapy, and inflammation in recent years, which involved the mechanism, comorbidities, and treatment (37–39). It indicated that, in addition to mechanistic exploration, treatment was still a hot research topic in the field of obesity and PCOS.

The co-occurrence keyword analysis of the past 2 years revealed mechanisms, comorbidities, drug therapy, and bariatric surgery as emerging topics. Among obese girls with PCOS, mitochondrial acylcarnitine (C4) was associated with valine and free fatty acids (40). According to Fatemeh et al. (41), PCOS may reduce the occurrence of silent coronary artery disease in a population-based cohort study. Compared with metformin, bariatric surgery should be prioritized for patients with obesity and PCOS in a Chinese prospective nonrandomized trial (42). In these studies, researchers were

TABLE 6 Analysis of keywords from 2021 to 2022.

Rank	Year	Frequency	Keyword
1	2021	4	Mitochondrial dysfunction
2	2021	3	Metabolic profile
3	2021	3	Sleeve gastrectomy
4	2022	3	Hypertensive disorder
5	2022	2	Fatty liver
6	2022	2	Chronic kidney disease
7	2022	2	Bifidobacterium
8	2022	2	Binding globulin
9	2022	2	Activated protein kinase
10	2022	2	Androgen generation

able to identify the research hotspots, which may shape future research directions.

5 Limitations

Without a doubt, the study had several limitations. Firstly, we only analyzed records from the Web of Science Core Collection and only included English articles and reviews, which might lead to selection bias. However, the number of retrieved papers was large enough to reflect the real research situation (43). Secondly, due to the diversity of keywords and the authors with the same name, it was hard to accurately retrieve all the records.

6 Conclusions

Utilizing the retrieved records about obesity and PCOS, we performed a bibliometric and visual analysis. The number of papers published had roughly risen and extensive cooperation was observed between different countries and institutions. In this field, comorbidities, mitochondrial dysfunction, binding globulin, and bariatric surgery were the frontiers of research. Bibliometric analysis of the literature in this field was helpful for researchers to understand the collaboration patterns, research hotspots, and frontiers.

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 in the article/ Supplementary Material.

Author contributions

PL and JKL designed the study. PZL, GHW, SZ, WZL, ZW, XLS, ZBF, XHY, LYZ, and SHZ searched and analyzed the data. All the authors contributed to writing and approving the final manuscript.

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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/fendo.2022.1011105/full#supplementary-material

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Early-life weight gain patterns of term small-for-gestational-age infants and the predictive ability for later childhood overweight/obesity: A prospective cohort study

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Objectives: We aimed to identify the weight gain patterns of small-for-gestational age (SGA) infants in early life and to explore the predictive value for later overweight/obesity in childhood.

Methods: We obtained data from a prospective cohort including term SGA infants born between January 2006 and November 2015 who received regular health care from birth to 5 years in West China Second University Hospital, Chengdu, China. A latent class growth analysis (LCGA) was applied to group children with similar growth trajectory patterns. Multiple logistic regression was performed to examine the association between weight gain patterns and later overweight/obesity.

Results: A total of 296 term SGA infants were finally included. Five weight gain trajectories were identified, including excessive rapid catch-up growth (ERCG) (class 1, 10.9%), rapid catch-up growth (RCG) (class 2, 17.9%), appropriate catch-up growth (ACG) (class 3, 53.0%), slow catch-up growth (SCG) (class 4, 13.4%) and almost no catch growth (NCG) (class 5, 4.8%). SGA infants in class 1 and class 2 had a higher BMI according to age- and sex-specific Z scores from 2–5 years of age. In addition, 25% of SGA infants in class 1 and 13.2% of SGA infants in class 2 were found to be overweight/obese at 2-5 years of age. After adjusting for confounders, we found that extremely rapid weight gain (class 1) in the first 2 years of life increased the risk of overweight/obesity by 2.1 times at 2 to 5 years of age (aOR=2.1, 95% CI: 1.3~4.8; P<0.05). Furthermore, the increment of ΔWAZ between 0 and 4 mo was prominently related to the risk of overweight/obesity at 2 to 5 years for term SGA infants (aOR=3.2, 95% CI: 1.7~8.1; P<0.001). A receiver operating characteristic (ROC) curve showed the area under curve (AUC) was 0.7, with a 95% confidence interval (CI) from 0.6 to 0.8 (P<0.001).

Conclusions: The extremely rapid weight gain pattern of term SGA infants in the first 2 years of life increased the risk of overweight/obesity at 2 to 5 years of age. It suggests monitoring weight gain across the infant period represents a first step towards primary prevention of childhood obesity.

KEYWORDS

infant, chidhood, small-for-gestational-age, obesity, weight gain

Introduction

Small-for-gestational-age (SGA) refers to newborns with birth weight (BW) below the 10th percentile according to the sex- and gestational age (GA)-specific reference (INTERGROWTH-21st Project) (1). Globally, approximately 16% of all infants are SGA, and this figure ranges from 7% in industrialized countries to 41.5% in South Asia (2, 3). It was reported that 32.4 million infants (27% of live births) were born SGA in low- and middle-income countries in 2010, and among them, 29.7 million were term SGA infants (3). In recent study, it was reported that a third of babies were born SGA (34%) and SGA accounts for a quarter (24%) of all neonatal deaths in South Asia (4). The number of SGAs was huge, and it had a significant impact on children's short-term and long-term health. Most term SGA infants showed significantly rapid weight gain or catch-up growth (CUG) compensating for intrauterine restraint within the first two years of life (5–7). However, associations between SGA and increased risks for disease in adulthood, such as metabolic syndrome, type 2 diabetes and cardiovascular disease, are now well established (8–11). Furthermore, growing evidence has suggested the greatest long-term risk of excessive adiposity and the accompanying comorbidities across life among infants who have been found to have intrauterine growth restriction followed by rapid weight gain in infancy (10, 12–15). Notably, increasing evidence has shown that children who experience rapid weight gain in early postnatal life are more prone to developing obesity and related diseases than those born SGA only (16).

Currently, obesity and accompanying comorbidities have become a global public health concern and have spread to the pediatric population (17, 18). In total, 35.1% of American children aged 2~19 years developed overweight/obesity from 2015~2016, and a sharp increase in obesity prevalence among children aged 2~5 years was detected (19). Treatment of overweight and obesity is notoriously difficult and often unsuccessful. Therefore, prevention-based strategies implemented as early as possible seem to have profound significance. As illustrated by the Developmental Origins of Health and Disease (DOHaD), obesity and accompanying comorbidities might originate very early from maternal, perinatal and early childhood factors; for example, SGA infants with postnatal accelerated weight gain were demonstrated to constitute an enormous high-risk group for developing overweight/obesity (20–22). Most studies have proposed that the most influential window for achieving catch-up growth is the first two years of life (5, 6, 23, 24). Thus, this window might be a critical period for setting the long-term growth trajectory and for the early prevention and intervention for overweight/obesity in later life.

To date, limited studies are available regarding the weight gain trajectories for SGA infants, especially in low-middle income areas, and a consensus has not been established regarding which period of weight gain contributes to future risks for overweight/obesity. Hence, we performed a prospective cohort study enriched with term SGA births to 1) identify weight growth patterns within the first 2 years of life for term SGA children; 2) explore the association of particular weight gain patterns with the development of overweight/obesity in later life; and 3) evaluate the predictive impact of differential rapid weight gain periods during infancy on later overweight/obesity.

Methods

Study design and subjects

Data were obtained from a cohort who had regular health care and birth data in the Children's Department of Health at West China Second University Hospital, Chengdu, China. Children were born between January 2006 and November 2015. Gestational age (GA), birth weight (BW), sex, gravidity and parity were collected at birth. Feeding patterns between 0~4 mo were documented. Parental weight, height and calculated body mass index, as well as the parental education were recorded. In the study, SGA was defined as birthweight < 10th percentile for sex and gestational age according to the Chinese Neonatal Network (1). Gestational age was determined by the mother's last menstrual period or ultrasound measurement during early pregnancy and was confirmed by physical

examination and ultrasonography when available. The flowchart of the study population is shown in Figure 1. All parents signed informed consent forms before participation. SGA infants born preterm (GA < 37 weeks) or postterm (GA > 42 weeks) and nonsingletons were excluded from the analysis. Besides, SGA infants with a dysmorphic features, congenital structural abnormalities of the organs, or chronic diseases had not been included. Additionally, individual measurements with unreasonable data were excluded in case of possible data-entry error. Only data from infants with anthropometric measurements for both weight and length at each of the follow-up age points were used in this analysis. Of these data, only those with at least one follow-up assessment during the period from 2–5 years were used. This study was approved by the Medical Ethics Committee of West China Second Hospital of Sichuan University.

Measurements

Weight and length (height) were prospectively measured once a month in the first six months, once every 2 months from 6 months to 10 months, once every 3 months from 1 to 2 years, and once every six months from 2 to 6 years. Trained researchers measured physical indicators following strict protocols. Weight was measured using an electronic scale (Seca376; Seca Measuring System Co. Ltd., Hong Kong, China). Length for children under 3 years old was measured in the supine position using a measurement bed (Seca416; Seca Measuring System Co. Ltd.). Height for children older than 3 years was measured in a standing position by a measurement stadiometer. A dedicated person calibrated the measuring appliances regularly. Weight and length were separately assessed to the nearest 100 g and 0.1 cm, respectively. Body mass index (BMI) was calculated as weight in kg divided by the square of length in m.

Growth trajectory

To group children with similar growth trajectory patterns according to their weight during the first 2 years of life, a latent class growth analysis (LCGA) was applied, which is a person-centered approach to classify individuals into distinct groups (25, 26). The 2006 World Health Organization (WHO) growth charts were used as a reference to calculate sex- and age-specific weight distributions prior to 2 years of age (27). Anthropometric indices of weight-for-age Z scores (WAZ) and height-for-age Z scores (HAZ) were calculated as indicators of the growth status of the children. The WAZ at each time point of 4 mo, 8 mo, 12 mo, and 24 mo was used for LCGA modeling. Participants were classified into 5 groups, WAZ < −1.28, − 1.28 ~ − 0.67, − 0.67~0.67, 0.67~1.28, and > 1.28, which corresponded to the 10th, 25th, 75th and 90th percentiles, respectively. The optimal number of growth trajectories (latent groups) was chosen according to the Bayesian information criterion. Lower Bayesian information criterion (BIC) values indicate a better

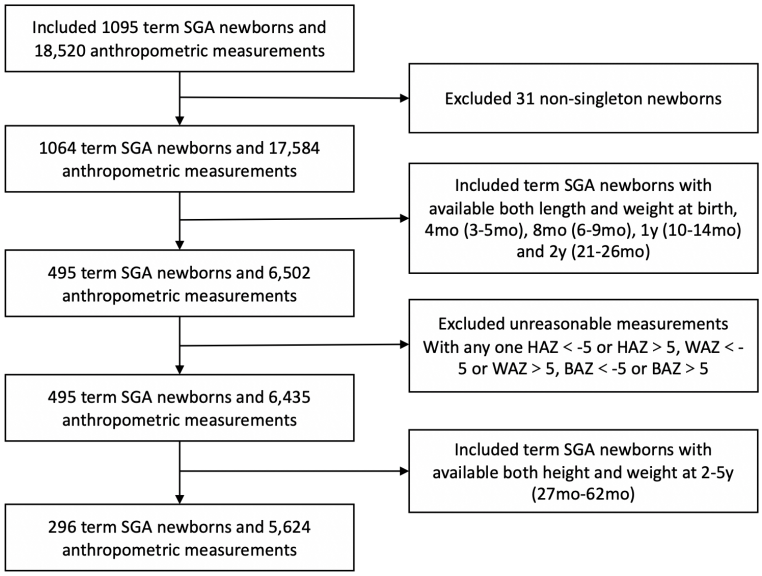

FIGURE 1
Flowchart of the study population.

fit. Moreover, the classification accuracy of the model was assessed with the entropy statistic. An entropy statistic >0.80 suggests sufficient accuracy of the model (range 0-1) (28).

Moreover, growth deviation was defined as being underweight or stunted, including children with Z score <−2 for weight or height, respectively, or being overweight or obese. BMI over the 85th percentile and 95th percentile was defined as overweight and obesity, respectively, in the current study. In addition, weight gain velocity between two target time points was indicated by different ΔWAZ degrees: i, ΔWAZ <−0.67 as crossing down one or more; ii, −0.67 ≤ ΔWAZ ≤0.67 as no crossing; iii, ΔWAZ > 0.67 to 1.28 as crossing up one; and iv, ΔWAZ >1.28 as crossing up two or more of the main weight percentiles of the WHO growth chart.

Statistical analysis

We used latent class growth analysis (LCGA) implemented in Mplus software (version 8.0) to identify weight gain trajectories from birth to 2 years (28, 29). Statistical analysis was performed using SPSS (version 26.0). Cochran–Mantel–Haenszel χ^2 was used to assess the differences in baseline characteristics among the 5 growth trajectory patterns. We used multiple logistic regression to examine the association between weight gain patterns and overweight/obesity adjusting for potential confounders, including sex, birthweight, gestational age, gravidity, parity, paternal height and BMI, feeding patterns between 0~4 mo. Furthermore, multiple logistic regression was applied to investigate the predictive impact of weight gain velocity from birth to 4 mo on overweight/obesity during 2-5 years with adjustment for the above confounding factors. Moreover, a receiver operating characteristic (ROC) curve was applied to test the sensitivity and specificity as well as the cutoff point for the predictive effect of weight gain in early life on later overweight/obesity. Statistical significance was defined as $P < 0.05$.

Results

There were 296 term SGA infants with available length and weight data at birth, 4 mo, 8 mo, 1 y, 2 y and 2-5 y were finally included in the study. Figure 1 shows the inclusion flow chart of the study population. Most demographic characteristics, including sex, gestation and birthweight, were not significantly different between SGA infants included and excluded from our study (eTable 1).

Based on the Bayesian information criterion in the LCGM, the BIC values were 4103.42, 3652.44, 3427.69, 3239.00, and 3293.68 when the population was divided into 2–6 categories, of which five were optimal grouping numbers for the minimum BIC. Five weight gain trajectories were identified, including excessive rapid catch-up growth (ERCG) (class 1, 10.9%), rapid catch-up growth (RCG) (class 2, 17.9%), appropriate catch-up growth (ACG) (class 3, 53.0%), slow catch-up growth (SCG) (class 4, 13.4%) and almost no catch growth (NCG) (class 5, 4.8%) (Figure 2). By comparing the baseline characteristics of SGAs in the 5 weight growth trajectories, we found that both birthweight and gestation played an important role (Table 1). SGAs with higher birthweight and late term were more prone to have weight gain trajectories from class 1 to 3 (P<0.01). Higher gravidity and parity were observed in the lower weight gain trajectories, but the difference was not significant (P>0.05). SGAs experienced class 1 and 2 weight gain trajectories were found having taller parents (P<0.01). Meanwhile SGAs with higher paternal BMI tend to have more rapid weight gain trajectories (P<0.01). Maternal BMI was highest in ERCG group, but with no significance (P>0.05). Additionally, feeding patterns between 0~4 mo was analyzed, and the proportion of formula feeding seemed to have a minor increase in class 1 and 2 weight gain trajectories. We further analyzed the disparities in ΔWAZ degrees during the first 4 mo of life among the five patterns. It was found that infants from classes 1 to 3 had a higher percentage of fast weight gain with ΔWAZ > 1.28 than the other two classes. Most infants in classes 4 and 5 had weight gain characterized by ΔWAZ from − 0.67 to 0.67 or ΔWAZ < − 0.67.

Furthermore, the study showed that weight gain classes in the first 2 years of life were associated with BMI for age z score and rate of overweight/obesity for SGA infants from 2-5 years (P<0.01). As shown in Figure 3, SGA infants in class 1 and class 2 had a higher BMI for age- and sex-specific Z score from 2–5 years. In addition, 25% of SGA infants in class 1 and 13.2% of SGA infants in class 2 were found to be overweight/obese at 2-5 years (Table 1). Nevertheless, 35.7% of SGA infants in class 5 were observed to be malnourished. After adjusting for confounding factors such as sex, birthweight, gestational age, gravidity and parity, paternal height and BMI, feeding patterns between 0~4 mo through multiple regression analysis, the results showed that the extremely rapid weight gain (class 1) of term SGA infants in the first 2 years of life increased the risk of overweight/obesity by 2.1 times at 2 to 5 years of age (aOR=2.1, 95% CI: 1.3~4.8; P<0.05). Besides, higher maternal height might be protective for childhood overweight/obesity at 2 to 5 years (aOR=0.823, 95% CI: 0.71~0.95; P<0.05).

We further explored the association of the ΔWAZ degrees of SGAs in early life with later overweight/obesity. When compared with the non-overweight/obese children, obviously higher ΔWAZ values from 0 to 4 mo (0.43 ± 0.12 vs. 0.26 ± 0.10, P<0.001) and 5 to 8 mo (0.07 ± 0.03 vs. 0.04 ± 0.02, P<0.01) were found in SGA infants with overweight/obesity in later life. After adjusting for confounding factors such as sex, birthweight, gestational age, gravidity and parity, paternal height and BMI, feeding patterns between 0~4 mo, the study showed that the risk of overweight/obesity between 2 and 5 years in term SGA infants was still related to the increment of ΔWAZ from 0 to 4 mo (aOR=3.2, 95% CI: 1.7~8.1; P<0.001). A receiver operating

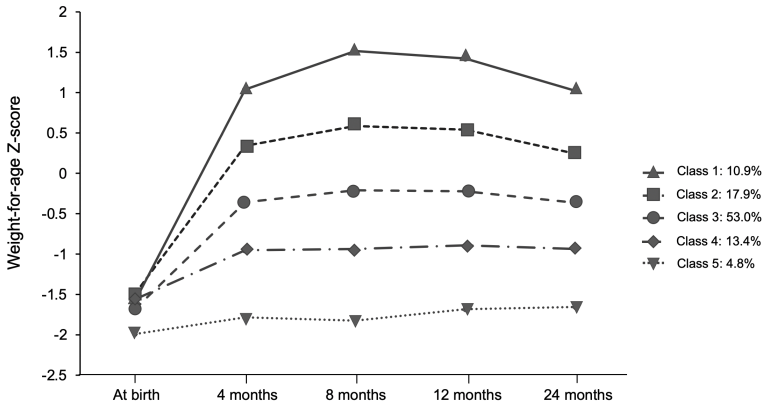

FIGURE 2
Trajectories of weight gain grouping classes in terms of SGA obtained by the latent class growth model (LCGA).

characteristic (ROC) curve was also established. The area under the curve (AUC) was 0.7, with a 95% CI from 0.6 to 0.8 (P<0.001) (Figure 4).

Discussion

The best growth and nutritional strategy for term SGA is currently unclear and is likely to differ in different populations. To our knowledge, this is the first prospective birth cohort study to investigate the temporal relationship between early life excessive weight gain and childhood overweight/obesity in relatively low-middle income areas in West China. After adjustment for sex, birthweight, gestational age, gravidity, parity, paternal height and BMI, feeding patterns between 0~4 mo, the excessive rapid weight gain pattern from birth to 2 years was associated with an increased risk of childhood overweight and obesity at 2-5 years of life. Higher maternal height might be a protective factor. In addition, our findings suggest that the increment of ΔWAZ from 0 to 4 mo is a potential predictor of childhood overweight and obesity from 2-5 years of life. It is possible that pediatricians and parents should pay more attention to and ensure optimal early life weight gain, especially in the first 4 mo of life.

The weight gain pattern of term SGA infants is likely to differ in different populations and different income areas (30). This might lead to different nutrition strategies for term SGA infants. In the study, five weight gain patterns for term SGA infants from birth to 2 y were established in a low-middle income area in West China. The weight gain pattern was similar to that in a study performed in Shanghai, the most developed area in China (31). However, it revealed that the combined proportion of slow catch-up growth (SCG) and almost no catch growth (NCG) was much higher (18.2% vs. 13.6%), and the combined proportion of excessive rapid catch-up growth (ERCG) and rapid catch-up growth (RCG) was slightly lower (28.8% vs. 30.5%) than that among term SGA infants in high-income areas. This difference might be attributed to the relatively limited medical/health conditions and socioeconomic disparities in low-middle income areas. This finding suggests that different catch-up growth strategies should be adopted in different areas.

There are also many factors that affect catch-up growth, including socio-economic factors, genetic factors, maternal prenatal factors, maternal pregnancy complications, and postnatal feeding, diseases, etc (7, 32–34). First of all, the subjects of this study are basically from urban areas, and there is no significant difference between parents' education levels. We further analyzed the effects of father's height and BMI, mother's height and prepregnancy BMI on SGA infants' catch-up growth. The results showed SGA infants whose parents were taller or had higher BMI slightly tended to experience rapid weight catch-up growth. In the multivariate analysis, the height of mother was discovered a potential protective factor for obesity in full-term SGA infants aged 2-5 years. The genetic gene of higher height is helpful to promote SGA linear catch-up growth, which may be the potential reason (35, 36). In addition, this study found that the formula feeding within 4 months of term SGA infants with fast weight catch-up growth was slightly higher. The exclusive breastfeeding rate was lowest in ERCG group. One newly study reported that SGA preterm infants fed preterm formula had significantly larger negative change in weight and length z-scores between birth and discharge, when compared with fortified mother's own milk (37). However, It was reported that breastfeeding may have positive effects on growth programming due to its nutrients' energetic efficiency (38). The short-term and long-term benefits are mainly due to the adaptation of nutrient proportion, regulation of immunity, regulation of intestinal flora, etc (39–41). In this study,

TABLE 1 The baseline characteristics of the SGA by weight gain class.

	Class 1 (ERCG)	Class 2 (RCG)	Class 3 (ACG)	Class 4 (SCG)	Class 5 (NCG)	*P* value
Number of children, n (%)	32 (10.8)	53 (17.9)	157 (53.0)	40 (13.5)	14 (4.7)	–
Male, n (%)	15 (46.9)	21 (39.6)	78 (49.7)	19 (47.5)	7 (50.0)	0.49
Birthweight, kg	2.55±0.24	2.54±0.23	2.51±0.24	2.45±0.22	2.31±0.26	<0.01
Birthweight Z score	-1.58±0.85	-1.49±0.49	-1.56±0.50	-1.68±0.47	-1.99±0.53	<0.01
Birthweight categories, n (%)						
BW<2.5kg	11 (34.3)	22 (41.5)	67 (42.7)	19 (47.5)	11 (78.6)	<0.01
2.5kg ≤ BW<3kg	21 (65.7)	31 (58.5)	90 (57.3)	21 (52.5)	3 (21.4)	
Gestation, wk.	39.00±1.21	39.02±1.28	38.88±1.21	38.64±1.15	38.28±1.13	<0.01
Gestation categories, n (%)						
Early term (37 wk. ≤ GA<40 wk.)	11 (34.4)	19 (35.8)	58 (36.9)	18 (45.0)	8 (57.1)	<0.01
Late term (40 wk. ≤ GA<42 wk.)	21 (65.6)	34 (64.2)	99 (63.1)	22 (55.0)	6 (42.9)	
Born type, n (%)						
Natural delivery	6 (18.8)	20 (37.7)	52 (33.1)	11 (27.5)	4 (28.6)	0.81
Cesarean	26 (81.2)	33 (62.3)	105 (66.9)	29 (72.5)	10 (71.4)	
Gravidity, n	1.20±0.63	1.30±0.82	1.41±0.81	1.30±0.63	1.47±1.10	0.34
Parity, n	1.00±0.00	1.04±0.19	1.07±0.26	1.09±0.29	1.14±0.48	0.13
Maternal height, centimeters	162.02±5.44	162.23±5.58	159.67±4.85	159.29±4.65	158.64±4.82	<0.01
Paternal height, centimeters	174.19±6.74	172.60±5.35	171.60±5.17	170.62±5.25	171.89±4.73	<0.05
Maternal BMI, kg/m^2	21.19±3.02	20.77±2.93	21.10±2.55	20.82±3.42	20.75±2.84	0.88
Paternal BMI, kg/m^2	23.55±2.34	24.30±3.27	23.83±2.57	22.80±3.58	22.81±2.75	<0.05
Feeding patterns between 0~4 mo						
Breast milk, n (%)	11 (35.6)	23 (43.4)	62 (39.7)	18 (45.4)	6 (39.3)	0.964
Mixed feeding, n (%)	15 (46.7)	21(39.6)	69 (44.1)	15 (37.8)	7 (46.4)	
Formula, n (%)	86 (17.8)	9 (17.0)	25 (16.2)	7 (16.8)	2 (14.3)	
ΔWAZ degrees between 4 mo. and birth, n (%)						
ΔWAZ <−0.67	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.5)	2 (14.3)	–
−0.67 < ΔWAZ< 0.67	0 (0.0)	0 (0.0)	23 (14.7)	20 (50.0)	9 (64.3)	
0.67 < ΔWAZ<1.28	1 (3.1)	9 (17.0)	52 (33.1)	13 (32.5)	3 (21.4)	
ΔWAZ > 1.28	31 (96.9)	44 (83.0)	82 (52.2)	6 (15.0)	0 (0.0)	
Adverse growth outcomes, n (%)						
Overweight/ obesity	8 (25.0)	7 (13.2)	14 (8.9)	1 (2.5)	0 (0.0)	<0.01
Malnutrition	0 (0.0)	1 (1.9)	5 (3.2)	6 (15.0)	5 (35.7)	

ERCG, excessively rapid catch-up growth; RCG, rapid catch-up growth; ACG, appropriate catch-up growth; SCG, slow catch-up growth; NCG, almost no catch-up growth; BW, birthweight; ΔWAZ, ΔWeight-for-age Z-score degrees.

suspected congenital syndrome (with obvious special facial features and severe growth and development disorders), congenital organ abnormalities and chronic diseases that may seriously affect the growth and development of SGA infants were not included. This is helpful to reduce the influence of confounding factors on SGA infants catch-up growth pattern discrimination. After correcting important confounding factors, the excessive rapid weight gain pattern from birth to 2 years was still associated with an increased risk of childhood overweight and obesity at 2-5 years of life.

It has been illustrated by the concept of the Developmental Origins of Health and Disease (DOHaD) that children born with low birth weight or SGA have an increased risk for obesity, insulin resistance, and ultimately impaired glucose tolerance, type 2 diabetes, and cardiovascular disease later in life (14, 15, 42, 43). In this study, we found that the extremely rapid weight gain pattern (ΔWAZ>1.28) of term SGA infants from birth to 2 years was significantly related to the increased risk of overweight/ obesity at 2-5 years in later life. The risk was approximately 2 times higher than that of term non-SGA infants. The pooled findings reviewed by Druet C et al. showed that each +1 unit increase in weight standard deviation (SD) scores between 0 and 1 year conferred a twofold higher risk of childhood obesity and a 23% higher risk of adult obesity, adjusted for sex, age and birthweight (44). A recent study from Denmark showed that rapid (ΔWAZ 0.67–1.34) and very rapid weight gain

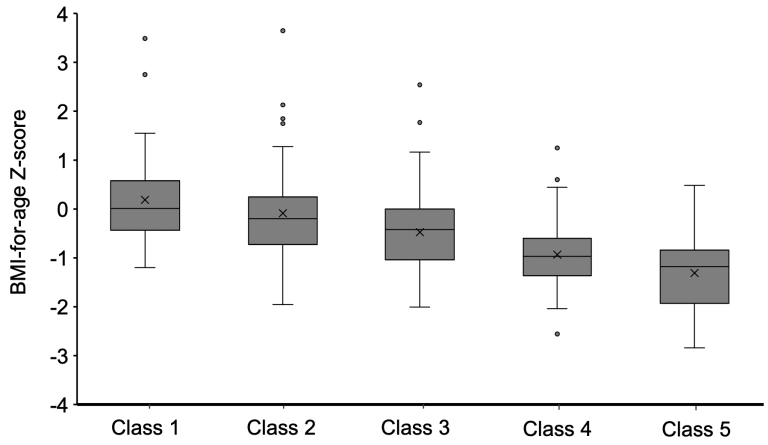

FIGURE 3
BMI Z score when participants aged 2–5 y were stratified by weight gain class in the first 2 years.

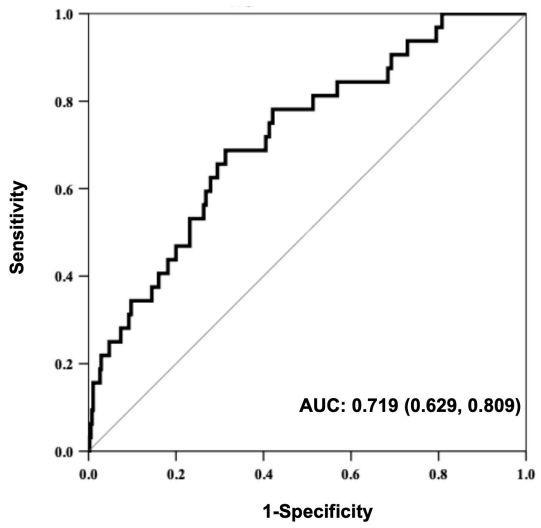

FIGURE 4
Receiver operating characteristic (ROC) curve for the predictive value of WAZ from birth to 4 months regarding overweight/obesity in SGA children aged 2~5 years.

(ΔWAZ>1.34) among infants between 0 and 8–10 months of age dramatically increased the risk of overweight and obesity at 2 years (22–26 months) (45). The risks for overweight and obesity were nearly 3 and 7 times higher, respectively. Although there were some disparities in risks due to the different study designs and populations, it was clear that extremely rapid weight gain in early life indeed significantly increased the risk for overweight/obesity in later life. However, the trigger mechanisms remain unknown. The body fat content of a healthy full-term infant rises sharply from 10 to 14% at birth to 25 to 30% at 6 mo of age. Children born SGA had higher central adiposity regardless of their body size. Being SGA at birth could program excess abdominal fat deposition in children, which is a major component in the clustering of cardiovascular disease risk factors defining metabolic syndrome (MetS) (46). It is known that the early time is a critical period for growth and nutrition programming. The effect of excessive fat gain in the early stages on long-term nutrient metabolism deserves further investigation.

Dynamic changes in body weight have long been recognized as important indicators of risk for human health. Many population-based observational studies have shown that rapid weight gain during infancy, including a catch-up growth phenomenon or adiposity rebound in early childhood, predisposes a person to the development of obesity, type 2 diabetes, and cardiovascular diseases later in life (15). However, the exact timing of the rapid weight gain that contributes to these long-term risks continues to be debated. In this prospective study, we found that the fast increment of ΔWAZ from 0 to 4 mo was significantly related to the high risk of overweight/obesity at 2 and 5 years in term SGA infants. The risk was almost 3.2 times higher among these individuals. Additionally, the ROC curve suggested that ΔWAZ from 0 to 4 mo might be a potential predictor of the overweight/obesity risk of preschool children born at term and SGA. Similarly, a multicenter cohort study in the U.S. discovered that weight gain in the first 4 mo was associated with the OR of obesity at 7 years (47). Stettler N et al. found that African American infants gaining ≥1 WAZ unit in the first 4 mo were significantly more likely to be obese by age 20 (OR = 5.22) (48). In a Chinese cohort, an increase in WAZ in the first 3 mo was associated with BMI-Z at 7 years (49). Even increases in WAZ as early as the first 8 d of life have been associated with an increased risk of overweight and obesity in adulthood (50). The studies cited suggest that the first few weeks and months of life were

particularly associated with later weight status (51). The disparity in research outcomes may result in part from ethnic differences, inconsistencies in research design, a lack of longitudinal and intervention studies, and appropriate unified indicators for catch-up growth. Previous studies have emphasized absolute weight gain, rarely addressing changes in body composition and failing to address fat distribution (subcutaneous vs. visceral). As also reviewed by Cho WK, the early fat increase in SGA infants may be the key factor affecting the occurrence of long-term cardiovascular metabolic diseases (46). Further prospective cohort multicenter studies evaluating fat growth should be designed. With regard to the mechanism(s) driving the correlation between excess weight gain in the first 4 mo and later obesity remain unknown. These months may be a critical time when metabolic programming can occur, similar to the *in utero* period, because infants' organ systems still maintain considerable plasticity for adaptation to nutritional and environmental exposures. During the very early period, SGA infants are still malleable, and the gut is so permeable that milk/formulas can elicit significant endocrine responses. The combination of these factors potentially makes this time a vulnerable period (52).

Limitations and prospects

This research has a number of limitations as well as strengths. First, the study failed to collect detailed information on detailed nutrition, which limited our analysis of the effects of nutritional factors on growth and development in SGA infants. Besides, the maternal obesity and weight gain during pregnancy were not well recorded. Second, we only included term SGA infants in the analysis. Thus, the results may not be applicable to preterm SGA infants or overdue SGA infants. Infants in these groups are thought to have different growth trajectories. Third, due to the limited duration of long-term follow-up, we analyzed whether the children were overweight or obese during the age of 2-5 years as the outcome. A better research design is needed in the future to ensure the integrity of long-term follow-up data. To identify an ideal growth trajectory, a longer follow-up period and more critical time points will be needed to assess the long-term impact of catch-up growth in childhood. These limitations aside, the strength of this research lies in the prospective study design with a relatively long follow-up time. Economic, geographical and ethnic factors are all potential factors affecting the growth and development of SGA infants. The risk of long-term chronic diseases among SGA infants requires early detection and timely intervention. However, to our knowledge, no such prospective cohort study has been conducted in Southwest China. This study has filled this gap to some extent. This study provides an important reference for the early health management of term SGAs in Southwest China.

Conclusion

This study shows that excessive rapid catch-up weight growth in full-term SGA infants aged 0-2 years is an important risk factor for overweight/obesity at the age of 2-5 years. Scientific management of the weight increase in full-term SGA infants in early life, especially from 0-4 months, is the key to preventing the occurrence and development of overweight/obesity and its metabolic diseases in the later period. Children's health care physicians should pay extra attention to this finding. Regular monitoring weight gain across the infant period represents a first step towards primary prevention of childhood obesity.

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 author.

Ethics statement

The study protocol was approved by the medical ethics committee of West China Second University Hospital.

Author contributions

Data extraction and curation: PL, SY. Statistical analysis: PL. Methodology: PL, FY. Validation: PL, DQ. Writing—original draft: PL. Writing—review and editing: all authors. All authors contributed to the article and approved the submitted version.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1030216/full#supplementary-material

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Is the visceral adiposity index a potential indicator for the risk of kidney stones?

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Objective: To determine whether the visceral adiposity index (VAI) was linked to the risk of kidney stones (KS) in the representative U.S. adults.

Methods: We investigated 59842 participants who joined the 2007–2018 National Health and Nutrition Examination Survey. The association between the visceral adiposity index (VAI) and KS was identified by logistic regression analysis. Meanwhile, the subgroup analysis as well as the calculation of dose–response curves were also utilized to identify sensitive groups.

Results: Data from 29384 participants were available, including 2781 self-reported ever experiencing KS diseases. Overall, the VAI was 0.74 (0.70, 0.78) in the KS group, while 0.55 (0.52, 0.57) in the control group. After adjusting for confounders, the prevalence of KS increased by 13% for each unit of VAI increment (OR = 1.13, 95% CI: 1.08, 1.19). Moreover, a linear relationship was found between the VAI and the prevalence of KS. By subgroup analysis, we found that a positive correlation between VAI and the risk of KS both in male (OR=1.14, 95%CI:1.07, 1.22) and female (OR=1.14, 95%CI:1.05, 1.24), White (OR=1.20, 95%CI:1.11, 1.28) and other race, all aged subgroups, nonhypertensive (OR=1.06, 95%CI:1.08, 1.25) and nondiabetic subgroups (OR=1.14, 95%CI:1.07, 1.21).

Conclusions: Elevated VAI was strongly associated with KS in representative U.S. adults, which may be a promising indicator for the risk of kidney stones.

KEYWORDS

visceral adiposity index, kidney stones, risk factors, obesity, NHANES

Introduction

Kidney stones (KS) are widespread diseases of the urinary system, that clinically manifest as hematuria, renal colic, urinary tract infection, urinary tract obstruction, and, in severe cases may manifest as renal failure and are even life-threatening (1–3). Epidemiological studies show that KS diseases affect 1% to 20% of the population worldwide and the annual incidence of new cases is approximately 150-200 per 100,000 people, 25% of whom require hospitalization (2, 4, 5). Unfortunately, the prevalence of KS is on the rise worldwide and with a stone recurrence rate up to over 50% within five years (3, 6). To date, KS diseases have become a major public health problem, causing a serious economic burden to individuals and the country (7, 8). Therefore, how to prevent the occurrence of KS should be regarded as a high priority.

Currently, accumulated evidence suggests that metabolic syndrome (MS) is implicated in an elevated risk of KS (9–11), in which the role of obesity has attracted more attention from researchers (12, 13). Obesity has emerged as a public health problem in many countries worldwide (14, 15). A study conducted by Carbone et al. (16) showed that the prevalence of obesity in patients with KS was approximately 10-35%. Consistent with this, a higher rate of KS was also found in obese (11.2%) and overweight populations (9.1%) than in normal weight populations (6.1%) (17). Moreover, the risk of KS increased markedly with increasing body mass index (BMI) (18, 19). Although obesity was strongly associated with KS, reliable indicators of obesity to predict the risk of KS presence are extremely lacking.

Interestingly, researchers have recognized that adipose tissue has complex functions and that not all adipose tissue is harmful to the body, such as brown fat (20), so that high body weight and overall excess adiposity cannot simply be assumed to be poor health states. Therefore, defining and quantifying lipid accumulation in specific settings that may represent certain physiological risks can contribute to a deeper understanding of the role of adipose tissue in the pathophysiological processes of disease and its value in predicting the risk of disease occurrence. Several studies have shown that visceral adipose tissue is more strongly associated with diabetes, hypertension, cardiovascular disease and cardiometabolic risk factors than subcutaneous adipose tissue (21–25). However, traditional indicators of obesity, such as waist circumference (WC), BMI, waist-to-hip ratio, and waist-to-height ratio, did not differentiate visceral fat from subcutaneous fat. Although CT or MRI is considered the gold standard for the quantification of abdominal obesity, its expensive, complex and radiation-risky nature has prevented its large-scale implementation.

The visceral adiposity index (VAI) is an indicator of abdominal fat distribution and adipose tissue function proposed by Amato et al. (26). Its calculation includes not only anthropometric characteristics such as WC and BMI but also two metabolic indicators, triglycerides (TGs) and high-density lipoprotein cholesterol (HDL-C). These metrics and metabolism are strongly associated with the development of KS (18, 27) which allows us to more accurately assess the relationship between obesity and KS. Thus, we propose the hypothesis that VAI is associated with the risk of kidney stones. To explore this hypothesis, we investigated the link between VAI and KS diseases based on data from 29384 individuals.

Materials and methods

Study design and participants

The baseline clinical data included in this analysis were derived from the National Health and Nutrition Examination Survey (NHANES) from 2007 to 2018, in which only participants aged over 20 years participated in the KS questionnaire; we preserved information on people who answered affirmatively as to whether they had kidney stones. A total of 59842 participated in the questionnaire. After excluding 30098 nontargeted participants, 29384 participants were available in this work (Figure 1), including 2781 self-reported ever experiencing KS diseases.

Collection and definition of data

The VAI was developed as an exposure variable utilizing the below gender-specific equations. For males VAI=[WC(cm)/(39.68 + 1.88×BMI(kg/m2))]×(TG(mmol/L)/1.03)×(1.31/HDL(mmol/L)); for females VAI=[WC(cm)/(36.58 + 1.89×BMI(kg/m2))]×(TG(mmol/L)/0.81)×(1.52/HDL(mmol/L)) (28). Serum triglyceride concentrations were determined by Roche Cobas 6000 chemistry analysers. Questionnaires were used to assess the age at the time of surgery for the presence of KS, was the response to the questions of “Ever been told you have kidney stones?”. A variable designed to measure outcome was kidney stone occurrence.

In multivariate adjusted models, we summarized covariates that may confound the relationship between VAI and KS. The following factors were included in the study’s covariates: gender, race, age, education level, cholesterol concentration, marital status, alcohol intake, poverty-to-income ratio (PIR), smoking status, physical activity, diabetes, hypertension, and dietary intake factors. The 24-hour dietary recall was conducted on each participant in 2007 and 2018, and we will analyse the average consumption of both recalls. Detailed measurement protocols using the research variables are available at http://www.cdc.gov/nchs/nhanes/. All NHANES protocols were

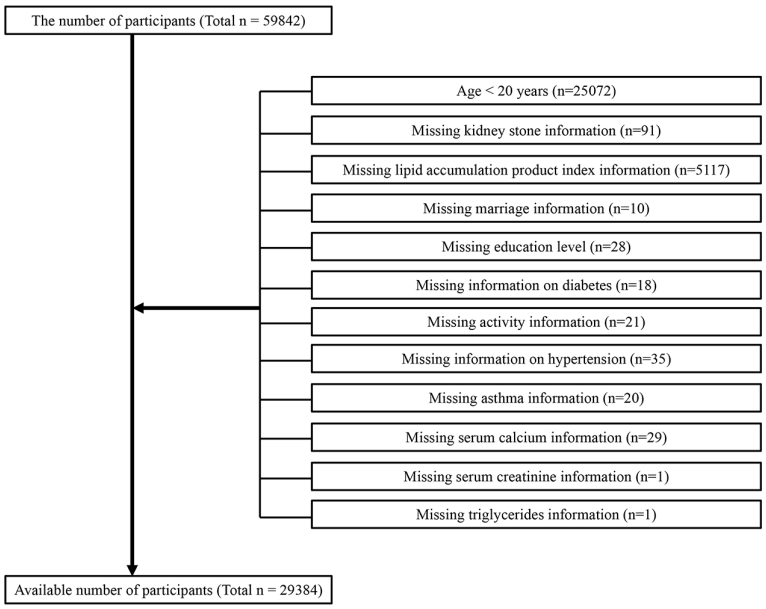

FIGURE 1
Flow chart of the participant selection process.

implemented following the Human Research Subject Protection Policy of the U.S and reviewed and standardized by the NCHS Research Ethics Review Committee. Informed consent forms were signed by all participants. There was no additional authorization or ethical review required for the release of NHANES data for this study.

Statistical methods

The NHANES sample weights, clustering and stratification were used in all statistical analyses to illustrate the elaborate sampling methodology used to select a representative sample of U.S. noninstitutionalized adults. Continuous variables are presented as weighted survey means and 95% CIs, while categorical variables are presented as weighted surveys and 95% CIs. As VAI have skewed distributions, LN transformations convert them into normal distributions. In our study, we performed VIF covariate screening and removed covariates if the VIF value was greater than 5. Based on the guidelines (29), a multiple logistic regression model was conducted to explore the VAI, different VAI tertile groups and the prevalence of KS in three different models. Covariates were not adjusted in model 1, while model 2 adjusted for gender, race, age and education level as well as matrimony status, model 3 adjusted for covariates in Model 2 + hypertension, diabetes, alcohol use, PIR, smoking, asthma, physical activity, total kcal, total water, total sugar, serum calcium, serum cholesterol and serum creatinine. The relationship between the VAI and KS was further assessed using generalized additive model regression as well as smoothed curve fitting. Whenever a nonlinear relationship was identified, the inflection points were calculated by likelihood ratio tests. Multiple regression analysis was next performed on a stratified basis by gender, race, age, diabetes mellitus and hypertension. In addition, a log-likelihood ratio was utilized to test for heterogeneity in subgroup associations. Statistical significance was defined as $P< 0.05$. Empower software® 4.0.2 was utilized for all analyses (www.empowerstats.com; X&Y Solutions, Inc., Boston, MA).

Results

Participant characteristics

The baseline demographic characteristics among the enrolled participants are summarized in Table 1. The VAI was 0.74 (0.70,0.78) in the kidney stones group, which was higher than that of 0.55 (0.52, 0.57) in the control group ($P < 0.0001$).

VAI in participants with KS

The VIF for all indices of covariate screening was less than 5, and all variables were analyzed in the final adjusted model. The results showed that a positive correlation was identified between the VAI and the prevalence of KS. This positive correlation remained stable in the model 3 (OR=1.13, 95%CI:1.08, 1.19),

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TABLE 1 Baseline characteristics of participants.

Characteristic	Nonstone formers (n=26603)	Stone formers (n=2781)	*P* value
Age(years)	46.60 (46.13, 47.06)	53.20 (52.57, 53.84)	<0.0001
Serum Cholesterol (mg/dl)	194.07 (193.08, 195.05)	192.28 (189.78, 194.79)	0.1434
Serum Calcium(mg/dl)	9.39 (9.38, 9.41)	9.37 (9.34, 9.40)	0.0587
Serum Creatinine(mg/dl)	0.87 (0.87, 0.88)	0.93 (0.91, 0.94)	<0.0001
VAI	0.55 (0.52, 0.57)	0.74 (0.70, 0.78)	<0.0001
Gender (%)			<0.0001
Male	47.78 (47.09, 48.47)	55.42 (52.73, 58.07)	
Female	52.22 (51.53, 52.91)	44.58 (41.93, 47.27)	
Race (%)			<0.0001
Mexican American	14.97 (13.08, 17.08)	11.35 (9.38, 13.66)	
White	65.69 (62.82, 68.45)	76.90 (73.77, 79.76)	
Black	11.13 (9.76, 12.67)	5.64 (4.65, 6.81)	
Other Race	8.21 (7.36, 9.15)	6.11 (4.91, 7.58)	
Education level (%)			0.0924
Less than high school	20.41 (19.00, 21.90)	20.08 (18.16, 22.15)	
High school	28.68 (27.50, 29.89)	31.34 (28.56, 34.27)	
More than high school	50.91 (49.04, 52.78)	48.57 (45.44, 51.72)	
Marital Status (%)			<0.0001
Cohabitation	63.36 (62.07, 64.63)	69.24 (66.67, 71.69)	
Solitude	36.64 (35.37, 37.93)	30.76 (28.31, 33.33)	
Alcohol (%)			0.7228
Yes	61.18 (59.71, 62.63)	59.99 (56.91, 62.99)	
No	18.51 (17.46, 19.61)	19.24 (17.05, 21.65)	
Unclear	20.31 (19.21, 21.46)	20.77 (18.05, 23.77)	
High Blood Pressure (%)			<0.0001
Yes	29.75 (28.75, 30.76)	46.35 (43.46, 49.27)	
No	70.25 (69.24, 71.25)	53.65 (50.73, 56.54)	
Diabetes (%)			<0.0001
Yes	8.54 (8.06, 9.05)	17.61 (15.87, 19.50)	
No	91.46 (90.95, 91.94)	82.39 (80.50, 84.13)	
Smoked (%)			<0.0001
Yes	43.65 (42.43, 44.87)	49.51 (46.67, 52.35)	
No	56.35 (55.13, 57.57)	50.49 (47.65, 53.33)	
Physical Activity (%)			0.0044
Never	26.05 (25.07, 27.07)	29.86 (27.65, 32.17)	
Moderate	31.94 (30.96, 32.95)	31.36 (29.06, 33.77)	
Vigorous	42.00 (40.88, 43.14)	38.77 (36.06, 41.56)	
Asthma (%)			0.0043
No	85.49 (84.77, 86.18)	82.72 (80.74, 84.54)	
Yes	14.51 (13.82,15.23)	17.28 (15.46,19.26)	
PIR			0.1303
<1.3	20.13 (18.90,21.43)	18.19 (16.46,20.07)	
≥1.3<3.5	32.50 (31.25,33.77)	35.03 (32.46,37.69)	
≥3.5	40.03 (38.15,41.93)	39.83 (36.56,43.18)	
3	7.34 (6.71,8.03)	6.95 (5.71,8.43)	
Total Kcal (%)			0.2454
Lower	39.08 (38.26,39.91)	40.26 (38.04,42.52)	
Higher	46.04 (45.04,47.04)	46.39 (43.69,49.12)	

(Continued)

TABLE 1 Continued

Characteristic	Nonstone formers (n=26603)	Stone formers (n=2781)	*P* value
Unclear	14.88 (14.09,15.72)	13.35 (11.62,15.29)	
Total Sugar (%)			0.9933
Lower	36.48 (35.63,37.35)	36.65 (33.86,39.54)	
Higher	37.22 (36.27,38.18)	37.11 (34.17,40.14)	
Unclear	26.30 (25.49,27.13)	26.24 (23.95,28.66)	
Total Water (%)			0.0507
Lower	38.88 (37.97,39.81)	37.34 (34.87,39.87)	
Higher	46.23 (45.28,47.18)	49.32 (46.55,52.09)	
Unclear	14.88 (14.09,15.72)	13.35 (11.62,15.29)	
Total Fat (%)			0.0507
Lower	38.88 (37.97,39.81)	37.34 (34.87,39.87)	
Higher	46.23 (45.28,47.18)	49.32 (46.55,52.09)	
Unclear	14.88 (14.09,15.72)	13.35 (11.62,15.29)	

Data of continuous variables are shown as the survey-weighted mean (95% CI), and the P value was calculated by survey-weighted linear regression. Data of categorical variables are shown as survey-weighted percentage (95% CI), P value was calculated by survey-weighted Chi-square test.

indicating that the prevalence of KS increased by 13% with each unit increase in the LN-transformed VAI. Meanwhile, we converted the VAI to a categorical variable (triple quantile) from a continuous variable for sensitivity analysis and found that a significant 0.25-fold increase in KS presence was observed in Tertile 3 (OR= 1.25, 1.13, 1.39) compared to the lowest VAI lowest tertile (Tertile 1), as shown in Table 2.

Subgroup analysis

To assess the robustness of the VAI-KS relationship, subgroup analyses were conducted (Table 3). We found that VAI was positively correlated with prevalence of KS both in male (OR=1.14, 95%CI:1.07, 1.22) and female (OR=1.14, 95%CI:1.05, 1.24), White (OR=1.20, 95%CI:1.11, 1.28) and other race (OR=1.26, 95%CI:1.07, 1.49), age ≤ 40 years (OR=1.21, 95% CI:1.09, 1.34), >40 ≤ 60 years (OR=1.11, 95% CI:1.02, 1.21) and >60 years groups (OR=1.09, 95% CI:1.00, 1.18), nonhypertensive (OR = 1.06, 95% CI: 1.08, 1.25) and nondiabetic subgroups (OR=1.14,95%CI:1.07,1.21) We also controlled for interactions between BMI, age, gender, diabetes and hypertension. The results showed that age and race have an interaction effect on the association between VAI and KS prevalence.

Dose–response and threshold effects analysis of the VAI on KS

Generalized additive model regression as well as smoothed curve fitting were applied to assess the relationship between the VAI and KS. Our results showed that VAI was linearly related to KS presence (Figure 2).

Discussion

Although obesity is involved in the high prevalence of KS (11, 19, 27), reliable indicators of obesity to predict the risk of KS remain lacking. In this study, based on the NHANES database we demonstrated that a higher VAI was linked to a higher

TABLE 2 Analysis between the VAI with KS prevalence.

Characteristic	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)
VAI Index	1.29 (1.23, 1.36)	1.20 (1.14, 1.26)	1.13 (1.08, 1.19)
Categories			
Tertile 1	1	1	1
Tertile 2	1.34 (1.21, 1.48)	1.21 (1.09, 1.35)	1.15 (1.04, 1.28)
Tertile 3	1.64 (1.49, 1.81)	1.40 (1.26, 1.55)	1.25 (1.13, 1.39)

Model 1 was adjusted for no covariates;
Model 2 was adjusted for race, gender, age, marital status and education;
Model 3 was adjusted for covariates in Model 2 + hypertension, diabetes, alcohol use, PIR, smoking, asthma, physical activity, total kcal, total water, total sugar, serum calcium, serum cholesterol and serum creatinine.

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TABLE 3 Subgroup analysis between the VAI and KS prevalence.

Subgroups	Model 1OR (95%CI)	Model 2OR (95%CI)	Model 3OR (95%CI)	*P* value for interaction*
Gender				0.9047
Male	1.25 (1.18, 1.33)	1.19 (1.11, 1.26)	1.14 (1.07, 1.22)	
Female	1.36 (1.27, 1.47)	1.26 (1.17, 1.37)	1.14 (1.05, 1.24)	
Race				0.0048
Mexican American	1.07 (0.97, 1.18)	1.02 (0.92, 1.13)	0.97 (0.88, 1.08)	
White	1.31 (1.22, 1.40)	1.27 (1.19, 1.36)	1.20 (1.11, 1.28)	
Black	1.23 (1.07, 1.41)	1.17 (1.01, 1.35)	1.12 (0.96, 1.29)	
Other Race	1.42 (1.22, 1.66)	1.34 (1.15, 1.57)	1.26 (1.07, 1.49)	
Age (years)				0.0075
20-39	1.40 (1.27, 1.54)	1.31 (1.19, 1.45)	1.21 (1.09, 1.34)	
40-59	1.27 (1.17, 1.37)	1.21 (1.11, 1.31)	1.11 (1.02, 1.21)	
60-80	1.15 (1.06, 1.24)	1.15 (1.05, 1.24)	1.09 (1.00, 1.18)	
Hypertension				0.0558
YES	1.17 (1.09, 1.25)	1.11 (1.03, 1.20)	1.07 (1.00, 1.16)	
NO	1.29 (1.21, 1.38)	1.19 (1.11, 1.28)	1.16 (1.08, 1.25)	
Diabetes				0.1713
YES	1.13 (1.02, 1.26)	1.08 (0.96, 1.21)	1.08 (0.96, 1.22)	
NO	1.26 (1.19, 1.33)	1.18 (1.11, 1.24)	1.14 (1.07, 1.21)	

Model 1 was adjusted for no covariates;
Model 2 was adjusted for race, gender and age;
Model 3 was adjusted for all covariates except the effect modifier;
* means only in model 3.

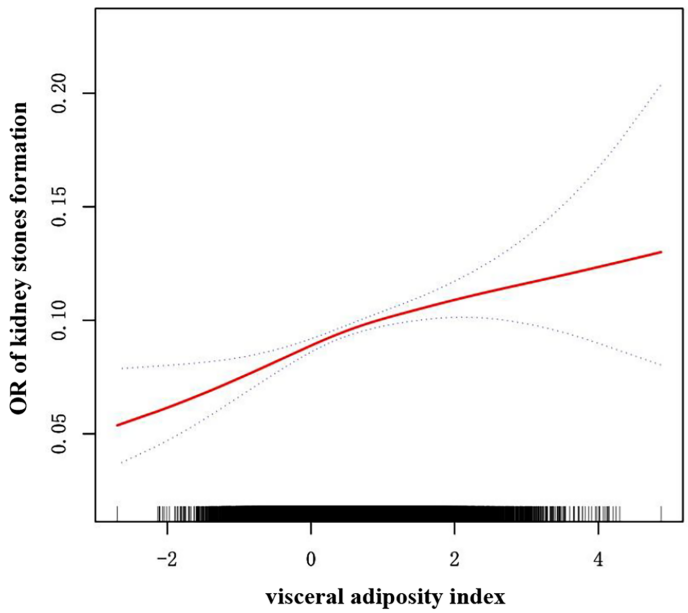

FIGURE 2
Density-dose-response correlation between the visceral adiposity index (VAI) and kidney stone formation. The area of the two blue dotted lines is indicated as the 95% CI. Each point illustrates the quantitative magnitude of the VAI index, which is linked into a continuous line. Adjustments were made for all individual covariates except effect modification factors.

prevalence of KS in the representative U.S. adults after adjusting for confounders and the prevalence of KS increases by 13% for each unit of VAI increment after normalizing the VAI by LN transformation. Moreover, by converting the VAI from a continuous variable to a trichotomous categorical variable, sensitivity analysis showed that the VAI was reliable, with a significantly greater incidence of KS in the highest VAI group (Tertile 3). We also discovered that VAI was linearly related to KS presence in the dose response and threshold effect analyses. Our findings suggest that VAI may be a promising predictor of KS.

VAI as a specific index of visceral adiposity disorders has been widely associated with cardiovascular diseases, hypertension, human purine metabolism, insulin resistance and thyroid function (30–32). Recently, a retrospective study reported by Sönmez et al. (33) concluded that the VAI was positively correlated with the creatinine levels in patients with KS. However, evidence regarding the association between the VAI and KS are limited. In a retrospective study of 1,698 patients with stones, Trinchieri et al. (34) found that overweight and obese patients were commonly associated with increased excretion of urinary calcium, urinary oxalate and uric acid, which are strongly associated with the development of KS (3). Excessive visceral adipose tissue can lead to disorders of fatty acid metabolism and release free fatty acids, which induce inflammatory responses in a variety of cells such as macrophages and adipocytes (35, 36). Taguchi et al. (12) reported that mice treated with a high-fat diet exhibited increased accumulation of lipids in the kidney and triggered an inflammatory response, which ultimately led to the development of kidney stones. Although the above reports do not directly point to the relationship between VAI and KS, there may be a link between visceral adiposity and KS, which may be an idea to explain the better development of KS in patients with a high VAI.

KS is a common urological disease with a high global prevalence but an unknown mechanism. On the one hand, the specific mechanism of its occurrence is still unclear, and there is no effective etiologic prevention method; on the other hand, the treatment method of KS is constantly adjusted according to the progress of the patient's stones, and early detection of KS can reduce the possibility of invasive operations for patients, reduce the pain suffered by patients and reduce the cost of treatment (37). Therefore, secondary prevention in the high-risk group is particularly important to achieve early detection, diagnosis, and treatment of KS. Our study showed that a high VAI is a statistically significant risk factor associated with KS. The VAI is related to the volume and area of visceral adipose tissue and is an index to evaluate the extent of visceral fat distribution and accumulation. Moreover, the VAI has higher sensitivity and specificity than traditional indicators such as waist circumference, BMI, and blood lipids (26). Our study supports that an elevated VAI was significantly associated with KS. Screening for VAI in high-risk groups provides new ideas for prevention and treatment of KS, which can prevent the occurrence of KS and provide early management of patients with KS.

Subgroup analysis showed that the relationship between the VAI and KS incidence was robust and generalized, and this positive correlation still existed in different gender, ethnicities, and age groups. This result needs to be supported by a larger sample size study. After further subgroup studies, the lowest VAI group (Tertile 1) showed a significant 25% increase in the prevalence of KS compared with the highest VAI group (Tertile 3), reflecting the considerable sensitivity of the VAI. A promising point in this study was that in different age subgroups, patients with a high VAI in the younger age group had a stronger correlation with KS presence than patients with a high VAI in the older age group, which gives us a new strategy for KS prevention, namely the management and control of the VAI in younger patients. We hypothesized that an elevated VAI is an important factor in the development of pro-KS and contributes to cardiovascular disease, metabolic syndrome and other disorders. However, the low age of the patient itself is a protective factor for cardiovascular disease, metabolic syndrome but has no significant protective effect on the development of KS, so the prediction of KS by the high VAI may have a higher specificity within younger individuals. No relevant reports in this regard have been published, and follow-up large-scale prospective studies are needed to confirm this causal relationship.

Our study has the following advantages. First, NHANES study participants are representative U.S. adults, which closely followed a well-designed study protocol with extensive guarantee and conformance. Second, we controlled for confounding variables and performed subgroup analyses to make our findings reliably and stably applicable to a wider range of individuals. Third, we further demonstrated that the VAI was linearly related to the prevalence of KS. However, the limitations of our study cannot be ignored. First, our cross-sectional investigation was based on the NHANES database, which prevented us from assessing a causal relationship between VAI and KS. Second, the prevalence of KS was obtained from patient self-reports, and recall bias was inevitable. Despite these limitations, our study clearly revealed a positive correlation between the VAI and KS presence.

Conclusion

We demonstrated that the elevated VAI was strongly associated with KS in representative U.S. adults in a cross-

sectional study. VAI may be a promising indicator for the risk of KS, which is beneficial for prevention guidance of KS.

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

The studies involving human participants were reviewed and approved by the NCHS Research Ethics Review Committee approved the NHANES survey protocol (https://www.cdc.gov/nchs/nhanes/irba98.htm). The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZH, MC and JX: Conceptualization, Methodology and Project administration; BH, QH and XS: Visualization, Investigation, Software and Writing - review & editing; YC, YX: Software and data collection. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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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

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Small RNA perspective of physical exercise-related improvement of male reproductive dysfunction due to obesity

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Purpose: To study whether physical exercise can effectively ameliorate obesity-induced abnormalities in male fertility and provide a new perspective on the role of small noncoding RNAs in spermatogenesis in obese male mice.

Methods: In this study, four-week-old C57/Bl6 male mice were randomly allocated to receive a control diet, a high-fat diet or physical exercise intervention for 40 weeks. Purified round spermatids and spermatozoa were obtained after intervention. Sperm motility, concentration, the ability of the sperm to undergo capacitation and acrosome reaction were assessed. Small RNA sequencing was conducted on round spermatids and spermatozoa. The small noncoding RNAs expression pattern was systematically analyzed.

Results: The spermatozoa concentration and percentage of motile spermatozoa, the capacitation and acrosome reaction, and the reproductive success rate, including mating success and pregnancy success, were decreased or delayed in the obesity group compared with controls. Physical exercise was able to restore the parameters to normal levels. Three microRNAs were consistently upregulated and 5 were downregulated in round spermatids and epididymal spermatozoa between the obesity and control groups.

Conclusions: This report provides evidence that the adverse effects of obesity could be offset after physical exercise. small noncoding RNAs, especially microRNAs in germ cells, may play an important role in the effects of obesity and physical exercise on spermatozoa.

KEYWORDS

male infertility, obesity, physical exercise, sncRNA, microRNA

Introduction

From 1975 to 2021, the obese male population tripled worldwide, and the proportion of obese men of childbearing age (20 to 40 years old) was approximately 11% (1, 2). Obviously, obesity is a complex disease of multifaceted etiology, with its own disabling capacities, pathophysiology and comorbidities. It remains a modifiable risk factor for diabetes mellitus, cardiovascular disease, obstructive pulmonary disease, arthritis, and cancer.

In parallel with rising male obesity, male sperm quality and fertility have declined in recent decades (3, 4). Despite considerable research efforts devoted to understanding the biology of obesity and energy balance, it has become obvious that, to date, our evolving scientific knowledge about the etiology of obesity has been of little help to evaluate the relationships between the obesity epidemic and spermatogenesis, sperm quality and fertility. A meta-analysis including data from 717 men revealed that, compared with men of normal weight, the odds ratio for oligozoospermia or azoospermia was 1.11 for overweight, 1.28 for obese and 2.04 for morbidly obese men (5). In addition, there is strong evidence of a negative relationship for testosterone, sex hormone binding globulin and free testosterone with increased body mass index (BMI) (6). Therefore, male obesity is considered to play an important role in the decline of male fertility (4).

The prescription of physical exercise has now been suggested to be an important component of obesity treatment. Although the effects of physical exercise on male fertility are uncertain, the positive effects of moderate exercise on spermatogenesis have been described by comparing sperm parameters in exercise and sedentary men (7); higher levels of FSH, LH and testosterone have been recognized in physically active subjects than in sedentary subjects. More recent reports have concluded that moderate training is associated with improvements in sperm DNA integrity and semen quality and with reduced expression of seminal markers of inflammation and oxidative stress (8–10). A randomized controlled trial including 200 obese men showed that physical exercise could significantly increase semen volume, semen concentration, semen mobility and the percentage of normal morphology (11). In rat testes, physical exercise effectively protected against the detrimental effect induced by obesity by downregulating stem cell factor, upregulating ghrelin and normalizing oxidative stress (12). In addition, physical exercise intervention improved testicular development in rats fed a high-fat diet by modulating KISS-1/GPR54 expression (13). Despite the painful progress made in the concept of improving obese male infertility through physical exercise, the role of physical exercise on infertility and spermatogenesis in obese men has not been adequately described from a holistic perspective.

Small noncoding RNAs (sncRNAs) are polymeric RNA molecules that are less than 200 nucleotides in length and are usually noncoding. They can be divided into several categories according to their origin, function and length, including microRNA (miRNA, 20-24 nt), PIWI-interacting RNA (piRNA, 29-30 nt), small interfering RNA (siRNA, 20-27 bp), small nucleolar RNA (snoRNA), small rDNA-derived RNA (srRNA) (10), tRNA-derived small RNA (tsRNA), and small nuclear RNA (snRNA), which is also commonly known as U-RNA. According to previous RNA-sequencing data, the small RNA population in mature mouse sperm is dominated by tRNA-derived small RNAs (tsRNAs), a smaller population of microRNAs (miRNAs) and PIWI-interacting RNAs, and an appreciable amount of ribosomal RNA (rRNA)-derived small RNAs (rsRNAs) (14). Many related mammalian studies have shown that sncRNAs may be involved in the intergenerational inheritance of environment-induced phenotypes. In a paternal mouse model given a high-fat diet, Chen et al. showed that a subset of sperm tsRNAs, mainly from 5′ transfer RNA halves and ranging in size from 30 to 34 nucleotides, exhibited changes in expression profiles (15). Furthermore, injecting sncRNAs from obese males' sperm into healthy fertilized embryos can induce offspring to fully or partially mimic paternal phenotypes, including behavioral changes, obesity and glucose metabolism disorder (15–17). In addition, there is now compelling evidence to support that these sncRNAs, especially microRNAs, are known to interact with gene expression, chromatin remodeling and genome protection against transposition during spermatogenesis and seem to affect male fertility (18).

This study focused on whether physical exercise could effectively ameliorate high-fat diet-induced abnormalities in male fertility, investigated sncRNAs in spermatogenesis in obese mice from a new perspective, and identified certain sncRNAs as biologically active molecules that suggest male fertility.

Materials and methods

Animals and intervention

All mice were obtained from Shanghai Model Organisms, Shanghai, China. The animal ethics committee of Shanghai Model Organisms approved all experiments (Protocol Number: IACUC 2019-0016). All mice had free access to water and food and were maintained at the Shanghai Model Organisms animal house at 24 °C on a 12-h light, 12-h dark illumination cycle. Four-week-old C57BL/6 male mice were allocated into three groups. Group 1 (control, n = 5) received a control diet containing 4.3% fat, 19.2% protein and 67.3% carbohydrate (D12450B). Groups 2 (obesity, n=6) and 3 (exercise, n=5) received a high-fat diet providing 60% fat, 20% protein and 20% carbohydrate (D12492). Males were housed two or three per cage and maintained on these diet for 10 weeks until obesity model completion. The diet of Group 3 was then replaced with a control diet, and a running wheel with a lap counter was placed in the breeding cage. Males were housed two or three per cage and kept on these diet for 30 weeks until intervention completion (Figure 1A). Subsequently, each male was caged with four 7-week-old female rats for one week, and the vaginal plug was checked every day during this period. If the vaginal plug was found, mating success was confirmed, and the plugged female was moved to an individual cage. After 12 days, pregnancy success was confirmed. Littler size was counted on the delivery day. The reproduction experiment was carried out at 20 weeks and 30 weeks of physical exercise intervention. All experiments were performed with the evaluator blinded to the diet group and were performed by the same evaluator throughout the study.

Body weight and body component measurements

Body weight for individual males was measured weekly. At the completion of intervention, body components were measured by the Body Composition Analyzer (QMR12-060H-I, Santan, Shanghai, China). Then, the mice were anesthetized with 1% pentobarbital sodium (50mg/kg body weight, intraperitoneal injection) for imaging with a magnetic resonance imager (NM21-060H-I, Santan, Shanghai, China). Then, the testes, seminal vesicles and gonadal white adipose tissue of the mice were separated and weighed.

Serum hormones and lipids analyses

The mice were anesthetized, and their eyeballs were taken for blood. The serum was obtained by centrifugation at 1000 × g for 15 min after agglutination at room temperature for 1 hour. Testosterone and estradiol were measured by a mouse testosterone ELISA kit and a mouse estradiol ELISA kit according to the manufacturer's instructions (Hu21207, Hu20201, BioTSZ, US). Serum cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and non-esterified fatty acids were measured by an automatic biochemical analyzer (Toshiba120, Japan).

Glucose tolerance test and insulin tolerance test

For GTT, mice were fasted for 16 h (1700 to 0900 h) with free access to drinking water. A baseline blood sample was collected from the tails of fully conscious mice, followed by an intraperitoneal injection of glucose (0.75 g glucose/kg body weight), and blood was taken from the tails for glucose measurements at 0, 15, 30, 60, and 120 min. For insulin tolerance tests, mice were fasted for 6 h (0900 to 1500 h), and baseline blood samples were collected from the tails of fully conscious mice. Insulin (1 unit/kg body weight) (Humulin; Eli Lilly and Company, Indianapolis, IN, US) was administered by intraperitoneal injection, and blood samples were taken from the tail at 0, 30, 60, 90 and 120 min postinjection. Blood was collected from the retroorbital sinus after an overnight fast (16 h) to measure plasma insulin levels using a mouse ELISA kit (Crystal Chem Inc.).

CASA for semen analysis

Computer-aided semen analysis (CASA) was performed using the Hamilton-Thorne Research IVOS sperm motility analysis system and the previously described version 10 software (Hamilton-Thorne, Danvers, MA) (19). CASA was performed on the epididymal semen sample placed in a Leja standard counting fixed cover glass slide (Leja Products B.V., Nieuw-Vennep, The Netherlands) with a depth of 20 microns. Using Olympus "negative phase" optics, the analysis is limited to 15-100 tracking points at a 60 Hz frame rate. The standard kinematics are calculated by the CASA program. Cells exhibiting an average path velocity of less than 10 μm·s-1 are considered nonmoving.

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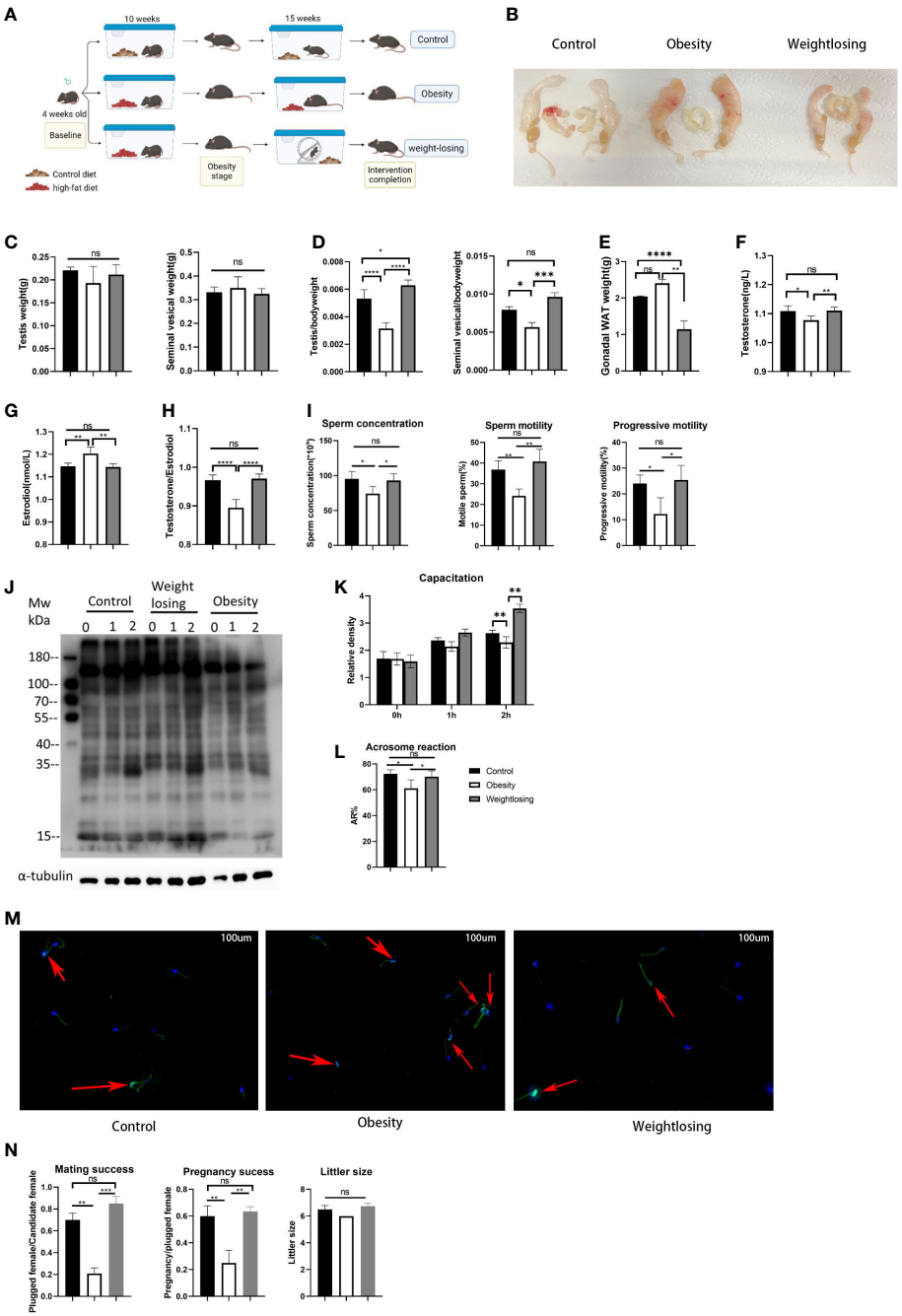

FIGURE 1
Effects of obesity and physical exercise intervention on reproductive morphology, reproductive hormones, sperm function and reproductive success. **(A)**, Schematic diagram of mouse breeding and intervention. **(B)**, Morphology of the mouse reproductive system. **(C)**, Testis weight (left) and testis index = testis weight/body weight (right). **(D)**, Seminal vesicle weight (left) and seminal vesicle index (right). **(E)**, Gonadal white adipose tissue weight. **(F)**, Serum testosterone (ng/L) levels. **(G)**, Serum estradiol (nm/L) levels. **(H)**, T/E2 and gonadal white adipose tissue weight (below). **(I)**, Analysis of semen traits include sperm concentration, motility and progressive motility. **(J)**, Western blot of sperm protein tyrosine phosphorylation to evaluate capacitation and its density. **(K)**, The relative density of the Western blot (p-tyrosine/α-tubulin). **(L)**, The proportion of sperm without intact acrosomes in the three groups. **(M)**. FITC-PNA was used to show the sperm acrosome and the sperm flagellum sheath. The red arrows indicate sperm with intact acrosomes that have not yet undergone acrosome reactions in the three groups. **(N)**. Mating success rate (left), pregnancy success (middle), litter size (right). One-way ANOVA was used to compare the differences among the three groups. Control: n=5, obesity: n=6, exercise: n=5. ns, $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$. Data are expressed as the mean ± SEM.

Western blot of protein tyrosine phosphorylation

After male mice were anesthetized, their epididymal tails were removed, cut into pieces with ophthalmic scissors, and placed in PBS. This liquid was placed in a 37°C incubator for 10 min for the spermatozoa to swim up. Using a large pipette, the liquid containing the spermatozoa was aspirated and placed in a centrifuge tube. After centrifugation at 1000 × g for 1 minute, the supernatant was aspirated, an appropriate amount of PBS was added to mix, and the sample was centrifuged again at the same speed and time as before. Washed spermatozoa were diluted with 1 ml of BWW (Solarbio, G2585) and quickly divided into three equal parts. One part was quickly added to the protein denaturation solution (Tris-HCl 0.0625 M, SDS 2%, glycerin 10%, β-mercaptoethanol 5%) and heated for denaturation, and the other two parts were incubated at 37°C for 60 min and 120 min, respectively, to achieve sperm capacitation. After incubation, the other two parts were centrifuged as before, and the upper layer was aspirated. Then, protein denaturation solution was added and heated to denature. Then, the samples were separated by SDS-PAGE and transferred to a PVDF (polyvinylidene fluoride) membrane. Next, the membranes were blocked with 5% skimmed milk powder and separately probed with rabbit anti Phospho-Tyrosine (8954, CST, USA) and rabbit anti α-tubulin (10094-1-AP, Proteintech, Wuhan, China) overnight at 4°C with a final dilution of 1:1000 in 5% milk. After three washes, the membranes were incubated with goat anti-rabbit secondary antibodies (SA00001-2, Proteintech, Wuhan, China) at a 1:2000 dilution at 37°C for 1.5 h. Images of the membranes treated with ECL (enhanced chemiluminescence) were captured by a Western Blotting Detection System (Tiangen, Beijing, China).

Acrosome reaction by immunofluorescence

After the male mice were anesthetized, their epididymal tails were removed, cut into pieces with ophthalmic scissors, and placed in PBS. The liquid was placed in a 37°C incubator for 10 min for the spermatozoa to swim up. Using a large pipette, the liquid containing the spermatozoa was aspirated and placed in a centrifuge tube. After centrifugation at 1000 × g for 1 minute, the supernatant was aspirated, an appropriate amount of PBS was added to mix and wash the spermatozoa, and the samples were centrifuged again at the same speed and time as before. Washed spermatozoa were diluted with PBS to adjust the final sperm concentration to 1×10^4 spermatozoa/ml. Add 20 μl of the diluted liquid to each smear so that the number of sperm on each smear is 200. Then, the cells were fixed with 4% PFA and stained with 0.5 mM PNA-FITC (L7381, Sigma-Aldrich) and DAPI (D9542, Sigma-Aldrich) as previously described. The stained spermatozoa were then covered with a cover slip (24 × 50 mm) (20).

Acquisition of spermatids at different stages

As in previous research (21), the testes of the anesthetized mice were collected in PBS and placed on ice. After removing the tunica albuginea, the testis was placed in 5 mL of PBS containing 120 U/mL type I collagenase preheated at 37°C and gently stirred for 5 minutes. The dispersed seminiferous tubules were further digested with 5 mL 0.25% trypsin and 0.1 mL DNase I (5 mg/mL), gently pipetted several times at 37°C for 8 minutes, and then stopped by adding 0.5 mL fetal bovine serum (FBS) to inactivate trypsin. After the two-step enzyme digestion, the separated testicular cell suspension was filtered through a PBS prewet cell filter with a pore size of 70 μm. The cell suspension was centrifuged at 500 × g for 5 minutes at 4°C, and the supernatant was carefully removed from the pellet. The cells in the pellet were resuspended in DMEM containing Hoechst 33342 (3 mg/mL) and 5 μl DNase I at a concentration of 1×10^6 cells/mL. The cell suspension was rotated at 10 r.p.m./min for 20 minutes at room temperature, centrifuged at 500×g for 5 minutes at 4°C, and resuspended in 0.3-1 mL DMEM for sorting. The cell population was collected based on the fluorescent label stained with Hoechst 33342 by fluorescence-activated cell sorting (FACS).

Small RNA-seq

The RNAs of round spermatids and spermatozoa were extracted using the miRNeasy Serum/Plasma Kit (217184, Qiagen, Germany) according to the manufacturer's protocol. The quantity and quality of RNA were assessed using a spectrophotometer (Nanodrop 2000, Thermo).

In this experiment, the single-end 50 bp sequencing mode of the Illumina HiSeq platform was used for high-throughput sequencing of samples. The original data were removed by primers and adaptor sequences, and the sequenced fragments with reliable quality were selected after inspecting sequence quality and length. Then, the type (unique) and quantity (total) of small RNA (sRNA) were counted, and length distribution analysis of small RNA was performed. We used fastx_Clipper to remove the splice sequence from the original data, filter out the low-quality sequence and select the sequence with a length of 14-40 nt for downstream analysis. Fastqc was used to evaluate the quality of the sequence, including mass density distribution, length distribution, redundancy statistics, etc. For all sequences after pretreatment and for the unique sequence after the duplication of each sample, Bowtie software was used for comparison with the reference genome, Rfam sequence database, RepBase sequence database and miRBase database of the species. The sequence alignment wass set to allow only one mismatch, and the comparison were statistically analyzed. The reads of each sample were compared to the existing miRNA

database (miRBase) and the results of the new miRNA prediction to calculate miRNA expression (miRNA expression calculation uses CPM, counts per million). The meaning of CPM is to use the paired sequence per million ratio as an indicator of miRNA expression, where the total ratio of paired reads is used to normalize the expression value. DESeq software was used to analyze the differential expression of the Control and Case sample groups, screen the differentially expressed miRNAs, calculate the expression level of each sample and the intragroup mean, calculate the fold change difference between the groups, and then calculate log2(fold change) for subsequent use. Screening for differentially expressed genes, when p value <= 0.05 and log2(fold change)> = 1, we believe that such genes are significantly different between groups. The miRanda database was used to perform target prediction on differentially expressed miRNAs.

GO annotation and KEGG pathway enrichment analysis

Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were conducted to investigate the roles of target genes of consistently differentially expressed miRNAs. GO analysis was performed to assess the biological implications of the target genes (22). We downloaded the GO annotations from NCBI (http://www.ncbi.nlm.nih.gov/), UniProt (http://www.UniProt.org/), and GO (http://www.geneontology.org/). Pathway analysis was performed to explore the significant pathways of target genes based on the KEGG database (23). The significant GO categories and KEGG terms were identified using Fisher's exact test. The threshold of significance was defined by a P value < 0.01 for GO analysis and a P value < 0.05 for KEGG analysis. The GO-Tree is a directed acyclic graph, and each term has defined relationships to one or more terms (24). The mutual regulation relationships between enriched KEGG pathways were illustrated by pathway-act networks (25).

Real-time quantitative PCR

First-strand cDNAs of small RNAs from round spermatids and spermatozoa were synthesized by a Bulge-Loop miRNA qRT-PCR Starter Kit (C10211, RIBOBIO, Guangzhou, China) according to the manufacturer's instructions. qPCR was carried out by using SYBR Green PCR Master Mix (TaKaRa, Dalian, China) and a LightCycler 480II Real-Time PCR System. The forward primers of small RNAs and universal reverse primers were designed based on the mature sequences of small RNAs (Supplementary Table 1). The expression levels of small RNAs were normalized with 5S to obtain the relative expression by the comparative CT method.

Statistical analysis

We adopted α=0.05 (both sides) and β=0.20 as standard values, the proportion of Obesity Group and Control Group is 1:1 following the formula for comparison of two means to calculate the sample size (26). Normality of the data was tested using the Shapiro-Wilk normality test. Nonparametric data with multiple comparisons were analyzed by Kruskal-Wallis one-way analysis of variance followed by Holm's Stepdown Bonferroni and non-paired analysis procedure for adjusted p-values. Data with normal distribution were analyzed by one-way ANOVA with Dunnett's post-test or Tukey's correction for multiple comparisons. Statistical analysis was conducted by GraphPad Prism V8.0 (GraphPad Software, San Diego, California, USA). All values are presented as the mean ± SD, and P values less than 0.05 (*), 0.01 (**), 0.001 (***), and 0.0001 (****) were considered significant differences.

Results

Effects of physical exercise on the reproductive system in obese mice

Male mice fed a high-fat diet for 10 weeks showed significant weight gain (obesity stage: control = 29.6 g vs. obesity = 36.6 g vs. exercise = 37.8 g, P<0.001), which means that the obese male mouse model was successfully constructed (Supplementary Figure 1A). After that, the exercise group began to perform physical exercise using an autonomous running wheel, and the obesity group continued to be fed a high-fat diet. At the end of 30 weeks of intervention, the weight of the mice in the exercise group was far lower than that of the obesity group and even significantly lower than the weight of the control group (control = 42.0 g vs. obesity = 61.0 g vs. exercise = 33.6 g, P<0.0001) (Supplementary Figure 1A).

When evaluating the effect of obesity and physical exercise intervention on reproductive organ morphology, we found that the testes and seminal vesicles of obese male mice were clearly reduced in volume relative to the overall reproductive system (Figure 1B). Then, we weighed the testes and seminal vesicles and found that the weights of the testes and seminal vesicles were not significantly different among the three groups (testis: control = 0.22 g vs. obesity = 0.19 g vs. exercise = 0.21 g, P=0.23; seminal vesicles: control = 0.33 g vs. obesity = 0.35 g vs. exercise = 0.32 g, P=0.87) (Figure 1C). However, when the weight of the testes and seminal vesicles was normalized for body weight, it was found that the relative weight of the testes or seminal vesicles was significantly reduced in the obesity group, and physical exercise intervention could restore it nearly to the control level (testis index: control = 0.5% vs. obesity = 0.3% vs. exercise = 0.6%, P<0.0001; seminal vesicles index: control = 0.8%

vs. obesity = 0.6% vs. exercise =. 1.0%, P<0.001) (Figure 1D). These results indicated that obesity or exercise did not change the morphology of the reproductive organs themselves but affected the relative proportion of reproductive organs in the body.

To evaluate the effects of obesity and physical exercise intervention on sex hormones, we measured the concentrations of testosterone and estradiol in peripheral blood serum. Obese male mice had significantly lower testosterone levels and higher estradiol levels than control mice, and physical exercise intervention could restore the serum reproductive hormones (testosterone: control = 1.11 vs. obesity = 1.08 vs. exercise = 1.11, P<0.01; estradiol: control = 1.15 vs. obesity = 1.20 vs. exercise = 1.14, P<0.001) (Figures 1F, G). The ratio of testosterone to estradiol represents aromatase activity; the lower the ratio is, the stronger the aromatase activity. Obesity significantly reduced this ratio in male mice, while physical exercise restored it to the control level (control = 0.97 vs. obesity = 0.90 vs. exercise = 0.97, P<0.0001) (Figure 1H). In addition, gonadal white adipose tissue is rich in aromatase P450, the weight of which also reflects the potential activity of aromatase in male mice. The weight of gonadal white adipose tissue was significantly increased in the obesity group compared with the control group but significantly decreased in the exercise group (control = 2.04 vs. obesity = 2.40 vs. exercise = 1.15, P<0.0001) (Figure 1E). Not surprisingly, physical exercise could significantly reduce gonadal white adipose tissue accumulation due to obesity. These results suggested that physical exercise could effectively restore the sex hormone disorder caused by obesity by reducing the stock of aromatase.

To evaluate the effects of obesity and physical exercise intervention on sperm function, we used CASA to analyze semen traits. The sperm concentration was significantly lower in the obesity group than in the control (almost 78% lower than the control), while physical exercise restored it to normal levels (control = $95.3*10^6$/ml vs. obesity = $74.1*10^6$/ml vs. exercise = $93.0*10^6$/ml, P=0.01). In addition, the obesity group demonstrated a notably lower percentage of motile spermatozoa than the control group, mainly due to the decrease in spermatozoa with progressive motility. The exercise group showed a similar concentration of motile and progressive motile spermatozoa as the control group (motility: control = 36.8% vs. obesity = 24.2% vs. exercise = 40.8%, P=0.001; progressive motility: control = 24.0% vs. obesity = 12.2%vs. exercise =25.4%, P=0.01), which indicated that physical exercise could increase sperm concentration and improve sperm motility in obese males back to normal levels (Figure 1I).

In mammalian species, the acquisition of sperm fertilization competence is dependent on sperm capacitation. One of the key elements of capacitation is protein tyrosine phosphorylation (TP) in various regions of the sperm membrane. To evaluate the capacitation of spermatozoa, we detected tyrosine phosphorylation by western blotting. Sperm from the tail of the epididymis of mice were incubated for 0, 1 and 2 hours in complete culture medium supporting capacitation. Then, the proteins in sperm were extracted immediately, separated by SDS-PAGE electrophoresis and incubated with tyrosine phosphorylated antibody. This experiment was carried out five times with similar results. The most representative figure is shown in Figure 1J; the other figures were detected density and are shown in Figure 1K. The process of sperm capacitation in obese male mice was slower, which was reflected in the lower tyrosine phosphorylation level at 1 hour of incubation than that in the control group. After incubation for 2 hours, tyrosine phosphorylation differed even more from that of the control group. However, the tyrosine phosphorylation of the exercise group was similar to that of the control group. This suggested that obesity could hinder sperm capacitation, while physical exercise in obese men could restore sperm capacitation.

To further evaluate the sperm acrosome reaction, we detected the retention of intact sperm acrosomes by immunofluorescence. The sperm were removed from the tail of the epididymis and washed twice with PBS. The washed sperm were diluted with PBS and fixed on the smear with paraformaldehyde so that there were approximately 200 sperm on each smear.The acrosome and flagellum sheath were labeled green, and the nucleus was stained blue under a fluorescence microscope after incubation with PNA-FITC and DAPI, respectively. Figure 1M shows the most representative figure. Compared with the control group, the sperm intact acrosome retention rate of obese male mice was higher, so the incidence of acrosome reaction was lower. The exercise group showed an intact acrosome retention rate and acrosome reaction similar to those of the control group (rate of acrosome reaction: control = 72.3% vs. obesity = 61.1% vs. exercise = 70.2%, P=0.01) (Figure 1L). This indicated that spermatozoa in obese male mice showed a delayed acrosome reaction, while physical exercise could restore it.

To evaluate the effects of obesity and physical exercise intervention on reproductive success, we mated each male mouse in the three groups with four 7-week-old female mice for one week at 20 and 30 weeks of intervention and checked vaginal plugs every day to determine mating success. For successfully mated females, pregnancy was confirmed at Day 12 after mating. Finally, the number of pups per litter was counted on the first day of delivery. The results showed a negative overall effect of obesity on mating and fertilization success, but physical exercise could still restore impaired reproductive success (Figure 1N).

To demonstrate whether the alteration of the reproductive system is associated with body composition and metabolism, we compared several parameters between the three groups. Through the analysis of the body composition of mice, it was found that the body fat ratio of obese mice increased significantly (obesity = 45.1% vs. control = 28.5%, P=0.001), mainly due to the accumulation of visceral fat. The red area that

represents fat was significantly concentrated in the central area of the internal organs in the MRI imaging; this area significantly decreased after physical exercise intervention (exercise = 13.4% vs. obesity = 45.1%) (Supplementary Figures 1B, C). In addition, we found that the obese mice were glucose intolerant and insulin resistant, showing raised blood glucose at fasting and during a glucose tolerance test (Supplementary Figure 1D) and elevated serum insulin at fasting (Supplementary Figure 1F). The insulin tolerance test response was blunted (Supplementary Figure 1E). As expected, mice that had experienced physical exercise showed improvement in glucose intolerance and insulin resistance in their GTT and ITT (Supplementary Figures 1D–F). Obese mice showed impaired lipid metabolism—specifically, elevated serum total cholesterol (Supplementary Figure 1G), triglycerides (Supplementary Figure 1H), low-density lipoprotein (Supplementary Figure 1J), and non-esterified fatty acids (Supplementary Figure 1K), except high-density lipoprotein (Supplementary Figure 1I). Physical exercise intervention effectively reduced the blood lipids of obese mice and returned them to control level (Supplementary Figures 1G–K).

sncRNA dynamics in mouse round spermatids and epididymal spermatozoa

To uncover the expression profiles of sncRNAs in mouse spermatogenic cells, we collected round spermatids (haploid, RS), spermatocytes (tetraploid), Sertoli cells and Leydig cells (diploid), and elongated spermatids (haploid, ES) from the testis by flow cytometry and epididymal spermatozoa (SP) by the swimming-up method. After setting a gate by forward scatter/side scatter characteristics and DAPI characteristics, round spermatids, elongated spermatids and spermatocytes were isolated, accounting for 18.6%, 1.3% and 6.2% of the testicular cells, respectively (Supplementary Figure 2A). To determine the purity of the isolated RSs, the expression of the RS marker *PRM1* (27) was examined by qPCR. Analysis showed that *PRM1* was significantly highly expressed in the isolated round spermatids (Supplementary Figure 2B). Then, total RNA was extracted from RS and SP, and small RNA-seq was conducted. The expression levels of sncRNAs in RS and SP were compared. The distributions of the total expression of sncRNAs between RS and SP were different, as illustrated by the barplot analysis (Figure 2A).

The majority of sncRNAs in RS were miRNAs and piRNAs, which accounted for 6.0% and 72.8% of the total sncRNAs, respectively (Figure 2A). However, in SP, the proportion of miRNA and piRNA decreased, accounting for 0.4% and 9.1%, respectively, and the proportion of tsRNA increased significantly, accounting for 85.5% (Figure 2F), indicating that the sncRNAs underwent dramatic changes during sperm maturation.

To further explore the function of sncRNAs in male fertility, we focused on the analysis of miRNA changes. First, we compared the expression of miRNAs in RS from the obesity and control groups. Systematic variations in the expression of miRNAs among samples were visualized by heatmap clustering analysis (Figure 2B). A scatterplot was used to evaluate the variation in miRNA expression between the obesity and control groups (Figure 2C). The threshold for differential expression was set to a P value < 0.05. In total, we identified 47 significantly differentially expressed miRNAs in RS: 17 were upregulated, while 30 were downregulated (Figure 2D). A volcano plot was constructed to identify significantly upregulated and downregulated miRNAs in the obesity RS group relative to the control group (Figure 2E). Then, we compared the expression of miRNAs in SP from the obesity and control groups. The heatmap clustering analysis showed the systematic variations in the expression of miRNAs among samples (Figure 2G). The scatterplot showed the variation in miRNA expression between the obesity and control groups (Figure 2H). In total, we identified 30 significantly differentially expressed miRNAs in SP: 14 were upregulated, while 16 were downregulated (Figure 2I). A volcano plot was constructed to identify significantly upregulated and downregulated miRNAs in the obesity SP group relative to the control group (Figure 2J). The data suggest that the expression of miRNAs in RS and SP cells from the obesity group differed from controls.

GO analysis and KEGG pathway analysis of target genes of consistently differentially expressed miRNAs revealed significantly enriched biological process GO terms and signaling pathways

According to the above analysis, we found that there were 3 miRNAs (miR-6538, miR-129-1, and miR-7b) that were consistently upregulated in RS and SP. Five miRNAs (miR-196a-1, miR-872, miR-21a, miR-143, and miR-200a) were consistently downregulated in the RS and SP groups compared with the obesity and control groups. MicroRNAs have been extensively reported to execute their functions by binding to their targets. miRNA targets were predicted by miRanda and were considered potential miRNA targets.

To elicit the biological implications of consistently differentially expressed miRNAs in the etiology of impaired male fertility, we conducted GO functional annotation for the target genes of differentially expressed miRNAs. For BPs, the top five enriched GO terms associated with upregulated mRNAs were covalent chromatin modification, muscle tissue development, histone modification, axonogenesis, and striated muscle tissue development (Figure 3A). The 3 consistently upregulated genes were enriched in GO terms related to chromatin modification, neuromuscular development, and reproductive system development (Figure 3B). The top five enriched GO terms associated with downregulated miRNAs

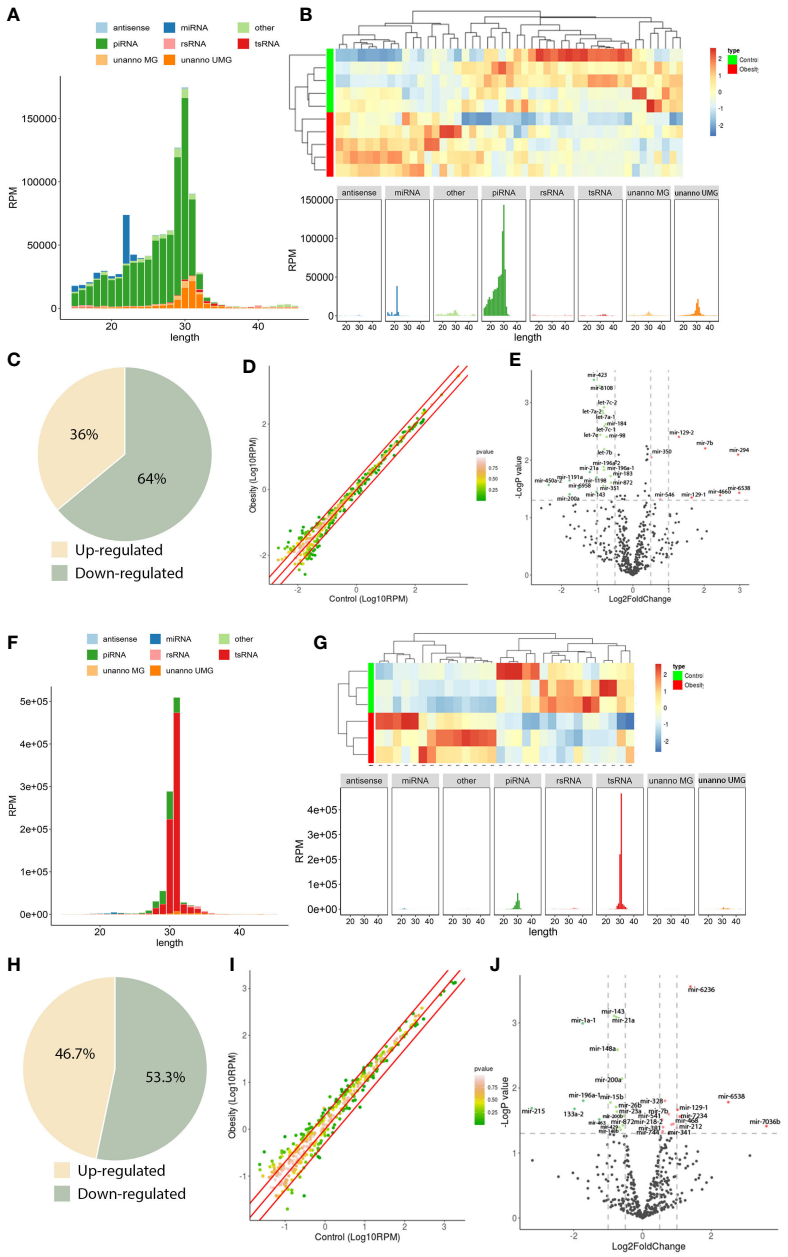

FIGURE 2
sncRNA dynamics in mouse round spermatids and epididymal spermatozoa. **(A)**, sncRNA length distribution in round spermatids. **(B)**, Heatmap of significantly differentially expressed miRNAs in round spermatids. Upregulated miRNAs are marked red, and downregulated miRNAs are marked blue. **(C)**, Scatterplot of miRNA expression variation between the obesity and control groups in round spermatids. The normalized expression values of each gene are shown on the x-axis and the y-axis. The miRNAs above the top red line or below the bottom red line had more than a twofold difference in expression between the two groups. **(D)**, The expression of a cohort of miRNAs in round spermatids from the obesity group differed from that in the control group. Of the 47 differentially expressed miRNAs, 17 were upregulated, and 30 were downregulated. **(E)**, Volcano plots of miRNA expression variation between the two groups in round spermatids. Red and green dots indicate significantly upregulated and downregulated miRNAs in the obesity group, respectively ($P < 0.01$). Gray dots represent nondifferentially expressed genes between the two groups. **(F)**, Length distribution of sncRNAs in epididymal spermatozoa. **(G)**, Heatmap of significantly differentially expressed miRNAs in epididymal spermatozoa. Upregulated miRNAs are marked red, and downregulated miRNAs are marked blue. **(H)**, Scatterplot of miRNA expression variation in epididymal spermatozoa between the obesity and control groups. The normalized expression values of each gene are shown on the x-axis and the y-axis. The miRNAs above the top red line or below the bottom red line had more than a twofold difference in expression between the two groups. **(I)**, The expression of a cohort of miRNAs in epididymal spermatozoa from the obesity group differed from that in the control group. Of the 30 differentially expressed miRNAs, 14 were upregulated, and 16 were downregulated. **(J)**, Volcano plots of miRNA expression variation between the two groups in epididymal spermatozoa. Red and green dots indicate significantly upregulated and downregulated miRNAs in the obesity group, respectively ($P < 0.01$). Gray dots represent nondifferentially expressed genes between the two groups.

included anoxogenesis, synapse organization, positive regulation of cell projection organization, regulation of neurogenesis, and forebrain development (Figure 3C). The 5 consistently downregulated genes were enriched in GO terms related to nervous system development (Figure 3D).

To further understand the core BP associated with male fertility and offspring development, GO-Tree analysis of upregulated and downregulated BP GO terms was performed based on their subordinate and interaction relationships (Figure 3E). The network indicates that BPs, including reproductive system development and reproductive structure development, may play a key role in paired male fertility. These BPs, such as central nervous system neuron differentiation, neuron migration, pallium development, cerebral cortex development and forebrain development, may play an important role in the development of the nervous system in offspring. BPs, such as covalent chromatin modification, histone modification, and peptidyl-lysine modification, may be involved in epigenetic inheritance across generations.

To indicate the signaling pathways associated with target genes of consistently differentially expressed miRNAs, KEGG pathway analysis of target genes was performed. The top five enriched pathways associated with upregulated miRNAs were human papillomavirus infection, focal adhesion, mTOR signaling pathway, axon guidance, and Wnt signaling pathway (Figure 3F). For downregulated miRNAs, the top five enriched pathways were the PI3K-Akt signaling pathway, MAPK signaling pathway, chemical carcinogenesis-receptor activation, tight junction, and dopaminergic synapse (Figure 3G).

A pathway-act network was explored to further investigate the mutual interactions of pathways and to obtain the hub pathways that may play a vital role in male fertility and offspring development (Figure 3H). The relaxin signaling pathway, FoxO signaling pathway, ErbB signaling pathway, longevity regulating pathway, and PI3K-Akt signaling pathway were the top five pathways showing the most interactions with other surrounding pathways. The relaxin signaling pathway is closely related to the reproductive system. The FoxO signaling pathway is involved in glucose metabolism. The longevity-regulating pathway includes the insulin signaling pathway, mTOR pathway, and AMPK signaling pathway coregulating glucose metabolism.

The consistently differentially expressed miRNAs and P values were further verified by quantitative real-time PCR. The results indicated that mir-6538, mir-129-1, and mir-7b were upregulated (Figure 3I) and that mir-196a-1, mir-872, mir-21a, mir-143, and mir-200a were decreased (Figure 3J) in the obesity and control groups in RS and SP. These results are consistent with the RNA-Seq profile data. In addition, physical exercise of obese mice could restore these changes induced by a high-fat diet in RS and SP (Figures 3I, J).

Discussion

Our research found strong, consistent evidence that a high-fat diet is detrimental for male fertility. Compared with controls, obese males fed a high-fat diet exhibit adverse changes in their reproductive system, including reduced testes and seminal vesicles relative to body size, and they produce fewer sperm and a lower proportion of motile sperm. In addition, obese males present reproductive hormone disorders. More importantly, males fed a high-fat diet have impaired sperm function and reduced reproductive success. Accordingly, physical exercise could partially or even completely offset the adverse effects of obesity, but it needs to have the prerequisites of body weight and body fat rate significantly below the normal level. It is a serious issue which is worth discussing to explore its reason, and among these, the changes of microRNA in round spermatids and spermatozoa may explain the effects of obesity and physical exercise on sperm function.

Previous human studies have little evidence supporting that increased BMI is related to reduced standard semen assessment traits (5, 6, 28), and yet our present results demonstrated that all measures of sperm quantity and quality are negatively impacted when obesity is experimentally induced with a high-fat diet, which was consistent with a high-quality meta-analysis including 52 animal studies (29). Moreover, the magnitude of the negative effects of a high-fat diet on sperm traits was increased when the difference in body mass of control and treatment animals was higher (29). Based on that, the outcome of male mating and pregnancy could be consequently affected by a high-fat diet; this is corroborated by our reproductive experiment, and importantly, the damage rates of mating and pregnancy were more likely to be amplified by long-term high-fat diet treatment. In addition to impaired sperm quantity and quality, male behavior, lack of libido or inability to mount might partly explain the reduced mating success. Abnormal male behavior might be attributed to reproductive hormone disorder, reduced testosterone and elevated estradiol. Apart from the influence of behavioral factors on pregnancy, how can the influence of obesity on reduced fertilization success be considered? The answer can be found in the IVF population. Several population studies have shown that the clinical pregnancy rate of IVF in couples with obese husbands is lower, which may be related to the sperm itself, such as delayed capacitation and acrosome reaction, rather than the number or concentration of spermatozoa (30). The higher proportion of obese men in infertile couples who choose intracytoplasmic sperm injection (ICSI) can also explain this finding because ICSI can prevent clinical pregnancy failure caused by insufficient sperm quantity, poor motility and delayed capacitation and acrosome reaction in IVF.

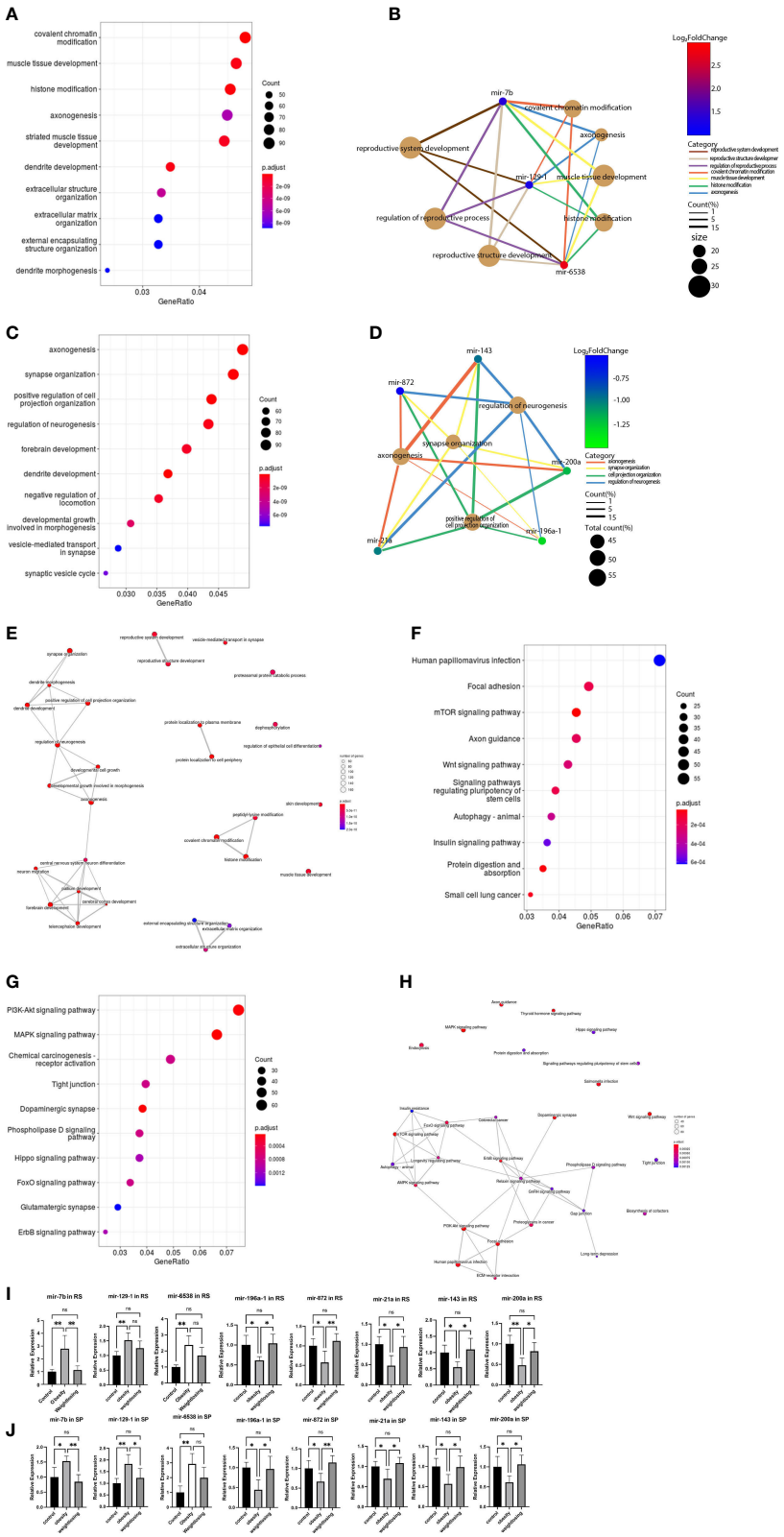

FIGURE 3 (Continued)

FIGURE 3 (Continued)
GO analysis and KEGG pathway analysis of target genes of consistently differentially expressed miRNAs revealed significantly enriched biological process GO terms and signaling pathways. **(A)**, The top 10 significantly enriched GO terms in the biological process (BP) category associated with target genes of 3 consistently upregulated miRNAs. **(B)**, Gene-concept network for the 3 consistently upregulated miRNAs with the associated top 4 and reproductive system-enriched BP terms as a net. **(C)**, The top 10 significantly enriched BP GO terms associated with target genes of 5 consistently downregulated miRNAs. **(D)**, Gene-concept network for the 5 consistently downregulated miRNAs with the related top 4 enriched BP terms as a net. **(E)**, GO-Tree network analysis based on the interaction relationship of enriched BP terms. **(F)**, The top 10 enriched signaling pathways associated with target genes of 3 consistently upregulated miRNAs. **(G)**, The top 10 enriched signaling pathways associated with 5 consistently downregulated miRNAs. **(H)**, Pathway-act network analysis illustrated mutual interactions between pathway terms. **(I)**, The relative expression levels of 3 consistently upregulated miRNAs and 5 downregulated miRNAs in round spermatids were validated by real-time PCR. **(J)**, The relative expression levels of 3 consistently upregulated miRNAs and 5 downregulated miRNAs in epididymal spermatozoa were validated by real-time PCR. One-way ANOVA was used to compare the differences among the three groups. Control: n=5, obesity: n=5, exercise: n=5. ns: no significant, p value > 0.05, *: $P < 0.05$, **: $P < 0.01$. Data are expressed as the mean $\pm$ SD.

Do epigenetic changes or oxidative stress damage in spermatozoa caused by obesity affect clinical pregnancy? A study showed that among couples undergoing intracytoplasmic sperm injection, the odds of live births for couples with obese male partners were 84% lower than the odds in couples with men with normal BMI (31). Another study also demonstrated that overweight men with nonobstructive azoospermia have worse pregnancy outcomes after testicular sperm aspiration (TESA) and ICSI (32). Data from investigating sperm epigenetic molecular status and embryo morphokinetics show the negative impacts of obesity on motile spermatozoa molecular composition and the possible risk of disturbing early embryonic cell cycle kinetics in the context of paternal obesity (33). Even so, there is conflicting evidence. A retrospective study supported no evidence for a relationship between male BMI and treatment outcomes of ICSI (34). In patients with nonobstructive azoospermia who underwent TESA-ICSI, a Chinese study showed that embryo quality and clinical outcomes were not influenced by high BMI levels (35). Therefore, this question warrants further investigation. Significantly, in our present study, it is clear that obesity causes a decreased pregnancy success rate in mice, which is effectively improved by physical exercise.

The next concept is whether a high-fat diet could affect spermatozoa by activating small RNA molecules. There is a widely accepted view that miRNAs may act as fine-tuners of large gene networks (36–38). miRNAs ensure accurate gene expression in the adult testis by keeping levels within the required thresholds, thus playing a crucial role in testis homeostasis (39). In our present study, we used small RNA-Seq data to reveal that the round spermatids and epididymal spermatozoa expression patterns of miRNAs are significantly altered in the obesity group compared with the controls. For the first time, we found several miRNAs that were consistently differentially expressed both in RS and SP between the two groups. The target genes regulated by these miRNAs were found to be involved in metabolic regulation, nervous system development regulation and chromosome modification according to GO and KEGG analyses. Why were these miRNAs consistently differentially expressed? miRNAs are involved in the regulation of gene expression during the postmeiotic stages of spermatogenesis. Specifically, miRNAs can repress the transcription of transition protein 2 (40), a marker for round spermatids, suggesting that miRNA functions affect postmeiotic germ cells. Translin (also known as testis-brain RNA-binding protein) has been demonstrated to bind to miRNAs and thus increase the *in vivo* stability of miRNAs (28). Furthermore, elongated spermatids exhibit abnormal morphology and motility, and consequently, male infertility occurs in Dicer1-knockout mice, indicating that both Dicer1 and miRNAs play crucial roles in proper differentiation during spermatogenesis. Rao et al. (29) demonstrated that the Wilms' tumor 1 (WT1) transcription factor, which could be repressed by miRNAs, plays an essential role in the control of germ cell survival and spermatogenesis. WT1 knockdown mice suffered from increased germ cell apoptosis, a loss of the adherent junction complex between germ cells and Sertoli cells, and impaired fertility. In addition, prevailing data demonstrate that proteins and miRNAs in the epididymal fluid associated with post testicular sperm maturation are transferred to the sperm by EVs (30). These differentially expressed miRNAs due to the high-fat diet treatment may be contained in extracellular vesicles and transferred to mature sperm through the Sertoli cells of the testis and epididymal fluid. Taken together, these studies further indicate that normal miRNA biogenesis is required for round spermatids to become epididymal spermatozoa.

Interestingly, in our present research, some altered miRNAs induced by high-fat diet are recovered *via* physical exercise including 3 consistently upregulated miRNAs (miR-6538, miR-129-1, and miR-7b) and 5 consistently downregulated (miR-143, miR-872, miR-21a, miR-196a-1, and miR-200a), revealing that physical exercise could participate remodeling of reproductive system development and regulation of reproductive processes. A previous study showed that Ppp2r2b, Rgs8 and Atp1b2, as the target genes of mir-7b, showed a complex interaction within a biological process leading to sperm dysfunction upon exposure to fluorosis (41). MiR-7b interacts with the 3' untranslated region of the

immediate-early gene Fos mRNA and inhibits Fos translation. It shows rapid induction and dimerization to form transcription factor activator protein 1 (AP-1) after stress stimulation, such as diet alteration, which is known as a pivotal regulator of major biological events, such as cell proliferation, differentiation, organogenesis, memory formation and apoptosis, in germ cells to regulate fertility (31). Increased miR-129 was previously proven to significantly reduce the expression of the mitochondrial Cox family, together with that of MyHC I, and knockdown of miR-129 conversely increased the expression of cox genes and MyHC I. Mature spermatozoa are rich in mitochondria, especially in the tail sheath, and the inhibition of mitochondrial genes by the mir-129 family significantly affects sperm motility (32). Additionally, it is important to note that the altered expression profiling of miRNAs in round spermatids and epididymal spermatozoa induced by a high-fat diet in our present data could affect the adult health condition in offspring *via* patterns that are not yet clear. It is well known that the nutritional status of the father affects the development of the offspring. In rodent studies, increased anxiety-like behavior was documented in offspring sired by males that consumed a high-fat diet (33). In humans, paternal BMI was found to be inversely associated with child IQ (34). It is certain that physical exercise to lose weight can partly counteract the effect of an obese father on the metabolic abnormalities of the offspring, mainly through increasing the absorption and utilization of glucose by skeletal muscle in the offspring (35, 36). These results are consistent with our findings that these altered miRNA profiles are involved in metabolic regulation, nervous system development regulation and chromosome modification according to GO and KEGG analyses, suggesting their potential additional adverse effects and involvement in the intergenerational transmission of obesity through gametes.

Epigenetic modifications, including functional sncRNAs in spermatogenesis, are directly involved in the etiopathology of adult diseases transmitted by sperm. To determine whether sperm ncRNA is the determinant of intergenerational heredity, Grandjean et al. microinjected ncRNA of obese mice spermatozoa into fertilized eggs from normal parents. It was found that the offspring had more weight gain and impaired glucose tolerance and insulin resistance in adulthood, although they kept a normal diet (37). This is consistent with the results of Fullston et al. (38). Chen et al. microinjected tRFs (tRNA-derived fragment, one of sncRNAs) extracted from the spermatozoa of obese male mice into normal fertilized eggs fed a normal diet and found that in adulthood, they showed impaired glucose tolerance but no insulin resistance (15). The above evidence supported that sncRNAs can undertake part of the transmission from father to offspring. Our present findings reveal the upregulated levels of miR-143, miR-872, miR-200a, miR-21 and miR-196a in obese mice rescued by physical exercise implying that physical exercise can restore the possibility of disease transmission from father to offspring. It is well known that miR-143 can control Notch receptor expression by binding and releasing lncRNAs. In addition, overexpression of miR-143 can also reduce cell proliferation and induce neuronal differentiation. MiR-143-mediated lncRNA function in the developing mouse cortex leads to an expansion of PAX6+ RGCs. In addition, miR-872 can directly target the protein SOD-1, a copper/zinc superoxide dismutase, and its overexpression in neurons is associated with increased cell death through apoptosis (39). mir-200a regulates neural induction by targeting zinc finger E-box-binding homeobox (ZEB) transcription factors, allowing embryonic stem cells to differentiate into neuroectodermal precursors rather than epidermal cells (41). Inducing the expression of mir-21 can improve cognitive function in patients with Alzheimer's disease, thus reflecting its active participation in neuromodulation processes, integrating cognitive signaling pathways, and strongly affecting cognitive development (42). miR-196a can directly interact with the IκBα 3'-UTR to inhibit IκBα expression and subsequently promote the activation of NF-κB, thereby promoting neuronal proliferation and inhibiting apoptosis *in vivo* and *in vitro*, thereby regulating neural development (43). These findings support that miRNAs contribute to the expansion of the cerebral cortex and the development of the nervous system (44), and it is reasonable to speculate that these functional microRNAs affect the nervous system development of offspring across generations to offspring through sperm according to our present study.

In this study, we also found that Sin3a and Rnf40, which are targeted by mir-7b, participate in the regulation of histone modification. MiR-129-5p can regulate HDACi, which is responsible for histone deacetylase inhibitors (45). In addition, Per1 is targeted by miR-6538, and its expression can be regulated by HDACi (46). Therefore, these microRNAs coordinate histone modifications. In mammalian sperm, the majority of nucleosomes are replaced with protamines to facilitate the compaction of the paternal genome (47). Nevertheless, a small percentage of nucleosomes and their associated histone modifications are retained, thereby forming a potential platform for the intergenerational transmission of regulatory states (48, 49). Both active (H3K4me2 and H3K4me3) and repressive (H3K27me3, H3K9me3 and H4K20me3) histone modifications have been detected in mammalian sperm. For example, in humans, H3K9me3-marked and H4K20me3-marked nucleosomes are transmitted through the sperm into the zygote, where they participate in the build-up of constitutive heterochromatin (50). Notably, these changes promoted developmental defects in the offspring and were transmitted across three generations.

In summary, this study clearly shows that obesity due to paternal diet can cause significant impairment of many sperm function parameters, including decreased motility and delayed capacitation and acrosome reaction. When the weight and body fat rate after physical exercise are lower than normal, the adverse effects of obesity could be offset. Small noncoding RNAs in spermatogenic cells may play an important role in the effects of

obesity and physical exercise on male fertility and offspring development. Our findings provide new evidence of the relationship between obesity, physical exercise, male infertility and offspring development.

Data availability statement

The original contributions presented in the study are publicly available. This data can be found here: https://www.ncbi.nlm.nih.gov/sra/PRJNA904533.

Ethics statement

The animal study was reviewed and approved by the animal ethics committee of Shanghai Model Organisms.

Author contributions

HH, XL devised the study concept and design. TL and SZ drafted the manuscript, completed the experiment, and collected data. YZ provided support in experimental technology. LW was responsible for the statistical analysis and quality control. All authors agreed with the final version of the article.

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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/fendo.2022.1038449/full#supplementary-material

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Association between metabolic scores for visceral fat and chronic kidney disease: A cross-sectional study

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Introduction: Central obesity is closely linked to the risk of chronic kidney disease (CKD). This study aimed to evaluate the association between the novel central obesity index- metabolic score for visceral fat (METS-VF) and the risk of CKD in a Chinese population, and to compare its ability to predict CKD with other central obesity indices including waist circumference (WC), waist-to-height ratio (WHtR), lipid accumulation product (LAP), visceral adiposity index (VAI), a body shape index (ABSI), body roundness index (BRI), and cardiometabolic index (CMI).

Methods: This cross-sectional study included 8866 individuals from China. Demographic information, lifestyle data, and medical history data were collected, and physical examinations, anthropometric measurements and laboratory tests were performed for each participant. CKD was defined as an estimated GFR< 60 ml/min/1.73m^2. Multivariate logistic regression models were used to evaluate the association between the METS-VF and the prevalence of CKD. Receiver operating characteristic (ROC) analyses were performed to assess and compare the predictive abilities of the central obesity indices and determine the optimal cut-off points.

Results: A graded increase in the prevalence of CKD was observed with increasing METS-VF tertiles. Moreover, the METS-VF was independently associated with the risk of CKD after adjustment for current smoking, current drinking, physical activity level, diabetes, hypertension, CVD history and BMI. Compared with participants with a METS-VF in the lowest tertile, the multivariate-adjusted ORs and 95% CIs for participants with a METS-VF in the highest tertile were 3.943 (2.435-6.385) in the overall population, 3.585 (1.585-8.109) for men and 4.627 (2.485-8.616) for women. Significant interactions were found between the METS-VF and the risk of CKD by age (P value for interaction = 0.023). In ROC analysis, the METS-VF had a higher AUC value than other indices for predicting CKD in men and had comparable or higher AUC than other indices for women. For predicting CKD, the optimal cut-off value of

the METS-VF was 6.891 for men and 6.744 for women. The METS-VF yielded the greatest Youden index among all indices for both sexes.

Conclusion: A higher METS-VF was independently associated with a greater risk of CKD. The METS-VF can be a useful clinical indicator for identifying CKD, as it had superior predictive power for CKD when compared with other central obesity indices.

KEYWORDS

CKD, central obesity, anthropometric measurement, METS-VF, predictor

Introduction

Chronic kidney disease (CKD) represents an enormous public health burden affecting 9.1% of the world's population (1). It is defined as an estimated glomerular filtration rate (eGFR) less than 60 ml/min per 1.73m^2 or demonstrated by markers of kidney damage that persist for at least 3 months. Approximately 2% of patients with CKD may progress into end-stage kidney disease (ESKD) (2). Moreover, the presence of impaired kidney function appears to have a marked impact on the risk of cardiovascular disease and its related mortality, and even a mild reduction in kidney function may have an adverse effect on cardiovascular health (3–5). Thus, the risk assessment of CKD in the general population is extremely important.

Obesity is a recognized risk factor for CKD. According to the 2011-2014 American National Health and Nutrition Examination Survey (NHANES), among individuals with CKD, 44% had obesity, 69% had elevated waist circumference, and the incidence rate of CKD paralleled the prevalence of obesity (6). Moreover, a global, collaborative meta-analysis that included more than five million individuals in 63 cohorts demonstrated that excessive adiposity is an independent risk factor for GFR decline (7). As a widely and frequently used index in obesity assessment, BMI has been most widely studied when assessing the relationship between obesity and CKD. For example, in the Framingham Offspring cohort, BMI was shown to be independently associated with the risk of CKD; for each 1 SD increase in BMI, a relative increase of 23.0% in the risk of CKD was observed (8). However, BMI has several limitations when assessing adiposity. First, BMI does not distinguish between lean and fat body mass, while sarcopenic obesity is highly prevalent among patients with CKD (9); thus, BMI may misclassify weight status among CKD patients. For example, the study performed by Dierkes et al. showed that 27.9% of the study participants were obese when using the BMI definition, while 48.8% of the study participants were obese when using the definition based on body fat percentage (which was measured by bioimpedance method) (10). Moreover, current studies indicate that the deleterious effect of obesity on kidney function is mainly attributed to excess visceral adiposity (11), whereas BMI is an index for overall obesity. Thus, indices that can provide accurate measurements of visceral obesity may be more helpful when assessing CKD risk, which is supported by a number of studies (12–16). For example, Oh et al. reported that central obesity indices such as WC, waist-to-hip ratio (WHR) and WHtR, but not BMI, were associated with the future risk of renal function decline (16).

The metabolic score for visceral fat (METS-VF) is a novel index for visceral adiposity, which was developed by nonlinear fits of an insulin resistance component (METS-IR), waist-to-height ratio (WHtR), age, and sex by using dual X-ray absorptiometry (DXA) as the reference. It has been validated by magnetic resonance imaging (MRI) and bioelectrical impedance analysis (BIA), which were used to measure visceral adipose tissue mass in an external population, and showed superiority over several other surrogate indices of visceral adiposity (17). However, the link between the METS-VF and the risk of CKD is still unknown. In this cross-sectional study, we therefore examined this association. At the same time, we aimed to compare the predictive ability of the METS-VF with other visceral adiposity indices, including WC, WHtR, LAP, VAI, ABSI, BRI, and CMI, for detecting CKD. This may help to determine the most appropriate visceral adiposity index for CKD risk prediction.

Methods

Study population

We used data from a subset population from the China Cardiometabolic Disease and Cancer Cohort study. The details of this cohort have been described elsewhere (18, 19). In brief, 10999 individuals aged over 40 years from Tianmen City, Hubei province were enrolled in 2011. Health, lifestyle, and sociodemographic data were collected through questionnaires

and interviews; participants also underwent a physical examination and provided blood samples. Written informed consent was obtained from each participant before the survey. Of the 10999 individuals, those with missing data regarding anthropometric measurements, blood pressure measurements or biochemical parameters were excluded. Moreover, as the METS-VF was derived from subjects with a BMI greater than 18.5 kg/m^2, individuals with a BMI less than 18.5 were also excluded. Finally, 8866 individuals were included in this cross-sectional study.

Central obesity assessment

Body weight was measured with a calibrated digital scale (Wuxi brand, RGZ120-RT) to the nearest 0.1 kg. Height was measured with a stadiometer to the nearest 0.1 cm without shoes. Waist circumference (WC) was measured at the midpoint between the last rib and iliac crest to the nearest 0.5 cm. The central obesity-related indices were calculated as follows:

(1) WHtR=WC (kg)/height (m^2)

(2) METS-IR and METS-VF (17, 20)

$$METS-IR=\frac{Ln((2\times G_0)+TG_0)\times BMI)}{Ln(HDL-C)}$$

$$METS-VF=4.466+0.011\left[(Ln(METS-IR))^3\right]+3.239\left[(Ln(WHtR))^3\right]+0.319(Sex)+0.594(Ln(Age)),$$

where G_0 is expressed in mg/dL, TG_0 in mg/dL, BMI in Kg/m^2, HDL-C in mg/dL, Age in years, and sex was a binary response variable (men=1, women=0).

(3) LAP (21)

$$LAP(men)=(WC(cm)-65)\times(TG(mmol/L))$$

$$LAP(women)=(WC(cm)-58)\times(TG(mmol/L))$$

(4) VAI (22)

$$VAI(men)=\left(\frac{WC}{39.68+(1.88\times BMI)}\right)\times\left(\frac{TG}{1.03}\right)\times\left(\frac{1.31}{HDL-C}\right)$$

$$VAI(women)=\left(\frac{WC}{36.58+(1.89\times BMI)}\right)\times\left(\frac{TG}{0.81}\right)\times\left(\frac{1.52}{HDL-C}\right),$$

where WC is expressed in cm, BMI in Kg/m^2, TG in mmol/L, and HDL in mmol/L.

(5) ABSI (23)

$$ABSI=\frac{WC}{BMI^{2/3}\times height^{1/2}},$$

where WC is expressed in m, BMI in Kg/m^2, height in m.

(6) BRI (24)

$$BRI=364.2-365.5\sqrt{1-\frac{\left(\frac{WC}{2\pi}\right)^2}{(0.5\times height)^2}},$$

where WC is expressed in m, height in m.

(7) CMI (25)

$$CMI=\left(\frac{TG}{HDL-C}\right)\times WHtR,$$

where TG is expressed in mg/dL, HDL-C in mg/dL.

Assessment of covariates and outcomes

Information on smoking habits, drinking habits, physical activity levels, and clinical history was collected through a standardized questionnaire. Current smoking was defined as smoking one or more cigarettes a day for at least six months. Current drinking was defined as having had one or more drinks of alcohol per week for at least six months. For physical activity, a metabolic equivalent (MET) value was assigned according to the compendium of activity energy costs for each activity in the questionnaire, and the total volume of physical activity was converted into MET-minutes per week (26); those who accumulated at least 600 MET-minutes of physical activity per week were classified as physically active. For medical history, CVD history was defined as having been diagnosed with myocardial infarction, coronary heart disease, stroke or peripheral artery disease.

Systolic and diastolic blood pressure were measured three times by using an Omron professional blood pressure monitor following a standardized protocol after the patients had been sitting for at least 5 minutes before measurement. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure (DBP) ≥90 mmHg, or self-reported use of taking antihypertensive medications.

The 75g oral glucose tolerance test 75 was performed to evaluate the glucose metabolism status of the study participants. Venous fasting and 2-hour postload plasma glucose levels were measured by the enzymatic hexokinase method. HbA1c was measured by using a high-performance liquid chromatography method. Diabetes was defined as fasting blood glucose ≥7.0 mmol/L, 2-hour postload plasma glucose concentrations ≥11.1 mmol/L, HbA1c ≥6.5%, or self-reported diagnosis of diabetes and the use of glucose-lowering medications. Total, HDL, and LDL cholesterol, triglycerides, and serum creatinine were measured using fasting blood samples. eGFR was calculated on

the basis of serum creatinine according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (27). CKD was defined as an eGFR<60 mL/min/1.73 m^2.

Statistical analysis

Normally distributed continuous variables are reported as the means (SDs), nonnormally distributed continuous variables are reported as median and interquartile ranges (IQRs). Categorical variables are presented as total numbers with corresponding percentages. Study population characteristics were compared between groups according to the presence of CKD. Differences between groups were evaluated by t test or one-way ANOVA for continuous variables and χ^2 test for categorical variables. Associations of baseline METS-VF with CKD were assessed with logistic regression models, and odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for participants in the highest tertile (T3) compared with participants in the two lower tertiles (T1–T2). Models were adjusted for current smoking, current drinking, physical activity level, diabetes, hypertension, CVD history and BMI. Stratified analyses by age (<60, ≥60 years), BMI (<24, ≥24), diabetes (no, yes), hypertension (no, yes) and history of CVD (no, yes) were also performed. Effect modification was tested by the likelihood ratio test comparing models with and without a multiplicative interaction term for the subgroup categories. Receiver operating characteristic (ROC) curve analysis was used to compare the predictive ability of these indices, and the areas under the ROC curve of different indices were compared using the method developed by DeLong et al. (28).The appropriate cut-off point of each index for the prediction of CKD was determined by using these indices as test variables and CKD as a state variable, and the optimal cut-off values were determined by maximizing the Youden index. All P values were two-sided and< 0.05 was considered statistically significant. The statistical analyses were performed using SPSS version 26.0 software (IBM Corporation, Chicago, IL) and R version 3.4.2 software.

Results

Baseline characteristics

Among the 8866 included participants, 35.2% were male, the mean (SD) age was 60.6 (10.1) years, and the mean eGFR was 94.4 (12.2) mL/min/1.73 m^2. Table 1 displays the baseline characteristics of all participants according to the presence of CKD. In the total population and among men and women, there was no significant difference in BMI between the CKD and non-CKD groups. However, the CKD group had significantly higher values for 8 central obesity indices (METS-VF, WC, WHtR, LAP, VAI, ABSI, BRI, and CMI) in the total population and among women; among men, the values for three (METS-VF, WHtR, and BRI) of the eight central obesity indices were higher in the CKD group than in the non-CKD group. At the same time, in the total population, the values for age, 2h-PG, HbA1c, TGs, HDL-C, and SBP and the proportions of individuals with hypertension, diabetes or a history of CVD were higher in the CKD group; among men, the values for 2h-PG and HDL-C and the proportion of individuals with a history of CVD were higher in the CKD group; among women, the values for HbA1c, TGs, HDL-C, and SBP and the proportions of individuals with hypertension or diabetes were higher in the CKD group.

Associations of the METS-VF with CKD risk

Table 2 shows the associations between the METS-VF and CKD. Positive associations were found between the METS-VF and the prevalence of CKD in the overall population and the population stratified by sex. When unadjusted (Model 1), the participants in the highest tertile of the METS-VF had a significantly higher risk of CKD than participants in the lowest tertile of the METS-VF (OR 2.838, 95% CI 1.888-4.264 for the total population; OR 3.063, 95% CI 1.539-6.093 for men; OR 3.058, 95% CI 1.787-5.233 for women). After adjusting for current smoking, current drinking, and physical activity (Model 2), the ORs showed little change. In Model 3, which was additionally adjusted for hypertension, diabetes, and history of CVD, the ORs in the highest tertile vs. the lowest tertile were as follows: 2.489 (1.635-3.789) for the overall population, 2.805 (1.389-5.663) for men, 2.585 (1.487-4.495) for women. In Model 4, we further adjusted for BMI and found that the OR value for the highest tertile vs. the lowest tertile increased; the corresponding ORs were 3.943 (2.435-6.385) for the overall population, 3.585 (1.585-8.109) for men, 4.627 (2.485-8.616) for women. In the fully adjusted model (Model 4), each SD increase in the METS-VF was associated with a 110.2% higher risk of CKD in the overall population, a 76.1% higher risk of CKD among men and with a 130.1% higher risk of CKD among women.

Subgroup analyses for the association between the METS-VF and CKD risk

In the subgroup analyses (Table 3), significant interactions were found between the METS-VF and risk of CKD by age (P value for interaction = 0.023). The association appeared to be significantly stronger among those aged over 60 years than younger individuals when comparing individuals in tertile 3 vs. tertile 1. When stratified by other factors, comparing individuals in tertile 3 vs. tertile 1, the association between METS-VF and risk of CKD was more pronounced among

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TABLE 1 Participant characteristics of CKD and non-CKD populations.

	Total (n = 8866)			Men (n = 3117)			Women (n = 5749)		
	eGFR ≥60 (n=8707)	eGFR<60 (n=159)	*P* value	eGFR ≥60 (n=3056)	eGFR<60 (n=61)	*P* value	eGFR ≥60 (n=5651)	eGFR<60 (n=98)	*P* value
Age (years)	60.46 ± 10.04	68.32 ± 10.08	<0.001	62.42 ± 9.80	68.05 ± 8.33	<0.001	59.40 ± 10.00	68.49 ± 11.06	<0.001
Current smoker (%)	13.9	9.4	0.105	38.4	23.0	0.014	0.7	1.0	0.490
Current drinker (%)	12.5	3.1	<0.001	33.1	6.6	<0.001	1.4	1.0	0.999
Physically active (%)	62.9	55.3	0.051	61.8	54.1	0.218	63.5	56.1	0.135
FPG (mmol/L)	5.53 ± 1.28	5.84 ± 2.02	0.059	5.53 ± 1.10	5.68 ± 1.34	0.301	5.53 ± 1.37	5.93 ± 2.34	0.092
2-h PG (mmol/L)	6.92 ± 5.02	7.83 ± 3.65	0.023	6.69 ± 2.59	7.45 ± 3.29	0.024	7.04 ± 5.93	8.07 ± 3.86	0.089
HbA1c (%)	5.81 ± 0.87	6.01 ± 1.03	0.004	5.73 ± 0.68	5.90 ± 0.94	0.051	5.85 ± 0.95	6.07 ± 1.08	0.023
TGs (mmol/L)	1.23 (0.89-1.77)	1.39 (1.02-1.92)	0.005	1.10 (0.81-1.64)	1.16 (0.96-1.58)	0.254	1.30 (0.95-1.82)	1.51 (1.16-2.12)	0.002
HDL-C (mmol/L)	1.50 ± 0.36	1.41 ± 0.33	0.002	1.52 ± 0.40	1.41 ± 0.31	0.044	1.50 ± 0.33	1.42 ± 0.34	0.016
SBP (mmHg)	150.62 ± 23.83	157.97 ± 26.37	<0.001	152.82 ± 23.51	155.93 ± 24.47	0.307	149.44 ± 23.92	159.23 ± 27.53	0.001
DBP (mmHg)	81.68 ± 12.40	82.45 ± 15.52	0.533	82.82 ± 13.38	84.36 ± 17.73	0.503	81.05 ± 11.79	81.26 ± 13.94	0.886
Hypertension (%)	68.1	81.8	<0.001	72.2	82.0	0.092	65.9	81.6	0.001
Diabetes (%)	12.5	19.5	0.009	12.0	16.4	0.294	12.8	21.4	0.012
CVD (%)	7.3	11.9	0.027	8.4	19.7	0.002	6.7	7.1	0.875
METS-VF	6.40 ± 0.59	6.66 ± 0.53	<0.001	6.52 ± 0.58	6.75 ± 0.55	0.001	6.34 ± 0.58	6.60 ± 0.51	<0.001
LAP	24.32 (13.92-41.60)	29.83 (17.02-53.60)	0.002	18.25 (9.90-32.76)	22.25 (12.32-33.91)	0.129	27.96 (16.68-46.00)	37.92 (21.54-59.55)	0.001
VAI	1.38 (0.86-2.25)	1.61 (1.00-2.70)	0.003	0.93 (0.61-1.58)	1.18 (0.74-1.54)	0.099	1.63 (1.08-2.55)	2.16 (1.41-3.27)	<0.001
ABSI	0.078 ± 0.007	0.081 ± 0.007	<0.001	0.078 ± 0.007	0.079 ± 0.005	0.237	0.078 ± 0.007	0.081 ± 0.007	<0.001
BRI	3.65 ± 1.17	4.04 ± 1.24	<0.001	3.40 ± 1.03	3.73 ± 1.03	0.012	3.79 ± 1.21	4.24 ± 1.33	<0.001
CMI	0.97 (0.63-1.59)	1.22 (0.73-1.95)	<0.001	0.86 (0.54-1.47)	1.07 (0.68-1.54)	0.058	1.04 (0.68-1.65)	1.39 (0.91-2.09)	<0.001
WHtR	0.51 ± 0.06	0.53 ± 0.06	<0.001	0.50 ± 0.05	0.52 ± 0.05	0.01	0.52 ± 0.06	0.54 ± 0.06	<0.001
WC (cm)	81.15 ± 9.20	83.47 ± 9.33	0.002	82.32 ± 9.07	84.18 ± 8.53	0.112	80.51 ± 9.21	83.03 ± 9.80	0.007
BMI (kg/m^2)	23.70 ± 3.09	23.86 ± 3.34	0.527	23.43 ± 2.99	24.01 ± 3.25	0.137	23.85 ± 3.13	23.77 ± 3.41	0.798

Continuous variables are expressed as the means (standard deviations) or medians (IQRs), and categorical variables are expressed as numbers (percentages). 2-h PG, 2-hour postprandial blood glucose; TGs, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; CVD, cardiovascular disease; METS-VF, metabolic score for visceral fat; LAP, lipid accumulation product; VAI, visceral adiposity index; ABSI, a body shape index; BRI, body roundness index; CMI, cardiometabolic index; WHtR, waist-to-height ratio; WC, waist circumference; BMI, body mass index.

individuals with overweight/obesity, individuals without diabetes, individuals without hypertension, and individuals without CVD history. However, none of these interaction terms reached statistical significance.

ROC analyses of the METS-VF and other central obesity indices with CKD risk

Table 4 and Figure 1 show the AUC scores (and 95% CIs) for the 8 central obesity indices for predicting CKD risk for total population and for both sexes. In the total population, both the METS-VF and seven other central obesity indices could identify CKD. The METS-VF had a higher AUC value than that of WC, WHtR, LAP, VAI and BRI and had a comparable value to that of ABSI and CMI. Among men, the METS-VF, WHtR, and BRI could identify CKD. The METS-VF had a higher AUC value than that of WHtR and BRI. Among women, all 8 indices could identify CKD. The METS-VF had a higher AUC value than that of WC, WHtR and BRI and a comparable value to that of LAP, VAI, ABSI and CMI.

In the total population, the optimal cut-off values were 6.705 for the METS-VF, 85.000 for WC, 0.535 for WHtR, 25.520 for LAP, 1.295 for the VAI, 0.077 for ABSI, 4.041 for BRI, and 0.892 for CMI. For men, the optimal cut-off values were 6.891 for the METS-VF, 0.519 for WHtR, and 3.713 for BRI. For women, the optimal cut-off values were 6.744 for the METS-VF, 85.000 for WC, 0.525 for WHtR, 33.920 for LAP, 1.610 for the VAI, 0.083 for ABSI, 3.846 for BRI, and 1.641 for CMI. In the total population and for both sexes, the METS-VF had the highest Youden index values for identifying CKD.

TABLE 2 Associations between METS-VF and CKD.

	METS-VF tertiles			P for trend	Per 1 SD increase
	Tertile 1	Tertile 2	Tertile 3		
Total					
Median (range)	5.84 (≤6.22)	6.48 (6.23-6.71)	6.97 (>6.71)		
Cases, n (%)	32 (1.1%)	38 (1.3%)	89 (3.0%)	<0.001	
Model 1	1	1.190 (0.742-1.910)	2.838 (1.888-4.264)	<0.001	1.689 (1.402-2.035)
Model 2	1	1.206 (0.751-1.937)	2.912 (1.930-4.393)	<0.001	1.699 (1.406-2.053)
Model 3	1	1.139 (0.708-1.832)	2.489 (1.635-3.789)	<0.001	1.568 (1.293-1.902)
Model 4	1	1.397 (0.857-2.275)	3.943 (2.435-6.385)	<0.001	2.102 (1.653-2.674)
Men					
Median (range)	5.99 (≤6.32)	6.58 (6.33-6.83)	7.07 (>6.83)		
Cases, n (%)	11 (1.1%)	17 (1.6%)	33 (3.2%)	<0.001	
Model 1	1	1.551 (0.723-3.329)	3.063 (1.539-6.093)	0.001	1.627 (1.208-2.192)
Model 2	1	1.567 (0.728-3.373)	3.100 (1.550-6.200)	0.001	1.613 (1.199-2.169)
Model 3	1	1.540 (0.714-3.322)	2.805 (1.389-5.663)	0.003	1.527 (1.132-2.060)
Model 4	1	1.698 (0.773-3.730)	3.585 (1.585-8.109)	0.002	1.761 (1.205-2.573)
Women					
Median (range)	5.78 (≤6.16)	6.42 (6.17-6.65)	6.90 (>6.65)		
Cases, n (%)	18 (0.9%)	26 (1.4%)	54 (2.8%)	<0.001	
Model 1	1	1.450 (0.792-2.653)	3.058 (1.787-5.233)	<0.001	1.717 (1.350-2.184)
Model 2	1	1.460 (0.798-2.672)	3.141 (1.833-5.380)	<0.001	1.740 (1.366-2.215)
Model 3	1	1.340 (0.730-2.460)	2.585 (1.487-4.495)	<0.001	1.582 (1.235-2.026)
Model 4	1	1.747 (0.937-3.254)	4.627 (2.485-8.616)	<0.001	2.301 (1.692-3.128)

Model 1: Unadjusted.
Model 2: Adjusted for sex (only in total population), current smoking, current drinking, and physical activity.
Model 3: Adjusted for sex (only in total population), current smoking, current drinking, physical activity, hypertension, diabetes, and CVD.
Model 4: Adjusted for sex (only in total population), current smoking, current drinking, physical activity, hypertension, diabetes, CVD and BMI.

TABLE 3 Subgroup analysis of the association between METS-VF and CKD.

	METS-VF tertiles			P for trend	P for interaction
Subgroup	Tertile 1	Tertile 2	Tertile 3		
Age (years)					
<60	1	2.073 (0.759-5.663)	4.880 (1.403-16.980)	0.015	0.023
≥60	1	1.062 (0.605-1.866)	2.492 (1.450-4.282)	0.001	
BMI					
Normal	1	1.434 (0.852-2.411)	3.339 (1.966-5.671)	<0.001	0.779
Overweight/Obesity	1	1.171 (0.251-5.459)	3.877 (0.931-16.142)	0.001	
Diabetes					
No	1	1.273 (0.739-2.194)	4.036 (2.381-6.839)	<0.001	0.359
Yes	1	1.786 (0.571-5.594)	3.255 (0.990-10.702)	0.048	
Hypertension					
No	1	2.252 (0.765-6.632)	14.578 (5.172-41.091)	<0.001	0.926
Yes	1	1.221 (0.707-2.108)	2.815 (1.641-4.831)	<0.001	
CVD history					
No	1	1.436 (0.852-2.420)	4.445 (2.665-7.413)	<0.001	0.847
Yes	1	1.260 (0.317-5.005)	1.479 (0.340-6.427)	0.601	

Adjusted for sex, current smoking, current drinking, physical activity, hypertension, diabetes, CVD and BMI, except for the stratifying factor.

TABLE 4 ROC analyses for the prediction of CKD by adiposity indices.

	AUC	95% CI	p value	Cut-off value	Sensitivity (%)	Specificity (%)	Youden index
Total							
METS-VF	0.634	0.589-0.680	<0.001	6.705	57.23	66.85	24.08
WC	0.571*	0.525-0.617	0.002	85.000	47.80	65.92	13.72
WHtR	0.595*	0.550-0.640	<0.001	0.535	49.69	67.64	17.33
LAP	0.572*	0.528-0.616	0.002	25.520	59.75	52.46	12.21
VAI	0.569*	0.525-0.612	0.003	1.295	66.04	46.56	12.60
ABSI	0.596	0.552-0.639	<0.001	0.077	70.44	44.02	14.46
BRI	0.595*	0.550-0.640	<0.001	4.041	49.69	67.64	17.33
CMI	0.589	0.547-0.631	<0.001	0.892	70.44	44.95	15.39
Men							
METS-VF	0.632	0.559-0.704	<0.001	6.891	52.46	71.47	23.93
WC	0.573	0.500-0.646	0.051	–	–	–	–
WHtR	0.605*	0.533-0.677	0.005	0.519	55.74	65.67	21.41
LAP	0.557	0.489-0.624	0.129	–	–	–	–
VAI	0.562	0.500-0.623	0.099	–	–	–	–
ABSI	0.555	0.487-0.623	0.144	–	–	–	–
BRI	0.605*	0.533-0.677	0.005	3.713	55.74	65.67	21.41
CMI	0.571	0.509-0.633	0.058	–	–	–	–
Women							
METS-VF	0.634	0.577-0.692	<0.001	6.744	52.04	73.47	25.51
WC	0.568*	0.509-0.626	0.021	85.000	44.90	68.11	13.01
WHtR	0.598*	0.539-0.657	0.001	0.525	61.22	57.10	18.32
LAP	0.599	0.543-0.655	0.001	33.920	59.18	60.77	19.95
VAI	0.608	0.553-0.663	<0.001	1.610	70.41	49.39	19.80
ABSI	0.619	0.563-0.675	<0.001	0.083	41.84	77.07	18.91
BRI	0.598*	0.539-0.657	0.001	3.846	61.22	57.10	18.32
CMI	0.610	0.555-0.665	<0.001	1.641	44.90	74.55	19.45

*P< 0.05 when comparing the AUC with METS-VF.
AUC, area under the curve; CI, confidence interval; METS-VF, metabolic score for visceral fat; LAP, lipid accumulation product; VAI, visceral adiposity index; ABSI, a body shape index; BRI, body roundness index; CMI, cardiometabolic index; WHtR, waist-to-height ratio; WC, waist circumference; BMI, body mass index.

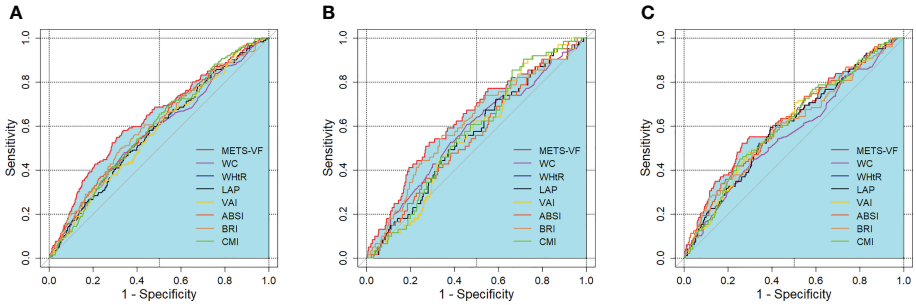

FIGURE 1
ROC curves for the prediction of CKD by adiposity indices. **(A)** total population, **(B)** men, **(C)** women. METS-VF, metabolic score for visceral fat; LAP, lipid accumulation product; VAI, visceral adiposity index; ABSI, a body shape index; BRI, body roundness index; CMI, cardiometabolic index; WHtR, waist-to-height ratio; WC, waist circumference; BMI, body mass index.

Discussion

In this cross-sectional study, we found that the METS-VF was positively associated with the risk of CKD, and this association was independent of current smoking, current drinking, physical activity, diabetes, hypertension, history of CKD and BMI. Moreover, the METS-VF is a good indicator for CKD compared with other central obesity indices, including WC, WHtR, LAP, VAI, ABSI, BRI, and CMI. The METS-VF showed better predictive ability for CKD among men and better or comparable predictive ability for CKD in the total population and among women. In the total population and for both sexes, the METS-VF had the highest Youden index.

The impact of visceral adiposity on kidney function has been evaluated by several studies. For example, Kang et al. used the multifrequency bioelectrical impedance analysis method to measure visceral body fat and found that a higher level of visceral adiposity was associated with a higher prevalence of CKD, and the association remained significant after adjusting for age, sex, diabetes, and hypertension (29). Visceral adiposity has also been associated with kidney disease progression. For example, Hiroshi et al. measured visceral fat and subcutaneous fat area by CT scan, and reported that the visceral-to-subcutaneous fat ratio was longitudinally associated with the risk of a more than 30% decline in eGFR among individuals with established CKD (30). These studies used bioelectrical impedance methods or imaging methods to measure visceral adiposity, which provide high measurement accuracy and effectively proved the role of excessive visceral adiposity in the pathogenesis or the progression of CKD. However, these methods are often not feasible in population-based epidemiological studies. Thus, using anthropometric indicators in CKD risk prediction is essential.

Waist circumference has long been used to assess central obesity and related disease risk; however, for individuals with similar WCs, WC may overestimate the risk for tall people and underestimate the risk for short people (31). To overcome the shortcomings of WC, indices including WHtR, ABSI and BRI adjusted for weight and/or BMI in their formulas. Moreover, the VAI, LAP and CMI integrate lipid parameters into their formulas, which enable them not only to assess of the mass of adipose tissue but also to reflect the dysfunction of adipose tissue. In our study, we focused on the newly invented-METS-VF index. The METS-VF algorithm mainly consists of three parts: an insulin resistance component (METS-IR), an anthropometric component (WHtR) and a demographic component (age, sex). The METS-IR component can reflect the degree of insulin resistance, and metabolic dysregulation in central obesity, which play critical roles in the pathogenesis of CKD. Moreover, the METS-VF was reported to have the ability to provide relatively accurate measurements of visceral adiposity and insulin resistance even in metabolically healthy obese individuals who do not have substantial laboratory disturbances (17). Meanwhile, the METS-VF has been demonstrated to be a strong predictor for hypertension and diabetes in Western and Chinese populations, and has stronger predictive power than several of the abovementioned indices (17, 32, 33). Overall, these characteristics and advantages of the METS-VF may facilitate its use in CKD risk prediction.

Through logistic analysis and ROC analysis, we proved the acceptable predictive ability of the METS-VF in CKD risk assessment. Moreover, we noted several points in exploring the relationship between the MRTS-VF and CKD in logistic analysis. First, sex differences were noted when we additionally adjusted for BMI in Model 4, which should be explained. The METS-VF is a measurement for visceral adipose tissue; for a given METS-VF, a higher BMI value may indicate elevations in lower body subcutaneous adipose tissue and muscle mass. It has been reported that BMI is positively correlated with visceral adipose tissue mass measured by magnetic resonance imaging when not adjusted for WC, but negatively correlated with visceral adipose tissue mass after adjusting for WC; thus, when assessing the association between central obesity and health outcomes, the strength of the association may not be fully realized until after adjustment for BMI (34, 35). In this study, after adjusting for BMI, the association was more evident among women than among men. Sex differences in fat distribution may partially account for this phenomenon; men tend to have relatively more visceral fat, while women have relatively more subcutaneous fat. The hyperplasia of subcutaneous adipose tissue can provide safe storage of excess lipids and reduce the spillover of excess lipids to visceral adipose tissue or other normally lean organs, contributing the maintenance of a metabolic health phenotype (36). Thus, subcutaneous tissue may modulate the association between the METS-VF and CKD risk to a higher degree among women than among men. Second, we found that sex can modify the association between the METS-VF and CKD risk. Among individuals aged less than 60 years, the association was significantly stronger, and the reasons behind this still need further investigation. In this study, the proportion of individuals with diabetes, hypertension and a history of CVD was significantly higher among those aged more than 60 years. Although our data did not include the duration of these comorbidities, it is likely that older individuals would have had these comorbidities for a longer period of time and that the kidneys would have tended to have more exposure to these risk factors, which might influence the relationship between visceral fat and CKD when setting these comorbidities as confounders.

Currently, a series of studies aimed at determining the best adiposity indices for predicting CKD in the Chinese population have been published. Several new indices, such as the VAI and LAP, were evaluated, as they have been reported to be better indicators for cardiovascular diseases or events than traditional central obesity indices such as WC and WHtR (22, 37). Dai et al. reported that the VAI and LAP were superior to BMI, WHtR

and WC in identifying CKD as defined by an estimated GFR< 60 ml/min/1.73m^2 for men but not for women aged more than 35 years (38). Chen et al. reported that the VAI had better discriminative ability for CKD defined by an estimated GFR< 60 ml/min/1.73m^2 or the presence of albuminuria than BMI and WC for women but not for men aged 50–90 years (39). In this study, we took the VAI and LAP into consideration when comparing the predictive performance of the METS-VF with other indices. Three other novel visceral adiposity indices (ABSI, BRI, CMI) were also considered, as they were reported be linked with CKD in other ethnic groups (40) or with other cardiometabolic diseases in the Chinese population (41). We found that the METS-VF had the best predictive power for CKD among all indices for men, as the METS-VF had the highest AUC value in the analysis. For women, its performance was also acceptable, as it had higher or similar AUC values than other indices. Thus, our study makes important contributions to the literature on this topic.

Our study has several strengths. First, this is the first study to explore the association between the METS-VF and CKD and compare its performance with several traditional central obesity indices. Second, this was a community population-based study with a relatively large sample size, and the results can be representative of the general population. Third, the use of a standardized protocol for anthropometric measurement guarantees the accuracy of the study results. However, our study has several limitations. First, the cross-sectional design could not provide an interpretation of the causation or directionality of the association. Second, CKD was defined on the basis of a single measurement of eGFR, and the presence of microalbuminuria was not included, as we did not collect urine specimens from the participants. Third, as physical activity is associated with the risk of incident CKD and CKD related outcomes (42, 43), we included physical activity levels as a confounder in the logistic analysis. However, it is not easy to objectively measure population-level physical activity levels and we used self-reported data, which may be subjected to recall bias. Fourth, the included participants in this study were aged more than 40 years and recruited from a Chinese population; thus, the generalizability of our findings to individuals younger than 40 years or of other ethnicities remains to be verified.

Conclusions

In conclusion, our study demonstrated that the METS-VF is closely associated with the risk of CKD after adjusting for potential confounders. Moreover, we found that the METS-VF has a superior ability to predict CKD than other indices (WC, WHtR, LAP, VAI, ABSI, BRI, CMI), and its advantage was particularly pronounced for men. The optimal cut-off values for the METS-VF in predicting CKD are 6.891 for men and 6.744 for women. The significant relationship between the METS-VF and the risk of CKD has important public health implications. This reminds us that in health management work, we should attach importance to visceral obesity in individuals at high risk of CKD, and interventions to reduce visceral adiposity should be adopted in CKD prevention.

Data availability statement

The datasets analysed in the current study are not publicly available due to the limits on the data-sharing agreement of the China Cardiometabolic Disease and Cancer Cohort study group.

Ethics statement

The study protocol was approved by the medical ethics committee of Ruijin Hospital, Shanghai Jiao Tong University. The patients/participants provided their written informed consent to participate in this study.

Author contributions

PY, XY contributed to the study conception and study design. PY performed the data analysis; PY, XM, RK and ZW interpreted the data; PY wrote the manuscript. All authors read and approved the final manuscript.

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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

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PDIA4, a new endoplasmic reticulum stress protein, modulates insulin resistance and inflammation in skeletal muscle

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Introduction: Endoplasmic reticulum (ER) stress has emerged as a key player in insulin resistance (IR) progression in skeletal muscle. Recent reports revealed that ER stress-induced the expression of protein disulfide isomerase family a member 4 (PDIA4), which may be involved in IR-related diseases. A previous study showed that metformin modulated ER stress-induced IR. However, it remained unclear whether metformin alleviated IR by regulating PDIA4 expression in skeletal muscle.

Methods: Herein, we used palmitate-induced IR in C2C12 cells and a high-fat diet-induced IR mouse model to document the relations between metformin, IR, and PDIA4.

Results: In C2C12 cells, palmitate-induced IR increased inflammatory cytokines and PDIA4 expression. Besides, knocking down PDIA4 decreased palmitate-induced IR and inflammation in C2C12 cells. Furthermore, metformin modulated PDIA4 expression and alleviated IR both in vitro and in vivo. In addition, serum PDIA4 concentrations are associated with IR and inflammatory cytokines levels in human subjects.

Discussion: Thus, this study is the first to demonstrate that PDIA4 participates in the metformin-induced effects on skeletal muscle IR and indicates that PDIA4 is a potential novel therapeutic target for directly alleviating IR.

KEYWORDS

Endoplasmic reticulum, insulin resistance, metformin, PDIA4, skeletal muscle

1 Introduction

Obesity is a triggering factor for diabetes associated with insulin resistance (IR). IR in obesity and type 2 diabetes is manifested by decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle and by impaired suppression of hepatic glucose output (1). IR increases lipolysis, resulting in the release of free fatty acids from fat and inhibits glucose uptake by muscle cells. In addition, elevated plasma free fatty acids (FFA) are risk factors for skeletal muscle IR (2). In addition, abnormal metabolites and local inflammation lead to disruption of insulin signaling, which plays a crucial role in the development of IR (3). Skeletal muscle-induced IR phenomenon plays a key role in the development of type 2 diabetes. Study indicates that endoplasmic reticulum (ER) stress as a key factor in the progression of skeletal muscle IR (4).

When the ER protein folding capacity is overwhelmed, cells undergo a condition defined as ER stress, characterized by misfolded proteins accumulated inside the ER lumen. ER stress status, such as nutrient deprivation, hypoxia, and calcium depletion. To overcome the imbalanced ER protein-folding capacity, cells have evolved an evolutionary conserved signal transduction pathway called unfolded protein response (UPR) (5). UPR included immunoglobulin heavy chain binding protein/glucose-regulated protein 78 (BiP/GRP78) in the ER lumen, PKR-like eukaryotic initiation factor 2α kinase (PERK), inositol-requiring enzyme 1α (IRE1α), activating transcription factor 6 in ER transmembrane (ATF6), activating transcription factor 4 (ATF4), tribbles homolog 3 (TRB3), C/EBP homolog protein (CHOP), box-binding protein in X cytoplasm 1 (XBP1) (6, 7). Liver and adipose tissue of obese mice found to highly express UPR markers (8), and improve insulin sensitivity by inhibiting BiP/GRP78 expression (9). ER stress induces expression of TRB3 in skeletal muscle, which results in impairment of insulin signaling and glucose uptake. Knockdown of TRB3 significantly blunts the effects of ER stress on glucose uptake and TRB3 knockout mice are protected from high fat diet-induced IR (10). Palmitate induces increased ER stress, which in turn induces IR in skeletal muscle (11). Altogether, these results indicate that palmitate-induced ER stress is a key step in IR development in skeletal muscle. However, the detailed regulation mechanisms remain unclear.

Protein disulfide isomerases (PDIs) are expressed in many tissues (12). PDI family members include ER-resident protein (ERp) 57, ERp29, ERp44, and PDI family A member 4 (PDIA4 or ERp72) (13). PDI is a well-known multifunctional protein and has been widely involved in many diseases, including neurodegenerative diseases, metabolic diseases, osteogenesis imperfecta, cancer, infectious diseases and cardiovascular diseases (14). PDIA1 assists insulin production by regulating insulin redox in the ER (15). PDIA4 function has been shown to improve diabetes, lower blood sugar, glycated hemoglobin (HbA1C) and reactive oxygen species (ROS), and increase insulin secretion (16). Previous clinical studies have found that the serum expression of PDIA4 from patients with metabolic disease is significantly higher than that of patients without metabolic disease, suggesting that PDIA4 has the potential as a new therapeutic target for metabolic disease or IR (17). However, the role of ER stress-induced PDIA4 expression in skeletal muscle IR remains poorly understood.

Metformin is an oral antidiabetic drug from the biguanide group and plays a first-line drug for treating type 2 diabetes. Metformin lowers blood glucose by inhibiting hepatic glucose production and increasing glucose uptake in peripheral tissues, mainly skeletal muscle (18). Metformin elevates insulin receptor tyrosine kinase activity, enhances glycogen synthesis, and increases glucose transporter type 4 (GLUT4) expression (19). Metformin inhibits the expression of saturated fatty acids-induced ER stress and ER stress proteins (20). Additionally, metformin inhibits ER chaperone protein, including BiP/GRP78, PDI, and ROS production (21). Finally, metformin regulates UPR by decreasing CHOP and caspase 3 expression in human islets (22), suggesting that metformin protected islet function partially by reducing ER stress. These results imply that ER stress might be one of the therapeutic targets of metformin.

Therefore, in this study, we first explored whether ER stress-induced PDIA4 was involved in skeletal muscle IR. Next, we investigated whether metformin regulated skeletal IR by modulating ER stress and PDIA4 expression.

2 Materials and methods

2.1 Inclusion and exclusion criteria and laboratory measurements of human study subjects

The Internal Review Board of the Ethics Committee of the Tri-Service General Hospital approved this study (institutional review board approval number: 098-05-182), and all enrolled subjects provided written informed consent. The criteria for exclusion and inclusion were modified from our previous study (17). Finally, we excluded new diabetic patients and enrolled a total of 444 adults. We performed laboratory measurements (including PDIA4 levels) as described in detail in our previous study (17). We calculated the indices of β-cell function (HOMA-2β) and hepatic IR (HOMA-2IR) using HOMA Calculator v2.2.2. (23)

2.2 Cell culture and reagents

Mouse skeletal muscle cells (C2C12) were obtained from Bioresource Collection and Research Center (BCRC, Taiwan)

and cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco Laboratories, Grand Island, USA) containing 10% fetal bovine serum (FBS) (Gibco Laboratories) and antibiotics (24).

2.3 Cell differentiation

Mouse C2C12 myoblasts were maintained in DMEM supplemented with 10% FBS. C2C12 myotubes were obtained by culturing myoblasts in DMEM containing 2% heat-inactivated horse serum for at least four days. And use passages 3-5 to perform experiments at 80% confluency. We updated the medium before the experiment. Insulin and metformin were obtained from Sigma-Aldrich (St. Louis, MO, USA) for *in vitro* experiments (25).

2.4 Palmitate preparation

Prepare a 40 mmol/L palmitate (C16:0; Sigma) stock solution in ethanol. Before adding cells, dilute the palmitate solution in differentiation medium containing 1% (w/v) palmitate-free BSA (Sigma) to bind palmitate to bovine serum albumin (BSA) (26). We filter sterilize the solution before adding it to the cells. The control group in this study was BSA (27).

2.5 Cell glucose uptake

Cellular glucose uptake was measured using a glucose uptake assay kit (Abcam, Cambridge, MA, USA). C2C12 cells were seeded at 5×10^4 cells/well in 96-well plates (Corning, Inc., Corning, NY, USA) in DMEM containing 10% FBS. Use medium containing palmitate (250 μM) for 18 h. Afterwards, cells were treated with 10 nM insulin and 1 mM 2-deoxyglucose (2-DG) for 30 min. Finally, absorbance at 412 nm was measured using a BioTek Synergy HT plate reader (BioTek Instruments, Inc., Winooski, VT, USA) (28).

2.6 Cell viability assay

Cell viability was assessed using the MTT (3-(4,5)-dimethylthiahiazole-2-y1)-3,5-diphenytetrazolium bromide) assay. Set the MTT concentration to 5 mg/mL. Add MTT and incubate for 2 hours. The assay was terminated with DMSO. Finally measure the absorbance at 570 nm using an ELISA plate reader (29).

2.7 Lentiviral shRNA transfection and adenoviral infections

We obtained lentiviral-based PDIA4 and control shRNA from RNAi core of Academia Sinica, Taipei, Taiwan. We transfected the cells using PolyJet *In Vitro* DNA Transfection Reagent (SignaGen, Frederick, MD, USA) according to the manufacturer's protocol (30).

2.8 Quantitative PCR analysis

We extracted total RNA using Trizol (Invitrogen, Grand Island, NY, USA). We synthesized complementary DNA (cDNA) using a SuperScript® III Reverse Transcriptase kit (Invitrogen, Grand Island, NY, USA). We then performed real-time PCR for the genes of interest using SYBR green dye (Thermo, Wilmington, DE, USA) and the LightCycler® 480 System (Roche). Table 1 lists the primer sequences. We preheated the reaction mixture containing reverse transcribed cDNAs for 7 min at 95°C to activate the Taq polymerase. Next, we performed 40 PCR cycles, each consisting of a 10 s denaturation step at 95°C and a 30 s annealing step at 60°C (two-step RT-PCR) (31). Throughout the RT-PCR analysis, we confirmed product identities by melting curve analysis. The ratios of the amounts of target mRNA to the amount of the internal standard (GAPDH) mRNA was determined as an arbitrary unit.

2.9 ELISA

We incubated C2C12 cells (5×10^4 cells per well of a 96-well plate) with palmitate alone or combined with insulin. After incubation for 48 h, we collected the supernatants and quantified IL-6 (Catalog Number: 88-7064) and TNF-α (Catalog Number: 88-7324) using an ELISA Ready-Set-Go kit (eBioscience, San Diego, USA) according to the manufacturer's instructions (32).

2.10 Western blot analysis

We harvested whole cell lysates for western blotting using RIPA buffer (1% SDS and 10 mM Tris buffer, pH 7.4) containing protease inhibitors and a phosphatase inhibitor (Thermo, Wilmington, DE, USA). We quantified proteins in the supernatants using a Pierce BCA Protein Assay Kit (Thermo, Rockford, IL, USA). We separated 30 μg of proteins on a 5%–15% gradient SDS-PAGE gel and transferred them to polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA) by wet blotting using an electroblotter (Hoefer system). Next, we blocked the membranes for 1 h at 25°C with 2% BSA or 5% skim milk in tris buffered saline with tween 20 (TBST). We then incubated the membranes overnight at 4°C with appropriately diluted primary antibodies: PDIA4 (ERp72) antibody (2798 [1:1000 dilution]; Cell Signaling Technology, Danvers, MA, USA), Akt antibody (9272 [1:1000 dilution]; Cell Signaling Technology, Danvers, MA, USA), Phospho-Akt antibody (5724 [1:1000 dilution]; Cell Signaling Technology, Danvers, MA, USA), Akt antibody (9272

TABLE 1 Primer sequences used for real time RT-PCR.

IL-6	Forward sequence	GACAACTTTGGCATTGTGG
	Reverse sequence	ATGCAGGGATGATGTTCTG
TNF-α	Forward sequence	GCCTCTTCTCATTCCTGCTTG
	Reverse sequence	CTGATGAGAGGGAGGCCATT
PDIA4	Forward sequence	AAGGTGGTGGTGGGAAAG
	Reverse sequence	GATGTCGTTGGCAGTAGC
CHOP	Forward sequence	CTGCCTTT CACCTTGGAGAC
	Reverse sequence	CGTTTCCTGGGGATGA-GATA
XBP1	Forward sequence	GAATGGACACGCTGGATCCT
	Reverse sequence	GCCACCAGCCTTACTCCACTC
Bip	Forward sequence	TACATCTCATGGTGGAAAGTGTCTGTTTGA
	Reverse sequence	CATCCTCCTTCTTGTCCTCCTCCTCG
ATF4	Forward sequence	GAGCTTCCTGAACAGCGAAGTG
	Reverse sequence	TGGCCACCTCCAGATAGTCATC
GAPDH	Forward sequence	CCCATCACCATCTTCCAGGAGC
	Reverse sequence	CCAGTGAGCTTCCCGTTCAGC

[1:1000 dilution]; Cell Signaling Technology, Danvers, MA, USA), Phospho-IRS-1(Ser307) antibody (2381 [1:2000 dilution]; Cell Signaling Technology, Danvers, MA, USA), IRS-1 antibody (3407 [1:2000 dilution]; Cell Signaling Technology, Danvers, MA, USA), GAPDH antibody (5174 [1:2000 dilution]; Cell Signaling Technology, Danvers, MA, USA),. After washing them in TBST three times, we incubated the membranes for 60 min at 25°C with HRP-conjugated goat anti-rabbit or anti-mouse secondary antibodies. We visualized the signal using horseradish peroxidase-conjugated secondary antibodies and the enhanced chemiluminescence assay. Finally, we determined band intensities using a UVP imaging system (33).

2.11 Animal model

We obtained C57BL/6J mice from the National Laboratory Animal Center, bred them in-house, and used them in accordance with the guidelines of the National Defense Medical Center of the Laboratory Animal Center (NLAC, *Taipei*, Taiwan). We fed 8 week-old male C57BL6/J mice with a chow diet (10% kcal from fat), a HFD (60% kcal from fat) (34), or a HFD with metformin (200 mg/kg, intraperitoneal) for 16 weeks (n = 10 in each group).

2.12 Insulin tolerance test

ITT is designed to determine the whole body sensitivity of insulin receptors by measuring blood glucose levels changes before and after insulin administration. For the ITT, mice fasted for 6 h received an intraperitoneal injection of human insulin (1 U kg^{-1} body weight). We collected blood samples from the tail vein before the glucose challenge and 20, 40, 60, 80, and 100 min after it. We measured serum glucose levels using a Bayer Diabetes Care analyzer (35).

2.13 Immunohistochemistry

We deparaffinized and rehydrated 4 µm sections of formalin-fixed, paraffin-embedded tissues and retrieved the antigen. Next, we incubated the tissue sections with hydrogen peroxide for 10 min at room temperature to quench the endogenous peroxidase. After blocking in normal goat serum, we incubated them at 4°C overnight with the primary antibodies: PDIA4 antibody (A07267 [1:200 dilution]; Biocompare, CA, USA), Phospho-AMPK antibody (2535 [1:400 dilution] Cell Signaling Technology, Danvers, MA, USA), Phospho-Akt antibody (3787 [1:400 dilution] Cell Signaling Technology, Danvers, MA, USA), Phospho-IRS-1 antibody (2381 [1:200 dilution] Cell Signaling Technology, Danvers, MA, USA), following the manufacturers' recommendations. After washing the sections three times, we added the secondary biotinylated antibody, we incubated them for 30 min at 25°C, and added diaminobenzidine as a chromogen. Finally, we lightly counterstained these tissue sections with hematoxylin and examined them under an optical microscope. For each section examined, we counted the cells in five randomly selected fields.

We analyzed the results using Image-Pro Plus (Media Cybernetics, Crofton, MA, USA) (36).

2.14 Statistical analysis

All statistical analyses of human data were performed using SPSS version 20.0 for Windows (SPSS Inc., Chicago, IL, USA). A P-value inferior to 0.05 was considered to indicate statistical significance. HOMA-2IR levels were divided into tertiles, with cutoff values for the tertiles of 1.59 and 2.87. We compared these tertile groups using one-way analysis of variance and the chi-square test. Relationships between PDIA4 and other variables (both anthropometric and biochemical) were analyzed by Spearman's rank-order correlations. For *in vitro* data, represented as the means ± SEM, we used Student's t-test to compare group pairs and one-way ANOVA to compare multiple groups. Statistical significance was evaluated with GraphPad Prism 6.01. Statistical significance was assumed at the 5% α-error level ($P < 0.05$).

3 Results

3.1 Human serum PDIA4 concentrations are associated with IR and inflammatory cytokines in subjects with normal glucose tolerance and impaired glucose tolerance

Table 2 shows the characteristics of the participants separated by tertiles of homeostatic model assessment 2 (HOMA-2) IR levels. The three groups had significant differences in all anthropometric and basic biochemical values except for age, systolic blood pressure, total cholesterol, LDL, creatinine, high-sensitivity C-reactive protein (hs-CRP) and interleukin 6 (IL-6) levels. The patients in the second and third tertile of HOMA-2 IR levels had higher PDIA4 levels than those in the first tertile ($P < 0.01$). In addition, we evaluated the association between serum PDIA4 levels and all anthropometric values (Table 3). The serum PDIA4 levels showed a significant positive correlation with HOMA-2 IR and IL-6.

TABLE 2 Anthropometric and biochemical data of the participants (n = 444).

	HOMA-2IR, Tertile[a]			
	T1	T2	T3	P-value
	n = 148	n = 148	n = 148	
Age (years)	48.12 ± 14.06	49.31 ± 13.44	48.25 ± 15	0.731
BMI (kg/m^2)	22.72 ± 3.26	24.29 ± 3.12	26.46 ± 3.81	< 0.001
WC (cm)	80.09 ± 9.61	84.41 ± 8.41	88.84 ± 10.02	< 0.001
SBP (mmHg)	126.84 ± 16.9	125.77 ± 17.1	129.25 ± 16	0.186
DBP (mmHg)	79.3 ± 10.14	79.62 ± 10.91	82.51 ± 10.33	0.015
Glucose 0' (mg/dL)	89.58 ± 13.0	92.87 ± 11.71	101.06 ± 13.37	< 0.001
Glucose 120' (mg/dL)	129.03 ± 36.32	127.17 ± 34.77	144.98 ± 35.97	< 0.001
Insulin 0' (μIU/mL)	9.63 ± 4.92	12.78 ± 18.13	18.62 ± 14.29	< 0.001
Insulin 120' (μIU/mL)	56.97 ± 35.3	82.44 ± 54.03	124.18 ± 91.14	< 0.001
HOHA-2B	83.44 ± 124.82	140.34 ± 89.9	223.12 ± 182.57	< 0.001
HOMA-2IR	1.15 ± 0.31	2.14 ± 0.34	4.76 ± 2.04	< 0.001
HbA1C (%)	5.58 ± 0.43	5.66 ± 0.35	5.89 ± 0.52	< 0.001
TC (mg/dL)	190.58 ± 32.59	192.28 ± 39	194.11 ± 34.41	0.750
LDL-C (mg/dL)	129.41 ± 29.64	128.41 ± 40.42	130.1 ± 33.03	0.957
HDL-C (mg/dL)	59.17 ± 18.88	53.04 ± 15.3	51.39 ± 12.22	0.006
TG (mg/dL)	108.79 ± 59.1	124.63 ± 76.22	149.94 ± 77.72	< 0.001
Creatinine (mg/dL)	0.86 ± 0.59	0.83 ± 0.32	0.8 ± 0.17	0.523
Uric acid (mg/dL)	5.71 ± 1.66	5.58 ± 1.35	6.18 ± 1.34	0.015

(Continued)

TABLE 2 Continued

	HOMA-2IR, Tertile[a]			
	T1	T2	T3	P-value
ALT (U/L)	21.83 ± 12.19	27.56 ± 21.68	39.5 ± 59.32	< 0.001
hsCRP (ng/mL)	1.08 ± 1.58	1.91 ± 5.97	2.09 ± 2.46	0.054
IL-6 (pg/mL)	1.7 ± 3.08	1.58 ± 2.12	2.11 ± 3.14	0.240
PDIA4 (ng/mL)	11.89 ± 11.64	15.12 ± 17.03	22.1 ± 19.94	< 0.001

[a]HOMA-2IR tertiles according to cutoff values of 1.59 and 2.87.
Data are expressed as mean ± SD.
BMI, body mass index; WC, Waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1C, Glycated hemoglobin; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

3.2 Palmitate can induce IR through disturbed insulin signalling, glucose uptake and increased inflammatory cytokines in C2C12 cells

Several studies reported that palmitate-induced IR or ER stress in obese conditions. First, we starved C2C12 myotubes from serum for 4 h and then incubated them with 0.6 mM of palmitate for another 24 h to mimic IR conditions. Next, to assess insulin action, we stimulated the cells with 100 nM insulin for a further 15 min. Then, we examined the effects of insulin signaling. We found that palmitate impaired insulin signaling. In the presence of insulin, palmitate increased the levels of phosphorylated insulin receptor substrate-1 (p-IRS-1(307), phosphorylated on serine 307)—which might contribute to IR—and decreased phosphorylated-Akt (p-Akt), while palmitate alone did not (Figures 1A–C). In addition, palmitate decreased glucose uptake in the presence of insulin (Figure 1D), but it did not affect cell viability, regardless of the presence of insulin (Figure 1E). Next, we examined the levels of the inflammatory cytokines IL-6 and tumor necrosis factor-α (TNF-α). In the presence of insulin, palmitate significantly increased IL-6 and TNF-α gene expression compared with palmitate alone (Figures 1F, G). We observed similar results for the IL-6 and TNF-α protein expressions (Figures 1H, I). Thus, palmitate can induce IR through disturbed insulin signaling and glucose uptake and further increase inflammatory cytokines levels in C2C12 cells.

3.3 Increased PDIA4 expression and ER stress markers in C2C12 cells with palmitate-induced IR

Next, we explored the role of PDIA4 in palmitate-induced IR in C2C12 cells. Figure 2 shows that palmitate-treated cells had significantly higher PDIA4, BiP/GRP78, and ATF4 gene expression levels than the control group. In the presence of insulin, palmitate significantly increased PDIA4, CHOP, BiP/GRP78, and ATF4 gene expression compared with palmitate alone (Figure 2). We observed similar results for the protein expressions of PDIA4, BiP/GRP78, and ATF4 (Figure S1). Thus,

TABLE 3 Correlations between serum PDIA4 and clinical variables.

	Serum PDIA4	
	r	p
Age (years)	−0.045	0.349
BMI (kg/m^2)	0.405	<0.001
WC (cm)	0.39	<0.001
SBP (mmHg)	0.098	0.041
DBP (mmHg)	0.215	<0.001
Glucose 0' (mg/dL)	0.194	<0.001
Glucose 120' (mg/dL)	0.213	<0.001
Insulin 0' (μIU/mL)	0.37	<0.001
Insulin 120' (μIU/mL)	0.325	<0.001
HOHA-2B	0.078	0.103
HOMA-2IR	0.274	<0.001
HbA1C (%)	0.176	<0.001
TC (mg/dL)	0.036	0.502
LDL-C (mg/dL)	0.137	0.051
HDL-C (mg/dL)	−0.31	<0.001
TG (mg/dL)	0.327	<0.001
Creatinine (mg/dL)	0.048	0.367
Uric acid (mg/dL)	0.244	<0.001
ALT (U/L)	0.619	<0.001
hsCRP (ng/mL)	0.253	<0.001
IL-6 (pg/mL)	0.154	0.001

BMI, body mass index; WC, Waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1C, Glycated hemoglobin; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

cells incubated with insulin and palmitate expressed higher PDIA4 levels than those incubated with palmitate only, suggesting that PDIA4 may be an important ER stress marker in skeletal muscle IR.

3.4 PDIA4 knockdown decreased palmitate-induced IR and inflammation in C2C12 cells

To clarify the role of PDIA4 in IR, we inhibited PDIA4 expression in C2C12 cells with short hairpin RNA (shRNA). Figure 3A shows the PDIA4 knockdown efficiency. Then, we examined the effects on insulin signaling. PDIA4 knockdown cells incubated with insulin had expressed lower p-IRS-1(307) and higher IRS-1 and p-Akt levels than those incubated with palmitate alone (Figures 3B–E). However, in the absence of insulin, the PDIA4 knockdown decreased IRS-1 and p-Akt expression (Figure S2), suggesting that it restored insulin signaling. Next, we examined the glucose uptake ability. PDIA4 knockdown cells had a higher glucose uptake ability than those treated with palmitate alone (Figure 3F). Furthermore, knockdown PDIA4 cells had lower palmitate-induced IL-6 and TNF-α gene expressions than non-knockdown cells (Figures 3G, H). Besides, we observed similar results for the protein expressions of IL-6 and TNF-α (Figures 3I, J). Overall, knocking down PDIA4 mitigated IR and decreased inflammatory cytokines expression in C2C12 cells.

3.5 Metformin modulated PDIA4 expression and palmitate-induced IR in C2C12 cells

Metformin is a common clinical treatment for IR (37). Thus, we explored whether metformin mitigated IR through PDIA4. We treated C2C12 with 1, 3, or 5 mM metformin after incubating them with palmitate and insulin. Metformin significantly decreased PDIA4 expression in the doses of 3 and 5 mM (Figures 4A, B) and on the time of 60 minutes (Figures 4C, D). Furthermore, in cells treated with insulin and palmitate, metformin decreased p-IRS-1(307) and increased IRS-1 and p-Akt expression (Figures 4E–G). Next, we assessed the effect of metformin on IR through PDIA4 by treating PDIA4 knockdown C2C12 cells with metformin after culturing them with palmitate and insulin. The result showed PDIA4 knockdown, and metformin have mild additive effects on the suppression of PDIA4 expression (Figures 5A, B). PDIA4 knockdown cells were more sensitive to the metformin-induced decrease in p-IRS-1(307) and increase in IRS-1 and p-Akt expression than normal C2C12 cells (Figures 5A, C, D). Moreover, the metformin-induced increase in glucose uptake was higher in the PDIA4 knockdown cells than in normal C2C12 cells (Figure 5E). Collectively, these results suggested that metformin can strengthen the inhibition of PDIA4 expression and improve IR and glucose uptake.

3.6 Metformin decreased PDIA4 expression and mitigated IR in a high-fat diet-induced mouse obesity model

HFDs affect skeletal muscle function and cause IR in mice (38). Metformin inhibits the mitochondrial respiratory chain, activating the AMP-activated protein kinase (AMPK) and enhancing insulin sensitivity (39). To establish a causal relationship between metformin, obesity, and inflammation in an animal model, we administered metformin (200 mg/kg) through intraperitoneal injection to C57BL6/J mice with HFD-induced obesity (HFD, 60% kcal from fat) and mice fed a chow diet (10% kcal from fat) as a control group. As expected, metformin improved basal blood glucose levels in HFD mice (Figure 6A). Likewise, metformin-treated mice exhibited significantly lower glucose concentrations than untreated mice in the insulin tolerance test (ITT) (Figure 6B). Next, we examined the expression levels of p-IRS-1(307), p-Akt, PDIA4, and p-AMPK by immunohistochemistry in mice soleus muscle tissues. As expected, metformin decreased p-IRS-1(307) and PDIA4 expression and increased p-Akt and p-AMPK expression in HFD mice (Figures 6C, D), suggesting that metformin improved skeletal muscle IR may by increasing AMPK and decreasing PDIA4 expression. Based on the above results, palmitate-induced IR increased PDIA4 and inflammatory cytokines expression, impaired insulin signaling, and reduced glucose uptake. Meanwhile, the PDIA4 knockdown decreased inflammatory cytokines levels and mitigated IR. Furthermore, PDIA4 participates in the metformin-induced effects on skeletal muscle IR (Figure 7).

4 Discussion

Chronically elevated FFA levels may contribute to IR progression. FFA and their metabolites can also interfere with insulin signaling and inhibit insulin-stimulated glucose uptake and glycogen synthesis (40). Excessive FFA levels in the blood increase lipid metabolites accumulation in skeletal muscle, leading to IR (41). The skeletal muscle is the primary site for insulin-stimulated glucose disposal and is sensitive to the insulin action mediated by elevated fatty acid availability in the human body (42). In this study, we utilized a well-established IR model by treating C2C12 myotubes with palmitate and insulin (43). Some studies showed that palmitate increased serine phosphorylation of IRS-1 and apoptosis in rat insulinoma cells and decreased Akt phosphorylation and glucose uptake (43, 44).

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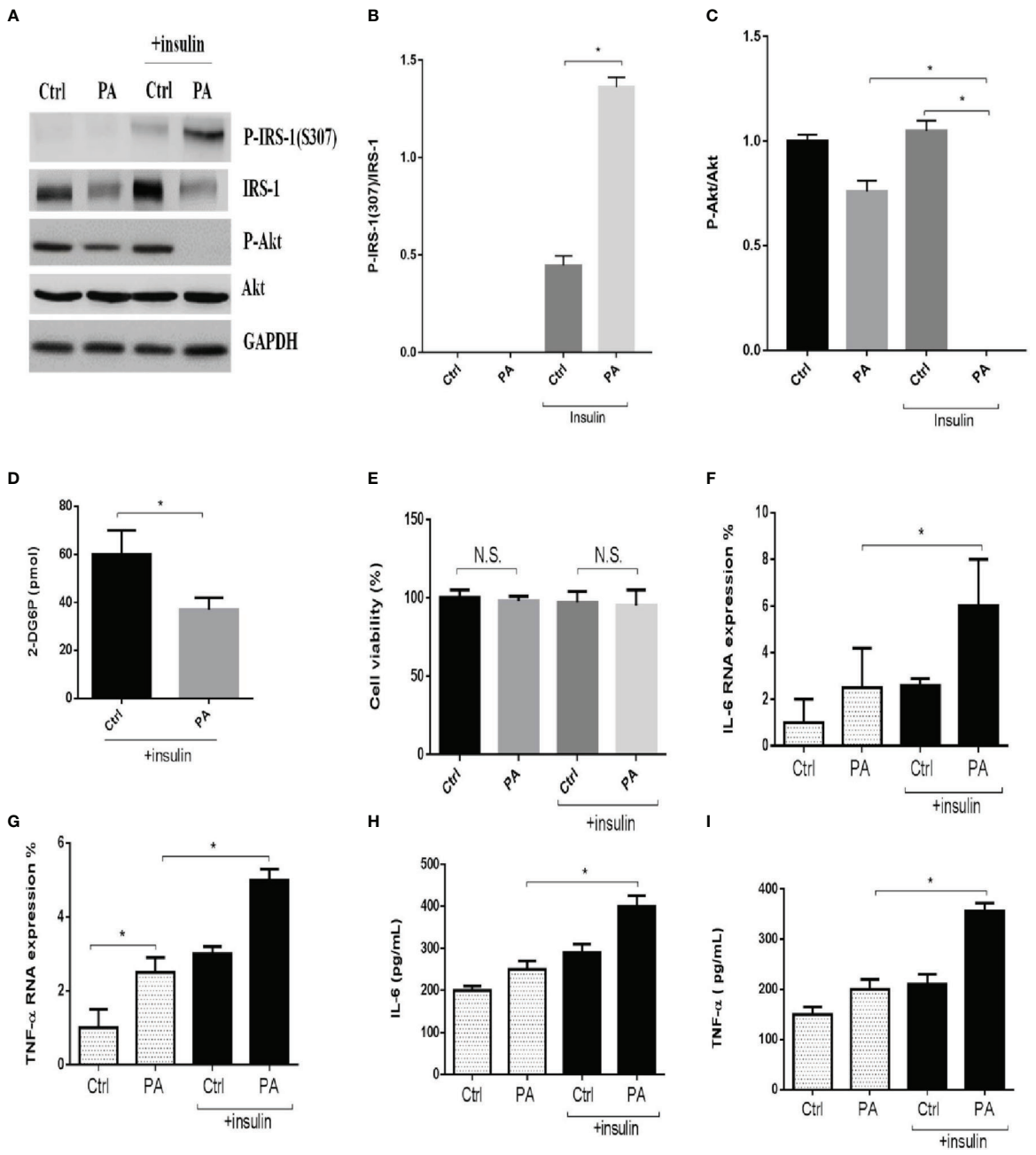

FIGURE 1
Palmitate disturbed insulin signaling and glucose uptake and increased inflammatory cytokines in C2C12 cells. The C2C12 myotubes treated with 0.6 mM of palmitate (PA) with or without 100 nM insulin, then estimated p-IRS-1(307), p-Akt by western blotting **(A-C)** and 2-Deoxyglucose (2-DG) uptake was assessed **(D)**. In addition, cell viability was measured by MTT **(E)**. The levels of **(F, G)** IL-6, TNF-α were analyzed by real-time PCR, **(H, I)** IL-6, TNF-α were analyzed by ELISA after C2C12 myotubes treated with 0.6 mM of palmitate (PA) with or without 100 nM insulin. All data are presented as the mean ± SD (n = 3 for each group); *P < 0.05. N.S, no significant.

Meanwhile, metformin attenuated the palmitate-induced increase in apoptosis and BiP/GRP78 and PDI expression (44).

In addition, c-Jun N-terminal kinase (JNK)-dependent serine (307) phosphorylation of IRS-1 is a key link between ER stress and IR (45). Chronic inflammation, which is related to IR and obesity, can be initiated by excessive lipid deposition in muscle, fat, and liver (46). The previous report showed palmitate-induced C/EBP homologous protein activation leads to NF-κB-mediated increase in β-site amyloid precursor protein cleaving enzyme 1 activity and amyloid beta genesis in neuroblastoma cells (47). Moreover, Tae

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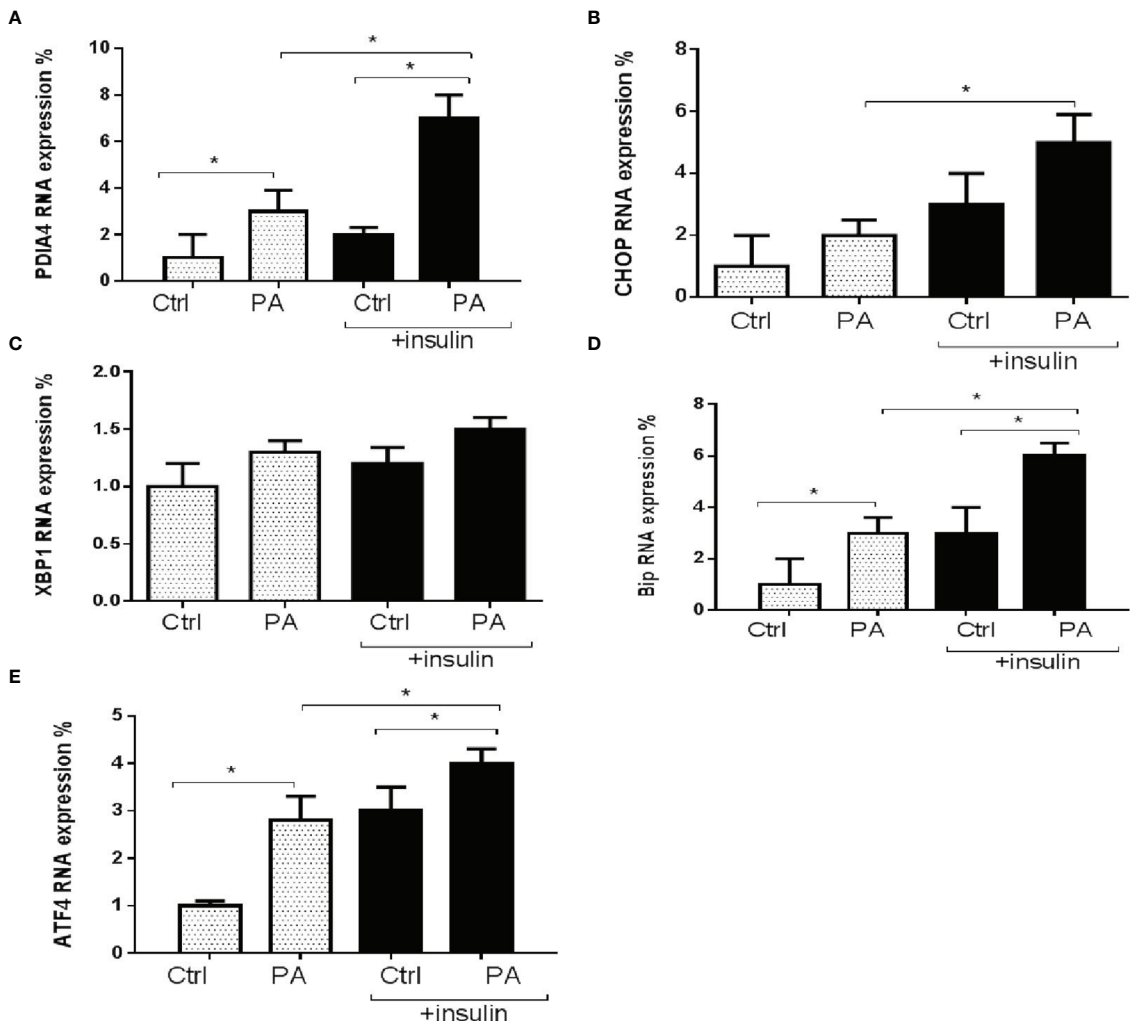

FIGURE 2
Palmitate increased PDIA4 and other ER stress markers in C2C12 cells. The levels of **(A)** PDIA4, **(B)** CHOP, **(C)** XBP1, **(D)** Bip, and **(E)** ATF4 mRNA were analyzed by real-time PCR after C2C12 myotubes treated with 0.6 mM of palmitate (PA) with or without 100 nM insulin. All data are presented as the mean ± SD (n = 3 for each group); *P < 0.05.

Woo Jung et al. revealed palmitate-induced aggravation of insulin signaling markers, such as IRS-1 and Akt phosphorylation, and inflammatory markers, such as NF-κB and IκB phosphorylation in C2C12 myocytes (48) . In our study, palmitate can induce IR through disturbed insulin signaling and glucose uptake and further increase inflammatory cytokines levels in C2C12 cells. The possible mechanisms by which palmitate induces inflammation in C2C12 cells, maybe through activating the NF-κB pathway. Several studies have demonstrated the role of ER stress to mediate palmitate-induced IR in muscle cells. These have shown that direct exposure of human primary myotubes, C2C12 myotubes, or L6 myotubes to palmitate can induce ER stress (49). Another study showed the palmitate-induced pathway driving the migration of the cancer cells through the loss of desmoplakin mediated by activation of the IRE1-XBP1 pathway and zinc finger E-box binding homeobox transcription factors (50). Palmitate disrupts erythropoietin production by activating the transcription factor ATF4, which is involved in the UPR (51). Besides, palmitate and insulin also increased ATF4, CHOP, XBP1, and BiP/GRP78 expressions in our skeletal muscle IR model. However, little is known about the link between FFA metabolism and ER stress-related effects on PDIA4 and IR. Our study showed that palmitate and insulin increased PDIA4, inflammatory cytokines, and p-IRS-1 levels and decreased p-Akt levels and 2-DG uptake in a skeletal muscle IR cell model. Furthermore, mice with HFD-induced IR had elevated expression levels of p-IRS-1(307) and PDIA4 in soleus muscle tissues.

The PDI family plays a role in mammalian development and in various diseases. The nine human PDI family members contain one to three CGHC active sites (52). Among them,

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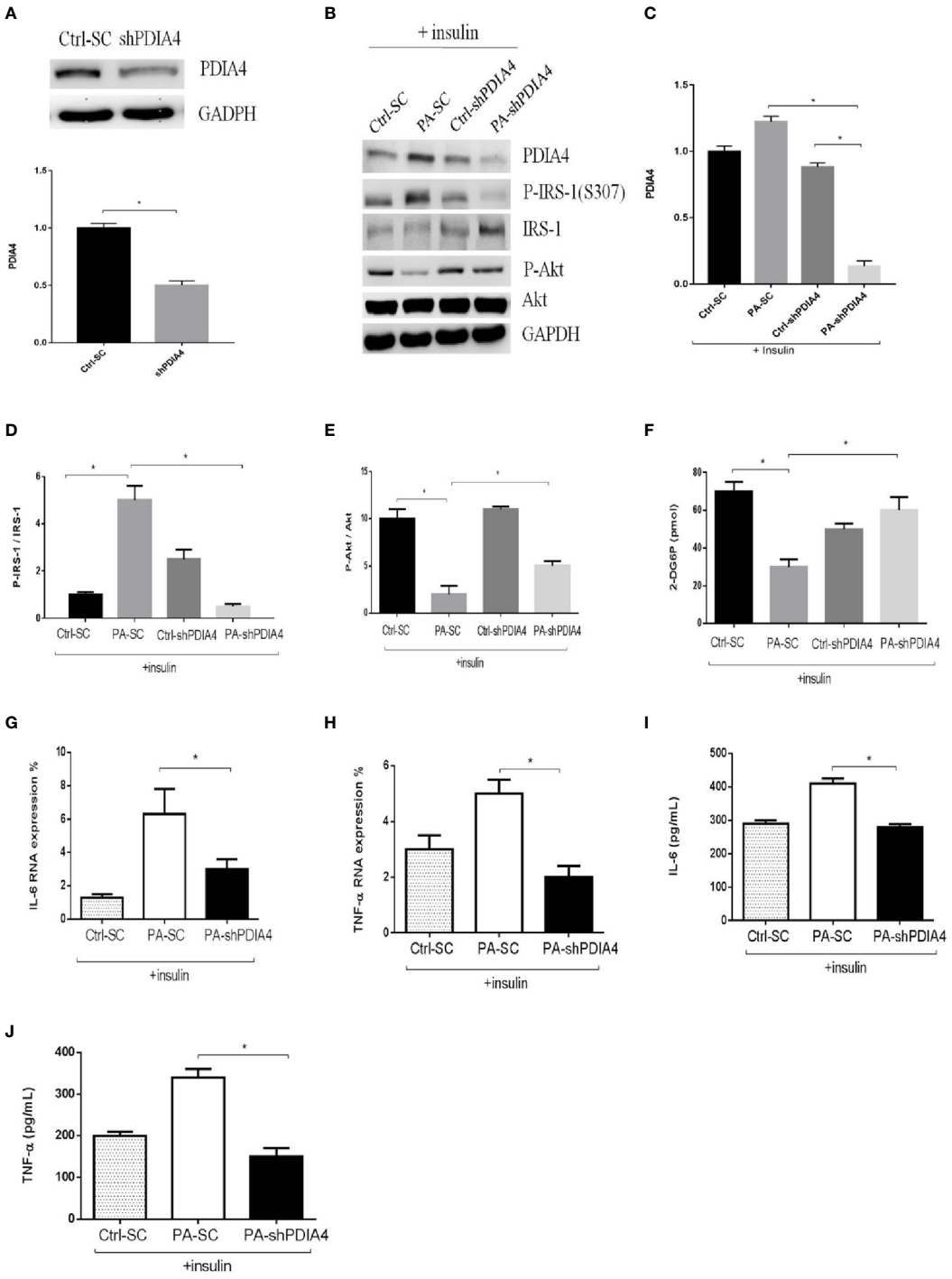

FIGURE 3
Knockdown PDIA4 inhibited palmitate induced IR, inflammatory cytokines and increased glucose uptake in C2C12 cells. **(A, B)** The protein levels of PDIA4 in C2C12 myotubes stably transfected with scrambled control (SC) or shRNA against PDIA4 (KD) were tested by western blot. **(C-E)** Knockdown PDIA4 in C2C12 myotubes, and treated with 0.6 mM of palmitate (PA) with 100 nM insulin, then estimated p-IRS-1(307), p-Akt by western blotting. **(F)** 2-Deoxyglucose (2-DG) uptake was assessed. The levels of **(G, H)** IL-6, TNF-αwere analyzed by real-time PCR, **(I, J)** IL-6, TNF-αwere analyzed by ELISA. All data are presented as the mean ± SD (n = 3 for each group); *P < 0.05.

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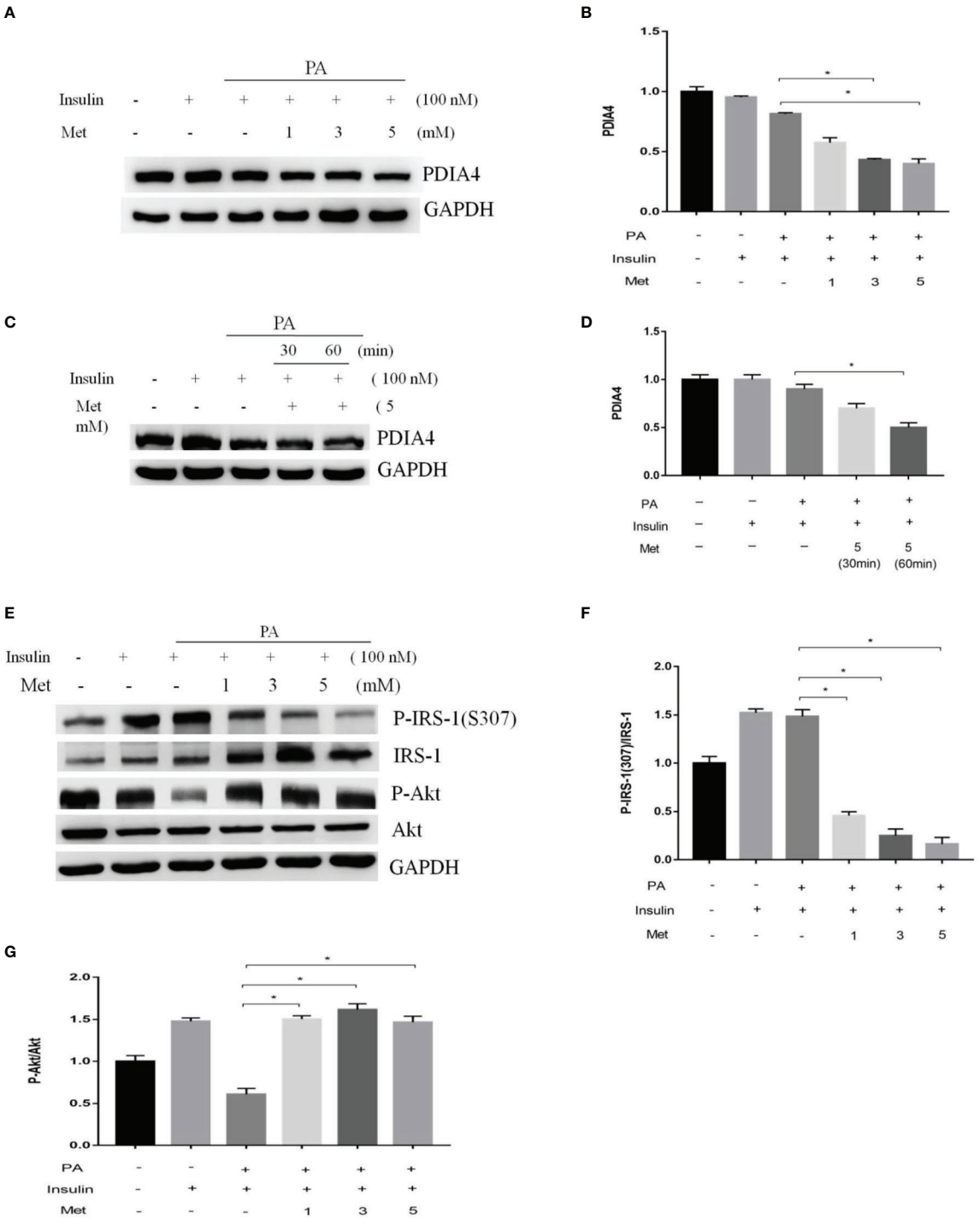

FIGURE 4
Metformin modulated PDIA4 expression and palmitate-induced IR in C2C12 cells. **(A, B)** The C2C12 myotubes treated 1,3,5 mM metformin with 100 nM insulin, then estimated PDIA4 expression by western blotting. **(C, D)** The C2C12 myotubes treated 5 mM metformin with palmitate and insulin, then estimated PDIA4 expression by western blotting. **(E–G)** The C2C12 myotubes treated 1,3,5 mM metformin with palmitate and insulin, then estimated p-IRS-1(307), p-Akt by western blotting. All data are presented as the mean ± SD (n = 3 for each group). *P < 0.05.

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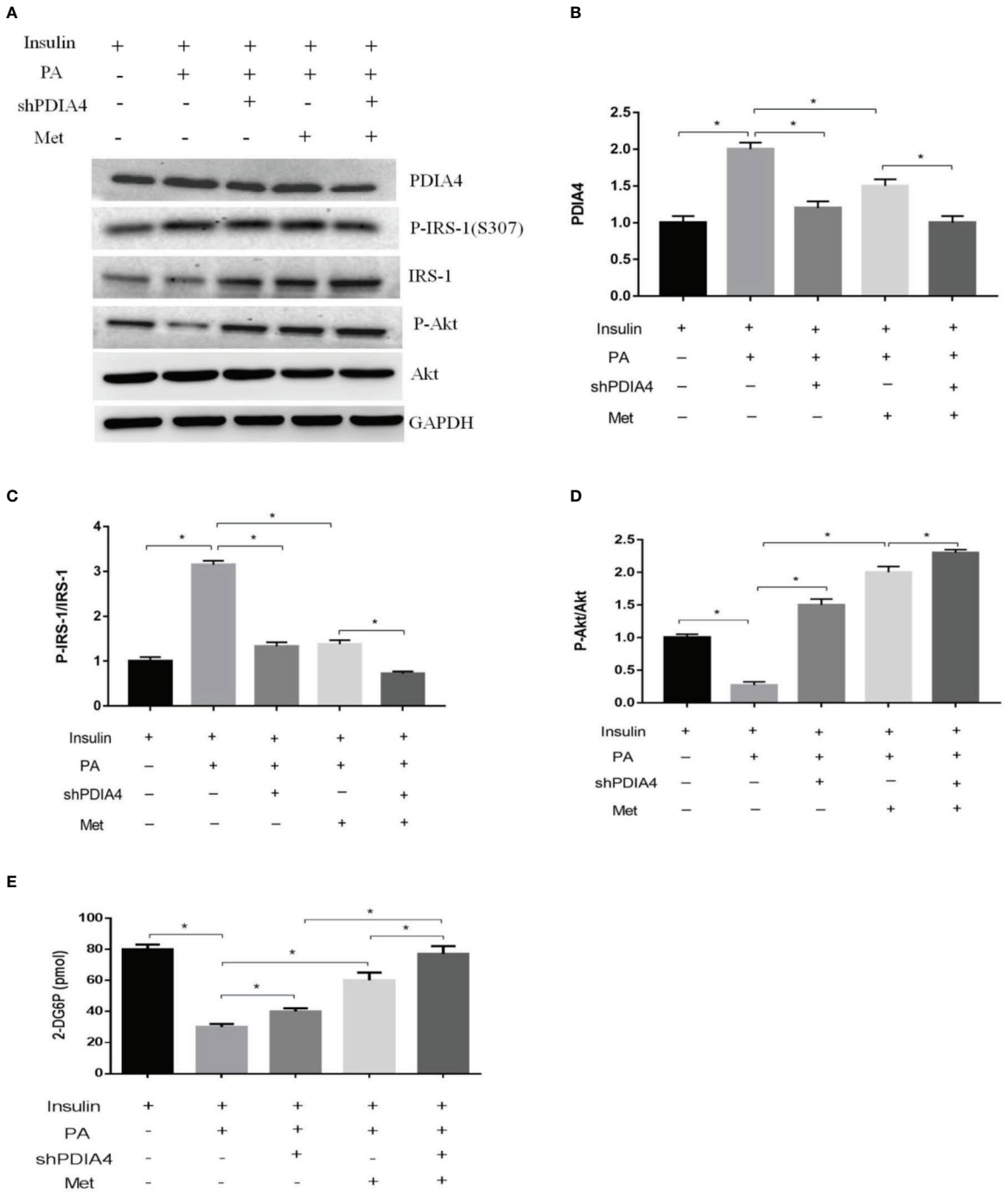

FIGURE 5
Metformin and PDIA4 knockdown in palmitate-induced IR and glucose uptake in C2C12 cells. **(A)** The PDIA4 knockdown of C2C12 myotubes treated metformin with palmitate and insulin, then estimated PDIA4, p-IRS-1(307), p-Akt by western blotting. **(B-D)** Quantization from western blot PDIA4, p-IRS-1(307), p-Akt. **(E)** 2-Deoxyglucose (2-DG) uptake was assessed knockdown PDIA4 in C2C12 myotubes treated metformin with palmitate and insulin. All data are presented as the mean ± SD (n = 3 for each group). *P < 0.05.

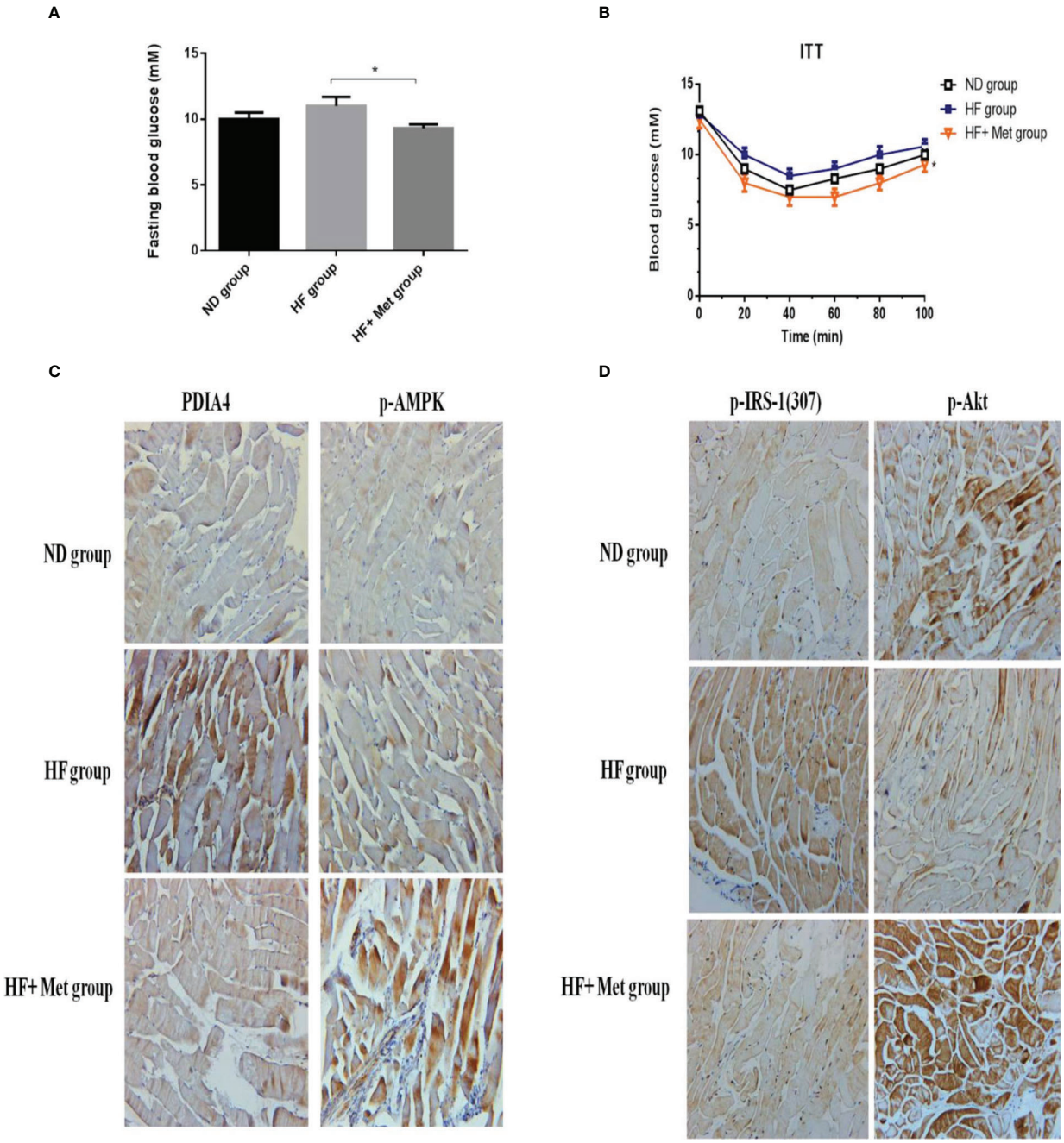

FIGURE 6
Metformin decreased PDIA4 and improved IR in high-fat diet induced obesity mice model. Administered metformin (200mg/kg) through intraperitoneal injection into high fat diet (HFD, 60% kcals from fat) induced obesity in C57BL6/J mice, and a chow diet (10% kcals from fat) as control group, and then examined **(A, B)** basal blood glucose, and insulin tolerance. **(C, D)** The mice soleus muscle tissues were examined PDIA4, P-AMPK, P-IRS-1(307), and P-Akt by immunohistochemistry, 20X. Representative data were shown from experiments independently performed at least three times. *P < 0.05.

only PDIA4 has three CGHC motifs. Most of the PDIs have an ER retention motif (53) . However, increasing data show that PDIs have also been found in locations outside the ER, including the cell surface, nucleus, cytoplasm, and extracellular space (54) and plasma of different cell types. Accordingly, PDI is a multifunctional protein including in a variety of redox-related intracellular and extracellular events and functions such as other ER chaperones (55, 56). Previous studies revealed BiP/GRP78,

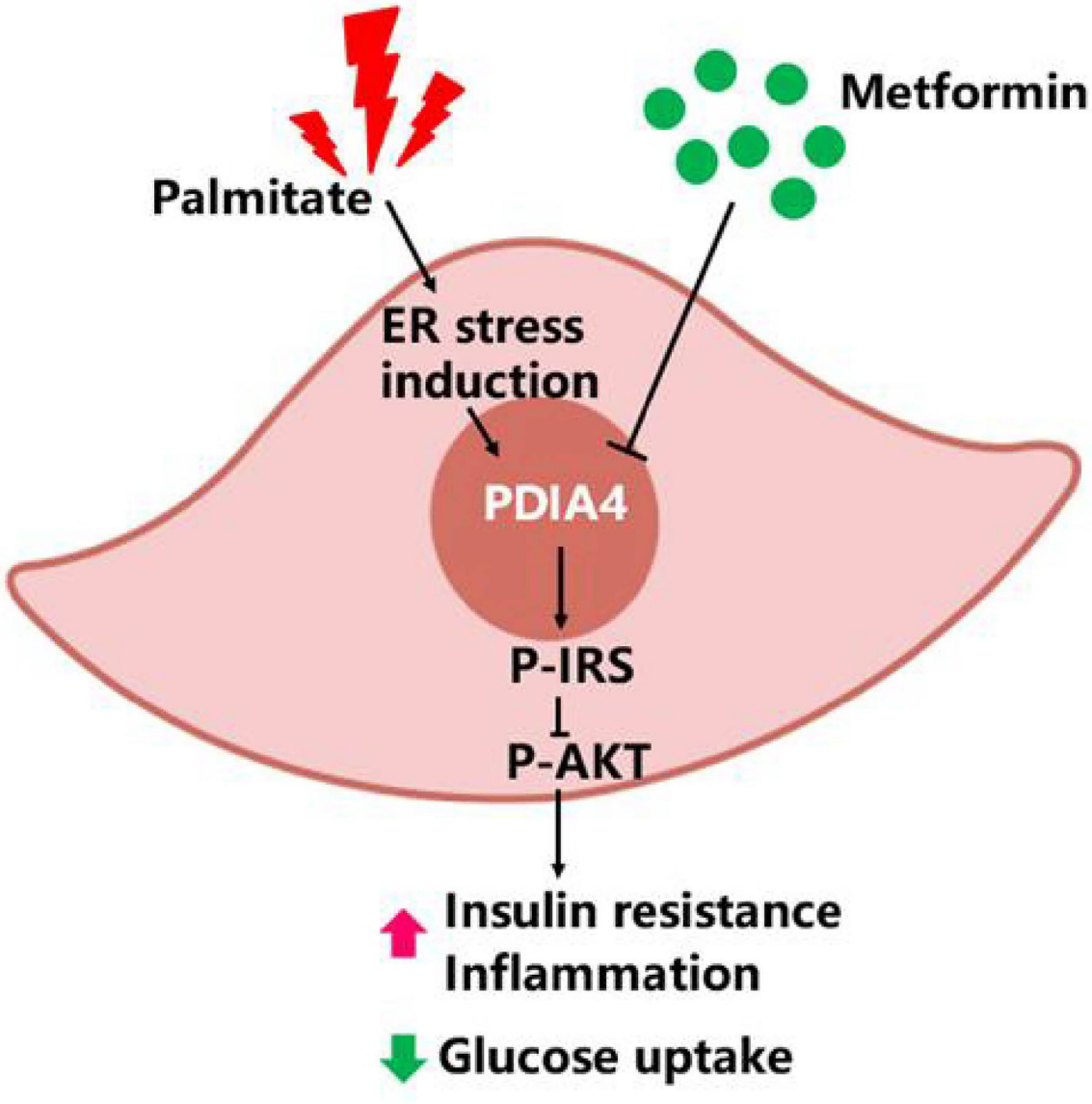

FIGURE 7
PDIA4 in skeletal muscle insulin resistance (IR). Palmitate induced IR increased PDIA4 and inflammatory cytokines, and then impaired insulin signaling and reduced glucose uptake. PDIA4 knockdown decreased inflammatory cytokines and improved IR. Furthermore, PDIA4 was involved in metformin improved skeletal muscle IR and suggested that PDIA4 may be a novel therapeutic target for directly alleviating IR.

an ER chaperone, has been detected in cell membranes, where it acts as a multireceptor and signal receptor transducer and mediates other functions (57). Bip/GRP78 was released into culture medium from challenged cells to induce ER stress. A soluble part of the BiP/GRP78 protein can be detected in circulation, probably due to active secretion rather than simply a result of cell necrosis or apoptosis (58, 59). Moreover, the circulating BiP/GRP78 levels are significantly increased in people with DM, obesity, and its associated metabolic alterations (60) . Recent study indicated that PDIA4 was distributed in the nuclei, cytosol, membrane, mitochondria, and ER of Min6 β-cells and serum PDIA4 also went up with diabetes development in HFD-fed B6 mice, and diabetic patients (16). Our previous report revealed that subjects with metabolic syndrome had significantly higher serum PDIA4 levels than those without metabolic syndrome. Furthermore, the individuals in the highest PDIA4 tertile had significantly higher waist circumference, blood pressure, fasting glucose concentration, and serum triglycerides than those in the lowest tertile (17). In this study, we used our previous data set but excluded new

diabetic patients. We found that serum PDIA4 levels were associated with IR and inflammatory cytokines levels in subjects with normal or impaired glucose tolerance. These results were consistent with our recent report (61). Moreover, individuals in the second and third tertile of HOMA-2 IR levels had higher PDIA4 levels than those in the first tertile. Furthermore, in this study's population, the serum PDIA4 levels had a significant positive correlation with HOMA-2 IR and IL-6 levels.

PDI regulates biological processes, such as protein folding, signal transmission, and cell communication, involving the interaction between PDI and substrate proteins (55, 62). Recently, PDIA1 has been characterized as a molecular chaperone to activate estrogen receptor *via* stabilizing the receptor (55, 63). Besides, ablating PDIA4 reduced the symptoms of diabetes, such as elevated blood sugar and HbA1C levels, in diabetic mice (16). However, the role of PDIA4 in skeletal muscle IR remained unknown. Our PDIA4 knockdown experiment results showed that PDIA4 participates in skeletal muscle IR. Indeed, knocking down PDIA4 decreased inflammatory cytokines and p-IRS-1 levels and increased Akt phosphorylation and 2-DG uptake in palmitate and insulin-treated C2C12 myotubes. In summary, knocking down PDIA4 expression mitigated palmitate-induced IR, glucose uptake and inflammation in C2C12 cells. IRS-1 is a substrate of the insulin receptor and the phosphorylation of serine residues in IRS-1 plays a critical role in the insulin-stimulated signaling pathway. The possible mechanisms of PDIA4 action in insulin signaling and GLUT4-mediate glucose uptake might be through the interaction between PDIA4 and IRS-1, Akt substrate proteins, or insulin receptor then disturbed the insulin signaling and glucose management in palmitate-treated C2C12 cells subsequently.

Metformin is an effective hypoglycemic drug (64) and a widely used insulin sensitizer that lowers blood glucose concentrations by decreasing hepatic glucose production and increasing glucose disposal in skeletal muscle. However, the molecular mechanism of metformin action is not well understood. Previous reports showed that metformin regulated systemic glycemia by stimulating AMPK in the liver and skeletal muscle and enhanced insulin signaling in skeletal muscle IR by increasing IRS-1 and Akt activity (19). The JNK-dependent phosphorylation of the serine 307 of IRS-1 is a key link between ER stress and IR. In rat insulinoma cells, metformin attenuated ER stress, IRS-1 phosphorylation, and apoptosis (44). Other studies indicated that metformin suppresses ER stress through the AMPK- PI3K-c-Jun NH2 pathway in NIT-1 cells (65), reduces ER stress-induced brain injury, and inhibits apoptosis by regulating the protein kinase R (PKR)-like ER kinase (PERK)-eIF2α-ATF4-CHOP pathway (66). Recently, our report revealed the clinical insulin sensitizer metformin modulates PDIA4 and adiponectin expression and improves obesity-associated conditions in both *in vitro* adipocytes and *in vivo* mouse models (67). However, the role of ER stress-related PDIA4 in the effect of metformin on skeletal muscle IR remained elusive. Our results demonstrated that metformin decreased PDIA4 expression in a time- and concentration-dependent manner, decreased p-IRS-1(307), and increased p-Akt in IR skeletal muscle. Moreover, our data revealed that knocking down PDIA4 potentiated the additive effect of metformin on IRS-1 and Akt phosphorylation and 2-DG uptake. However, it is known that metformin does decrease ER stress. Our study revealed that metformin may alleviate skeletal muscle ER and at least partially through inhibiting PDIA4 expression.

Skeletal muscle is the major site for insulin-stimulated glucose disposal, and muscle IR has many adverse health outcomes. Feeding rodents with an HFD for several weeks or months induces IR (68). Male C57BL/6J mice on an HFD providing 60 kcal energy from fat for nine weeks display impaired insulin sensitivity and chronic inflammation (69). Metformin alleviates skeletal muscle IR in an HFD-induced IR rat model (70). In our animal experiments, we observed substantial body weight differences between the HFD and HFD with metformin groups (data not shown), indicating that metformin had beneficial effects on body weight gain without affecting daily food intake (data not shown). Besides, the ITT experiment showed that metformin improved IR in HFD-fed mice. One mechanism mediating IR may involve the phosphorylation of serine residues in IRS-1, preventing IRS-1 from activating downstream PI3K-Akt-dependent pathways (71). IRS-1 is a substrate of the insulin receptor and plays a central role in the insulin-stimulated signal transduction pathway. Some studies showed that metformin does not influence β-cell secretion of insulin or IRS-1 in skeletal muscle, whereas one study reported decreased IRS-1 in soleus muscle after metformin treatment (72). In our animal experiments, metformin decreased p-IRS-1 and increased p-Akt in the soleus muscle. A previous report showed that the metformin-induced AMPK activation decreases cardiac injury during ER stress by preventing CHOP expression in C57BL/6 mice (73). Our *in vivo* animal experiments demonstrated that metformin increased AMPK and decreased PDIA4 expression in the soleus muscle. However, it is known that metformin does increase AMPK activation, the decreased PDIA4 may still be an indirect effect of the decrease in ER stress.

The present study has several limitations. First, our human clinical data only showed an association between PDIA4 and IR and inflammatory cytokines. Determining the effect of metformin on PDIA4 concentrations requires further studies. Second, an animal model with PDIA4 conditional knockout in skeletal muscle could confirm the direct causal relationship between metformin, PDIA4, and skeletal muscle IR. Third, in this study we only used genetic inhibition of PDIA4 in *in vitro* cell model. In the future, we will find and use PDIA4 specific inhibitor as pharmacological inhibition for future studies. Fourth, physiological insulin is well under 1nM, but the concentration of insulin added in this cell experiment is 100 nM.

The correlation between the results of the cell experiment and clinical application still needs more research to be carried out.

In conclusion the study is the first to explore the role of PDIA4 in IR skeletal muscle. We elucidated that palmitate-induced IR increased PDIA4 and inflammatory cytokines expression, impaired insulin signaling, and reduced glucose uptake in skeletal muscle cells. Conversely, knocking down PDIA4 decreased inflammatory cytokines expression and mitigated skeletal muscle IR. Furthermore, we demonstrated that metformin may mitigate skeletal muscle IR and at least partially through inhibiting PDIA4 expression. More importantly, our results suggest that PDIA4 is a novel therapeutic target for directly alleviating skeletal muscle IR. In addition,

Data availability statement

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

Ethics statement

Tri-Service General Hospital approved this study (institutional review board approval number: 098-05-182). The patients/participants provided their written informed consent to participate in this study. The animal study was reviewed and approved by National Defense Medical Center of the Laboratory Animal Center (NLAC, Taipei, Taiwan).

Author contributions

C-HsL, C-FC, Y-SS contributed to conception and design of the study. F-HL, F-CK, S-CS organized the database. C-LH, P-FL, J-SL performed the statistical analysis. C-FC wrote the first draft of the manuscript. C-HsL, C-HuL, C-HH, and Y-JH wrote sections of the manuscript. All authors contributed to the article and approved the submitted version.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.1053882/full#supplementary-material

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A cross-sectional analysis of association between visceral adiposity index and serum anti-aging protein Klotho in adults

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Background: The visceral adiposity index (VAI) is regarded as a reliable indicator to assess body fat distribution and dysfunction. Klotho protein is a hormone with anti-aging biological functions. However, the relationship between them has not been researched.

Objects: This study aimed to evaluate the association between VAI and serum anti-aging protein klotho in American adults.

Methods: A cross-sectional study of participants was conducted based on the National Health and Nutrition Examination Surveys (NHANES) 2007–2016. Visceral adiposity was determined using the VAI score, while the klotho protein concentration was measured by ELISA kit. After adjusting some possible confounding variables, multivariate regression model was conducted to estimate the relationship between VAI and klotho protein. Furthermore, the smooth curve fitting and the segmented regression model were applied to examine the threshold effect and to calculate the inflection point.

Result: In total, 6 252 adults were eligible, with a mean VAI of 2.04 ± 0.03 and a mean klotho protein concentration of 848.79 ± 6.98 pg/ml. Multivariate regression analysis indicated that serum klotho protein concentration was lower in participants with high VAI score. When VAI was divided into quartiles, participants in the fourth quartiles of higher VAI had lower klotho protein levels (Q4: -32.25 pg/ml) than participants in the lowest quartile (Q1) after full adjustment ($P < 0.05$). Segmented regression suggested that the turning point value of VAI was 3.21. A 1-unit increase in VAI was significantly associated with lower klotho protein levels by -18.61 pg/ml (95% CI: -28.87, -8.35; $P < 0.05$) when VAI ranged from 0.29 to 3.21(accounting for 83.7% of the participants), however, the association was not significant when VAI ranged from 3.21 to 11.81 ($P = 0.77$).

Conclusion: There was a nonlinear correlation between VAI score and the serum anti-aging protein klotho concentrations, showing a saturation effect. When VAI was less than 3.21, they were negatively correlated, and when VAI was greater than 3.21, they had no obvious correlation.

KEYWORDS

visceral adiposity index, Klotho, obesity, aging, NHANES

Introduction

With the changes in dietary nutrition structure and daily lifestyle, the prevalence of being overweight or obese has been increasing rapidly around the world (1). Obese individuals, especially those with excessive abnormal fat accumulation, are more susceptible to type 2 diabetes, hypertension, and atherosclerotic events, which affect human life span (2, 3). The visceral adiposity index (VAI) is an indicator that can reliably evaluate visceral fat distribution and function in adults (4). Compared with traditional adiposity indexes, such as body mass index (BMI) and waist circumference (WC), VAI has a better predictive capacity and stronger correlation with unhealthy metabolic phenotypes (5). Sensitive detection methods for visceral obesity, including computed tomography and magnetic resonance imaging, are characterized by high costs, time consumption and radiation hazards, making these techniques unsuitable for large-scale populations (6). VAI, which includes not only anthropometric (BMI and WC), but also metabolic parameters [triglyceride (TG) and high-density lipoprotein (HDL)], is regarded as a more easily applicable and reliable indicator to assess body fat distribution and dysfunction. Studies have shown that VAI, associated significantly with cardiovascular events and atherosclerosis, is also an independent risk factor for coronary artery disease, hypertension and diabetes (7).Increased insulin resistance and low-grade chronic inflammation, caused by an increase in visceral adipose tissue, are thought to be a possible cause of metabolic diseases (8).

Klotho protein, encoded by the klotho gene, possesses remarkable anti-aging abilities. Three members of the klotho family have been identified: α-, β-, and γ-isoforms (9). α-klotho acts as an obligate coreceptor for fibroblast growth factor 23, and can be released from cells into the blood after cleaved by secretases (10). Its transgenic overexpression has been revealed to extend lifespan up to 30% in transgenic mice (11). On the contrary, mice homozygous for mutations in the klotho gene exhibit many pathways of the aging process including skin atrophy, shortened lifespan, growth retardation, arteriosclerosis, and osteoporosis (12, 13). The expression of klotho protein is beneficial to cardiovascular diseases by improving endothelial dysfunction and alleviating arteriosclerosis (14, 15). Meanwhile, downregulation of this protein was also reported to involve the common aging-related disorders, such as cancer, metabolic syndrome, and chronic kidney disease (13, 16, 17). Some of the above diseases are related to obesity, such as arteriosclerosis and osteoporosis (18, 19). In addition, reduced levels of klotho in white adipose tissue were found to be associated with high fat-induced obesity in non-human primates (20). And it has been consistently reported that serum klotho level was inversely correlated with age in humans (21). As one of the robust markers of biological aging, shorter leukocyte telomere length was reported to be related with higher VAI score (22). Nevertheless, little research has been reported on the direct relationship between VAI and serum klotho protein in humans.

Diets inducing weight loss through caloric restriction, could improve metabolism and increase lifespan (23). But the exact mechanism by which weight control affects longevity remains unclear. Klotho expression levels may potentially be involved in the relationship between adiposity obesity and aging. Therefore, we used large population data from the National Health and Nutrition Examination Survey (NHANES) to analyze the association between VAI and klotho protein, to provide new ideas for exploring the mechanism. We hypothesized that higher VAI was associated with lower serum klotho protein concentrations.

Participants and methods

Study design and population

This study information is based on NHANES 2007–2016. NHANES is a program of studies aiming to investigate the health and nutritional status of American participants, and involves interviews, examinations, and laboratory components. The data consisted of 5 continuous cycles from 2007 to 2016 (other cycles did not include VAI or klotho protein). Participants who were pregnant (n=317) were excluded from the total included population (n=50588). Next, 43891 participants without data information of VAI or klotho protein were also excluded from the study. After the sensitivity analysis performed by removing extreme values (VAI>99% percentage or <1% percentage, n=128), 6252 eligible participants were included for further analyses. All the study protocols included here obtained written informed consent and were approved by the Research Ethics Review Board at the National Center for Health Statistics.

Outcome and exposure factors

The major exposure factor was VAI, calculated using the following sex-specific formula (24): Males: VAI = {WC/[39.68+(1.88*BMI)]} * (TG/1.03) * (1.31/HDL);

Females: VAI = {WC/[36.58+(1.89*BMI)]} * (TG/0.81) * (1.52/HDL).

WC is measured in cm, BMI in kg/m^2, TG and HDL in mmol/L.

The main outcome was serum klotho concentration. Serum specimens from participants were collected, transferred and stored at -80°C. Serum klotho concentrations were measured by a commercially available ELISA kit produced by IBL International, Japan. The assay sensitivity was 6 pg/mL. All study samples were run in duplicate, bisected and measured separately, and the average of the two concentrations was calculated as the result.

Covariates

Additional covariates were collected from each cycle of NHANES. The continuous variables included age, and poverty income ratio (PIR). Categorical variables included gender, age, race, education level, marital status, smoking and alcohol use. According to previous studies, several possible confounding variables were adjusted. Specifically, age was defined as 40–49, 50–59, and ≥60 years. BMI was divided into <25, 25–30, and ≥30 kg/m^2. Participants were stratified by PIR: ≤1.3, >1.3 & ≤3.5, >3.5%, and missing. Race was classified as Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other races. Marital status was divided into

married, widowed, divorced, separated, never married, and living with partner. Education level was divided into < 9th grade, 9–11th grade, high school graduate, some college, and college graduate or above. Physical activity was categorized as <500, ≥500 and missing. Smoking status included never (less than 100 cigarettes in lifetime), former (100 cigarettes or more but no currently smoking) and now (100 cigarettes or more and currently smoking), and alcohol consumption included <12 drinks/year, ≥12 drinks/year and missing. Participants with EGFR≤ 60 ml/min/1.73m^2 or urinary albumin/creatinine ratio ≥ 30 mg/g were defined as having chronic kidney disease. Self-reported medical conditions included cancer, diabetes, hypertension, stroke, cardiovascular disease (coronary heart disease, angina, congestive heart failure), and chronic obstructive pulmonary disease (all classified as Yes/No). When the value of the missing covariable was more than 2% of the total population, dummy variables were used instead.

Statistical analysis

The baseline characteristics of all participants are clearly described in Table 1, by the means of proportions or the mean ± standard error (SE). Particularly, categorical variables were presented by weighted chi-square analysis, and simultaneously, the continuous variables were evaluated by a weighted linear regression model. VAI was treated as not only a continuous independent variable, but also a categorical variable (divided into quartiles), with the lowest quartile used as the reference.

To study the independent relationship between VAI and klotho protein, multivariate generalized linear regression analyses were conducted. Model 1 was adjusted for no covariate. Model 2 was adjusted for age, gender, and race. Model 3 was adjusted for gender, age, race, PIR, BMI, education, marital status, physical activity, smoking, alcohol use, chronic kidney disease, cancer, diabetes, hypertension, stroke, chronic obstructive pulmonary disease, and cardiovascular disease, for the purpose of further subgroup analysis.

The smooth curve fitting and a generalized additive model were set up in order to explore the potential non-linear correlation. We further applied the segmented linear regression model to examine the threshold effect and to calculate the inflection point.

We used sampling weights recommended by the CDC guidelines to account for the complex study design in all the analysis except for the curve fitting (25). All the above statistical analyses were completed by using R 3.6.3 and EmpowerStats. A P-value <0.05 was considered to be of statistical significance (two-tailed).

Results

Participant characteristics

The participants' characteristics at the baseline according to the categories of VAI were presented in Table 1. A total of 6252 American adults were qualified to be enrolled in the study. Among all participants, 52.45% were females and 47.55% were males. The mean ± SE of VAI was 2.04 ± 0.03. The mean ± SE of klotho protein concentration was 848.79 ± 6.98 pg/ml. The serum klotho protein concentration in the last quartile was lowest (Q4: 820.42 ± 11.80 pg/ml), compared to the other three quartiles (Q1: 867.62 ± 10.82, Q2: 867.55 ± 11.14, Q3: 835.83 ± 9.42 pg/ml, p < 0.01).

Multivariate regression analysis

Table 2 demonstrated that VAI was negatively correlated with klotho protein concentration in the non-adjusted model [β(95%CI) = -12.95 (-18.95, -6.94)], minimally adjusted model [-12.22 (-18.45, -6.00)], and the fully adjusted model [-9.80 (-16.37, -3.22)]. Multivariate regression analysis indicated that serum klotho protein concentration was lower in participants with higher VAI score. When VAI was divided into quartiles, participants in fourth quartiles of VAI had lower klotho protein levels (Q4: -32.25 pg/ml), than participants in the lowest quartile (Q1) after full adjustment (P < 0.05). Although no negative significant difference was seen in the prevalence in the second quartile group, there was a significant relationship between VAI and klotho protein level in the fourth quartile group in all three models. Compared to participants with lower VAI (0.29–0.95) in the first quartile group, people with higher VAI (2.56–11.81) in the fourth quartile group, had a significantly lower level of klotho protein in model 1 [-47.20 (-75.94, -18.46)], model 2 [-42.20 (-71.62, -12.79)], and model 3 [-32.25 (-64.26, -0.25)]. P values for trend were less than 0.05 in all three models.

Non-linear analysis

We then performed a smooth curve fitting and a segmented regression to explore the non-linear association between VAI and serum klotho protein concentration (Figure 1; Table 3). The smooth curve after fully adjustment showed a non-linear relationship between VAI and klotho protein level (Figure 1). The segmented regression suggested that the turning point value of VAI was 3.21 (Table 3). A 1-unit increase in VAI was significantly associated with lower klotho protein levels by 18.61 pg/ml (95% CI: -28.87, -8.35; P < 0.01) when VAI ranged from 0.29 to 3.21 (5 233 individuals, accounting for 83.7% of the participants), however, the association was not significant when VAI ranged from 3.21 to 11.81 (P > 0.05, log likelihood ratio test = 0.017).

Discussion

To our knowledge, this is the first study to evaluate the relationship between VAI and serum anti-aging protein klotho concentration by analyzing a large population data in NHANES. There was a dose–response negative association: on the left side of the turning point, a higher VAI score was associated with a decreased level of serum klotho concentration. Further study demonstrated that klotho decreased 18.61 pg/ml for every 1 unit increase in VAI among Americans. However, on the right side of the turning point, the relationship was not significant.

Klotho is mainly expressed in kidney and brain tissues and has hormone-like functions. On the one hand, the anti-aging mechanism of klotho protein is related to the down-regulation of phosphate

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TABLE 1 Characteristics of participants by categories of visceral adiposity index (VAI) in NHANES 2007–2016[ab].

Characteristic	All	VAI quartiles				P value
		Q1(0.29-0.95)	Q2(0.95-1.55)	Q3(1.55-2.56)	Q4(2.56-11.81)	
N. of participants	6252	1563	1563	1563	1563	
Visceral adiposity index	2.04 ± 0.03	0.67 ± 0.01	1.23 ± 0.01	2.02 ± 0.01	4.24 ± 0.07	<0.0001
Klotho (pg/ml)	848.79 ± 6.98	867.62 ± 10.82	867.55 ± 11.14	835.83 ± 9.42	820.42 ± 11.80	<0.0001
Age (years)	56.30 ± 0.20	56.03 ± 0.47	55.84 ± 0.43	56.91 ± 0.27	56.47 ± 0.30	0.0103
40-49 (%)	31.22	33.50	33.07	27.72	30.54	
50-59(%)	30.87	26.30	31.38	34.56	30.87	
≥60 (%)	37.92	40.20	35.55	37.72	38.58	
Gender (%)						0.0145
Female	52.45	52.76	49.34	56.91	52.30	
Male	47.55	47.24	50.66	43.09	47.70	
Race (%)						<0.0001
Mexican American	6.44	4.34	5.36	8.86	7.31	
Other Hispanic	4.94	3.81	5.10	5.41	5.38	
Non-Hispanic white	73.78	71.69	74.02	72.40	77.34	
Non-Hispanic black	8.55	13.51	9.40	6.99	4.01	
Other races	6.29	6.65	6.13	6.34	5.97	
Poverty income ratio	3.25 ± 0.06	3.50 ± 0.08	3.35 ± 0.07	3.10 ± 0.07	3.05 ± 0.08	<0.0001
≤1.3 (%)	16.40	13.32	15.68	17.31	19.17	
>1.3 and ≤3.5 (%)	31.07	27.55	28.52	34.87	33.61	
>3.5 (%)	46.33	53.03	49.49	41.13	41.73	
Missing (%)	6.20	6.10	6.32	6.70	5.49	
BMI (kg/m^2)	29.51 ± 0.15	26.74 ± 0.20	28.68 ± 0.24	31.01 ± 0.26	31.83 ± 0.18	<0.0001
<25 (%)	24.43	43.84	26.96	15.96	9.73	
25-30 (%)	35.73	34.18	41.11	33.67	33.93	
≥30 (%)	39.85	21.98	31.93	50.37	56.34	
Education (%)						<0.0001
Less than 9th grade	6.22	4.94	5.61	7.21	7.02	
9-11th grade	10.48	7.71	9.88	11.45	12.71	
High school graduate	21.31	17.58	21.19	22.38	24.66	
Some college	30.23	27.13	30.66	31.58	32.24	
College graduate or above	31.77	42.64	32.67	27.38	23.37	
Marital Status (%)						0.3608
Married	66.21	69.72	65.51	65.02	64.91	
Widowed	5.79	4.79	5.71	5.78	7.17	
Divorced	13.51	11.11	15.18	13.18	13.79	
Separated	2.23	2.22	2.01	2.41	2.30	
Never married	7.46	7.60	6.77	8.52	6.97	
Living with partner	4.80	4.56	4.82	5.09	4.86	
Smoking (%)						<0.0001

(Continued)

TABLE 1 Continued

Characteristic	All	VAI quartiles				P value
		Q1(0.29-0.95)	Q2(0.95-1.55)	Q3(1.55-2.56)	Q4(2.56-11.81)	
<100 cigarettes in life	51.23	58.25	51.23	51.50	44.27	
≥100 cigarettes in life but no smoking now	30.83	28.54	29.80	32.23	32.89	
≥100 cigarettes while smoking now	17.94	13.22	18.97	16.27	22.84	
Alcohol (%)						0.0048
<12 drinks/year	22.03	19.30	21.62	22.68	25.53	
≥12 drinks/year	72.52	73.72	72.79	71.69	70.87	
Missing	5.45	6.98	5.59	5.63	3.59	
Physical activity(%)						<0.0001
<500	14.07	13.77	12.86	14.87	15.37	
≥500	61.77	69.61	66.02	55.72	54.78	
Missing	24.17	16.62	21.12	29.42	29.86	
Chronic kidney disease (%)						<0.0001
No	84.72	88.62	88.54	83.67	78.82	
Yes	15.28	11.38	11.46	16.33	21.18	
Cancer (%)						0.1782
No	87.24	88.10	87.63	87.92	84.83	
Yes	12.76	11.90	12.37	12.08	15.17	
Diabetes (%)						
No	78.32	89.14	83.13	76.13	65.85	<0.0001
Yes	21.68	10.86	16.87	23.87	34.15	
Hypertension (%)						
No	51.40	63.67	55.44	48.25	38.81	<0.0001
Yes	48.60	36.33	44.56	51.75	61.19	
Stroke (%)						
No	96.35	96.90	96.41	96.75	95.22	0.1130
Yes	3.65	3.10	3.59	3.25	4.78	
Cardiovascular disease(%)						
No	88.57	90.77	90.77	87.49	85.63	0.0002
Yes	11.43	9.23	9.23	12.51	14.37	
Chronic obstructive pulmonary disease(%)						
No	92.55	93.98	92.06	92.00	92.00	0.3239
Yes	7.45	6.02	7.94	8.00	8.00	

[a]Mean ± SE for continuous variables, and P value calculated by weighted t test.
[b]% for categorical variables, and P value calculated by weighted Chi-square test.

reabsorption and up-regulation of calcium reabsorption, thereby inhibiting the increase of vitamin D level (26). On the other hand, another mechanism is through inhibition of various Wnt ligands, of which overactivity is related to aging and tumorigenesis (27). Additionally, klotho can suppress inflammation and oxidative stress (28). In a recent study, negative associations were observed between α-klotho and some inflammatory markers (14). Therefore, this protein may play an important role in anti-aging through its anti-inflammatory effect.

According to 239 large, multinational, prospective studies performed in four continents, the relationship between overweight and obesity measured by BMI, and higher all-cause mortality was indisputable (29). Our study showed that visceral obesity measured by VAI was negatively correlated with anti-aging protein klotho. Obesity

TABLE 2 Association between visceral adiposity index (VAI) and serum anti-aging protein klotho.

Exposure	Model 1[a]	Model 2[b]	Model 3[c]
	β(95% CI) P value	β (95% CI) P value	β (95% CI) P value
VAI	-12.95 (-18.95, -6.94)	-12.22 (-18.45, -6.00)	-9.80 (-16.37, -3.22)
	0.00006	0.00026	0.00436
VAI quartile			
Q1	Ref	Ref	Ref
Q2	-0.07 (-25.40, 25.27)	2.17 (-23.01, 27.35)	7.46 (-18.44, 33.36)
	0.99585	0.86626	0.56386
Q3	-31.79 (-58.20, -5.38)	-32.40(-59.22, -5.58)	-25.49 (-54.06, 3.08)
	0.02089	0.02070	0.07873
Q4	-47.20 (-75.94, -18.46)	-42.20 (-71.62, -12.79)	-32.25 (-64.26, -0.25)
	0.00189	0.00641	0.04818
VAI quartile continuous	-17.35(-26.74,-7.96)	-16.12 (-25.79, -6.46)	-13.05 (-23.57, -2.53)
	0.00052	0.00166	0.01611

[a]Model 1: adjusted for no covariates.
[b]Model 2: adjusted for age, gender, race.
[c]Model 3: adjusted for gender, age, race, poverty income ratio, body mass index, education, marital status, physical activity, smoking, alcohol use, chronic kidney disease, cancer, diabetes, hypertension, stroke, chronic obstructive pulmonary disease, and cardiovascular disease.

is characterized by changes in the distribution of adipose tissue and the mass expansion of the body. Obesity can be divided into visceral obesity, excess adipose tissue accumulated in the abdomen, and subcutaneous obesity, accumulation of adipose tissue under the skin (30). Furthermore, surgical removal of visceral fat in rats was proved to be beneficial for improving insulin sensitivity, reducing the incidence of liver and kidney disease, and extending lifespan (31, 32). These studies demonstrated that visceral fat accumulation made contributions to the reduction in life expectancy in aging.

Previous studies have demonstrated that visceral obesity is a risk factor for various metabolic diseases (33), while subcutaneous obesity does not appear to be related with those metabolic syndromes (34). A relationship has been suggested between the accumulation of abdominal fat and a low-grade elevation of inflammatory mediators, such as c-reactive protein, tumor necrosis factor, in the circulating concentrations in the body fluids (35). Normally, lymphatic tissue and the liver are the main producer of these inflammatory mediators, but in obesity, adipose tissue becomes the main site of production, leading to a long-term and sustained environment of local and systemic inflammation (30). Recent study has shown that not only local but also systemic inflammation could decrease klotho expression in the kidneys (36). Additionally, Ma et al. found that chronic inflammation, caused by a pro-inflammatory diet pattern, can reduce serum klotho levels (37). Thus, as a characteristic of the obese state, the excessive proinflammatory products, mainly from adipose tissue, may in turn deplete the klotho protein in the serum, reducing its concentration.

A compelling study suggested that diets inducing weight loss through caloric restriction, could improve metabolism and increase lifespan by positively affecting adipose tissue (23). In addition, white adipose tissue is the main component of visceral tissue and can secrete adipokines. The increase of visceral fat leads to the accumulation of triglycerides, resulting in the dysregulation of adipokine secretion (38). Adipokines affect immune cell chemotaxis and promote the release of inflammatory cytokines, which ultimately affect klotho protein synthesis and promote cellular aging (38). Besides, visceral adiposity has a positive correlation with oxidative stress and insulin sensitivity, while klotho expression has a negative relation with oxidative stress (6, 39). Thus, oxidative stress, caused by excess visceral fat, may deplete some of this protein. More studies are needed to discover the detailed mechanism.

Recently, it has been demonstrated for the first time that the relationship between cerebrospinal fluid α-klotho and BMI was inverse (40). Likewise, the same held true for the association between the serum α-klotho and VAI in our study. These two studies prove the correlation between obesity and klotho protein from different angles. This protein concentration is closely related to lipid levels: negatively related to TG and positively related to HDL (41, 42). In addition to these two parameters, the VAI score includes waist circumference and BMI, which quantifies the severity of visceral obesity. We also determined the optimal VAI cut-off value to clarify the relationship between VAI and klotho protein in different cases, which provides a new perspective for the study of visceral obesity and aging.

There are some strengths in our study. Firstly, as far as we were aware, this was the first report of an association between visceral adiposity and serum anti-aging protein klotho level in humans. To a certain extent, this provides some novel and easy-to-practice insight into the resistance or delay of aging, which including proper weight control, especially visceral adiposity. Secondly, our study used a multi-ethnic, as well as a large multi-regional population based on a large population analysis from the NHANES and included a relatively large sample size of 6 252 Americans.

In the meanwhile, there are some limitations of this study. The primary limitation is that the causality between VAI and klotho

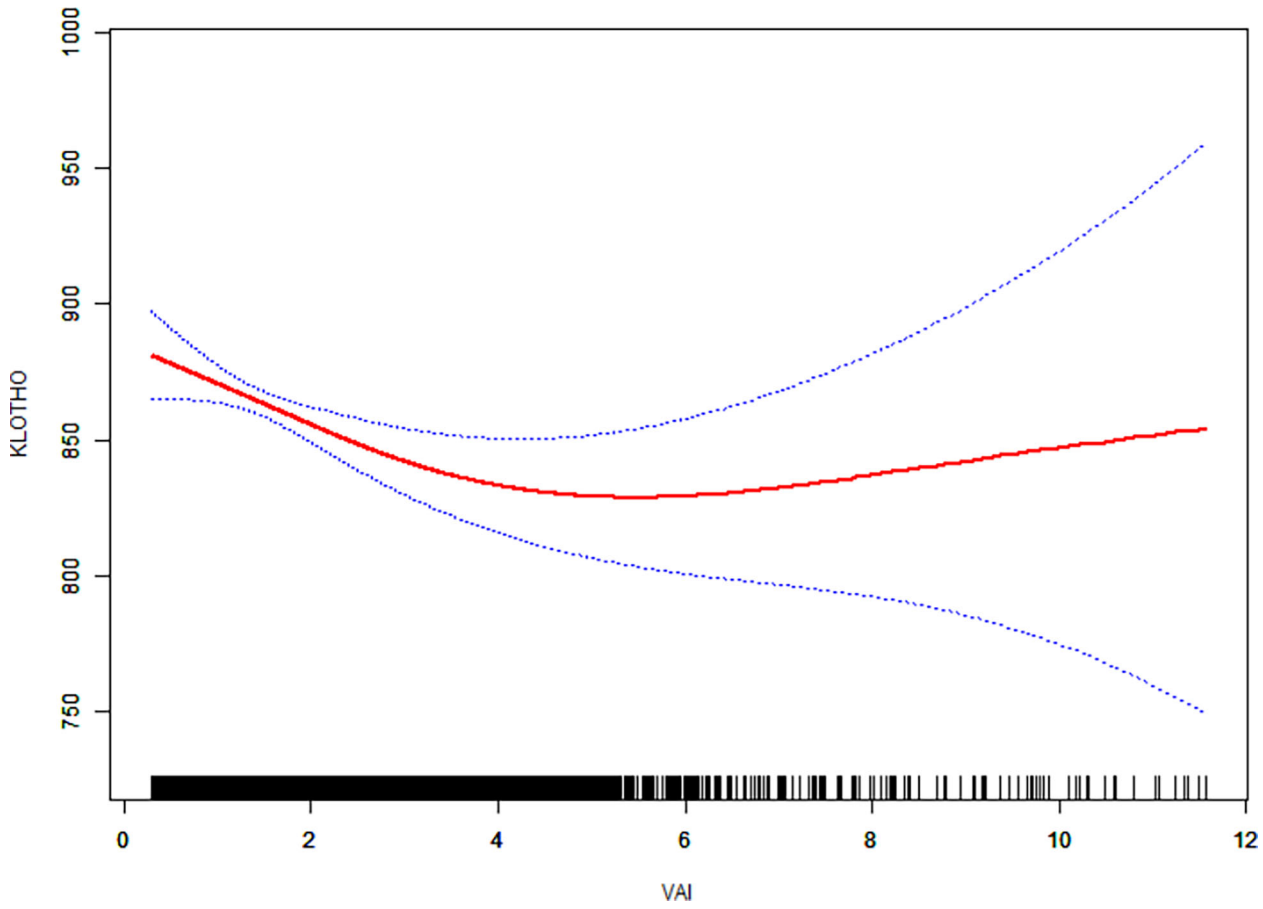

FIGURE 1
The relationship between visceral adiposity index (VAI) and serum anti-aging protein klotho. Solid line in the middle represents smooth curve fitting between variables. The dotted line on both sides represents a 95% confidence interval (CI) for the fit. The bottom black and white strip shows the population density according to VAI. All models were adjusted for gender, age, race, poverty income ratio, body mass index, education, marital status, physical activity, smoking, alcohol use, chronic kidney disease, cancer, diabetes, hypertension, stroke, chronic obstructive pulmonary disease, and cardiovascular disease.

TABLE 3 Threshold effect analysis for the relationship between visceral adiposity index (VAI) and serum anti-aging protein klotho in NHANES 2007–2016[a].

Model	serum klotho protein concentration	
	Adjusted β (95% CI)	P value
Model I		
the standard linear mode	-7.84 (-13.02, -2.65)	0.0030
Model II		
Turning point (K)	3.21	
VAI < 3.21 (accounting for 83.7% of the participants)	-18.61 (-28.87, -8.35)	0.0004
VAI > 3.21 (accounting for 16.3% of the participants)	1.40 (-7.79, 10.59)	0.7652
Log likelihood ratio test	0.017	

[a]All models were adjusted for gender, age, race, poverty income ratio, body mass index, education, marital status, physical activity, smoking, alcohol use, chronic kidney disease, cancer, diabetes, hypertension, stroke, chronic obstructive pulmonary disease, and cardiovascular disease.

protein cannot be determined, because of the characteristics of the cross-sectional study design. In other words, visceral adiposity may make the protein klotho level go down, but lower level of this protein may lead to obesity. However, there is indeed a convincing relationship between them. Within a certain range, a lower level of protein was associated with high VAI scores. Additionally, information about VAI came from questionnaires. Some participants might be reluctant to answer the relevant questions for a variety of reasons, resulting in no answers, while others might have omitted some information when answering the questionnaire, both of which could inevitably lead to bias. Finally, there were still potentially confounding factors which were not adjusted, leading to this affecting the association between them.

Conclusion

Based on the nationally representative population, this study demonstrated a non-linear association and a dose–response relationship between VAI and serum anti-aging protein klotho in American adults. Indeed, when VAI was less than 3.21, serum levels of klotho protein shown a significant downward trend as VAI

increased, and these individuals were more likely to develop aging-related syndromes. These results indicated that careful control of adiposity may have anti-aging and health benefits by increasing serum klotho concentrations. Future large and well-designed prospective studies are warranted to confirm the causal relationship and detailed mechanisms.

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

All the study protocol included here obtained written informed consent, and were approved by the Research Ethics Review Board at the National Center for Health Statistics.

Author contributions

Conceptualization and methodology: JiaW, YB, JC. Data acquisition: JC, ZY, JiahW. Data analysis and interpretation: JC, JiahW, ZY, SY, YX. Writing – original draft: JC. Writing – review & editing: all authors. Data curation and supervision: JC, YB. All authors contributed to the article and approved the submitted version.

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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.

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Visceral fat and attribute-based medicine in chronic kidney disease

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Visceral adipose tissue plays a central role in obesity and metabolic syndrome and is an independent risk factor for both cardiovascular and metabolic disorders. Increased visceral adipose tissue promotes adipokine dysregulation and insulin resistance, leading to several health issues, including systemic inflammation, oxidative stress, and activation of the renin-angiotensin-aldosterone system. Moreover, an increase in adipose tissue directly and indirectly affects the kidneys by increasing renal sodium reabsorption, causing glomerular hyperfiltration and hypertrophy, which leads to increased proteinuria and kidney fibrosis/dysfunction. Although the interest in the adverse effects of obesity on renal diseases has grown exponentially in recent years, the relationship between obesity and renal prognosis remains controversial. This may be attributed to the long clinical course of obesity, numerous obesity-related metabolic complications, and patients' attributes. Multiple individual attributes influencing the pathophysiology of fat accumulation make it difficult to understand obesity. In such cases, it may be effective to elucidate the pathophysiology by conducting research tailored to individual attributes from the perspective of attribute-based medicine/personalized medicine. We consider the appropriate use of clinical indicators necessary, according to attributes such as chronic kidney disease stage, level of visceral adipose tissue accumulation, age, and sex. Selecting treatments and clinical indicators based on individual attributes will allow for advancements in the clinical management of patients with obesity and chronic kidney disease. In the clinical setting of obesity-related nephropathy, it is first necessary to accumulate attribute-based studies resulting from the accurate evaluation of visceral fat accumulation to establish evidence for promoting personalized medicine.

KEYWORDS

visceral fat, patient-centered medicine, sex difference, personalized medicine, obesity, precision medicine, chronic kidney disease, attribute-based medicine

1 Introduction

Accumulated epidemiologic evidence indicates that being overweight and obese are risk factors for chronic kidney disease (CKD) (1–4) and end-stage kidney disease (ESKD) (5–8); additionally, the causal link between obesity and CKD has been extensively reviewed (9–11). Visceral fat accumulation is the central pathological condition in obesity/metabolic syndrome

(12–15) and is significantly associated with atherosclerosis (14), hypertension (16, 17), and metabolic impairments, including hyperglycemia/diabetes mellitus (17–19), hypertriglyceridemia (17), low high-density lipoprotein (HDL) cholesterol (17, 20), hyperuricemia (21, 22), high C-reactive protein concentration (14, 17), fatty liver (14), cardiovascular disease (CVD) (23), and kidney disease (24, 25). Nevertheless, at present, no clinical practice guidelines for obesity-related glomerulopathy (ORG) have been established. This narrative review provides an overview of visceral fat and obesity-related kidney disease and its clinical indicators, aiming to generate novel ideas for future studies and clinical applications focusing on attribute-based medicine/personalized medicine.

2 Literature review

2.1 Visceral fat is a major pathophysiological condition of obesity/metabolic syndrome

The pathophysiology of obesity/visceral fat accumulation is complex, with numerous interrelated aspects, including a sedentary lifestyle, individual dietary habits, genetic predisposition, and environmental factors (26–29). Visceral adipose tissue (VAT) plays a central role in being overweight and obese (30–34), whereas subcutaneous fat tissue is considered benign or protective (35, 36). Increased visceral fat accumulation causes adipose tissue inflammation and adipokine dysregulation (30–34), which can lead to dyslipidemia, insulin resistance (32, 37), chronic systemic inflammation (32, 38, 39), oxidative stress (30), brain melanocortin system stimulation (38, 40), sympathetic nervous system overactivation (40–42), renin-angiotensin-aldosterone system (RAAS) overactivation (43–47), mineralocorticoid receptor activation (48), sodium retention (49, 50), and extracellular fluid volume expansion (50–52). Increased visceral fat accumulation is also accompanied with perirenal and renal sinus fat accumulation, which causes high intrarenal pressure, which leads to compression of the vasa recta capillaries and thin loops of Henle, reduced blood flow in the renal medulla, increased sodium reabsorption in the loop of Henle, RAAS activation, and increased sodium reabsorption (50, 53, 54). These pathological conditions interact in a complex manner, ultimately damaging the kidneys by causing glomerular hyperfiltration (55, 56) and inflammation (57, 58), both of which are characteristics of obesity-related kidney disease (50, 53, 54, 59–61).

2.2 The complex pathophysiology of obesity

Numerous studies in the last 20 years have investigated obesity, significantly elucidating the systemic pathology associated with visceral adiposity/obesity and the mechanism of kidney injury in patients with obesity (2, 62). However, while the number of patients with obesity and patients with ORG has continued to increase, treatment strategies for ORG generally have remained ineffective in clinical practice (63, 64). Although patients and medical staff understand that weight loss is a simple solution to obesity-related diseases, the clinical prognostic indicators for ORG are poorly established, as is the optimal treatment for individual patients, with no clinical practice guidelines for ORG (63, 65, 66).

The following issues may have led to some confusion in studies and the creation of a knowledge gap regarding obesity-related neuropathy (1): the concept of the "obesity paradox," in which protective effects of obesity have been observed in certain patient populations [e.g., ESKD patients (67, 68)] (2); the idea of a "metabolically healthy obesity phenotype" (69, 70) (3); the biphasic clinical change in the estimated glomerular filtration rate (eGFR) based on hyperfiltration during CKD progression in patients with obesity (56, 71) (4); the biphasic course of glomerular size during glomerular damage (72, 73) (5); the presence of many obesity-related complications and the long clinical course of obesity; and (6) the lack of evidence based on the precise measurement of visceral fat. Among these, though the concept of the "obesity paradox" remains controversial (67, 74–76), its existence has recently been questioned owing to concerns about the limitation of epidemiological studies (i.e., selection biases, confounding factors, influence of malnutrition), the inherent limitations of anthropometric measures, such as the body mass index (BMI), and the limitations of studies with short periods of observation (74, 77–82). As Kramer et al. (83) reported, multiple residual confounders and biases strongly affect the "obesity paradox." Indeed, bariatric surgery, effective for multiple residual confounders, such as obesity-related complications, solves the "obesity paradox" by decreasing visceral fat accumulation and glomerular hyperfiltration, which are essential pathophysiological conditions of obesity (84, 85). Furthermore, the concept of a "metabolically healthy obesity phenotype" is also questioned by an accumulation of results considering the long clinical course of obesity. The "metabolically healthy obesity phenotype" is reportedly associated with low levels of VAT/ectopic fat, high levels of lower body subcutaneous fat storage, younger age, insulin sensitivity, increased adiponectin, a favorable lipoprotein profile, and non-Hispanic black race/ethnicity (18, 86). Meta-analyses of studies with a follow-up duration >10 years reported that individuals with "metabolically healthy obesity phenotype" are at an increased risk for CVD events (87, 88), with this risk increasing with a longer follow-up duration (89). The accumulation of studies that precisely evaluates visceral fat is an issue that remains to be addressed by researchers and clinicians worldwide.

2.3 Precise visceral fat measurement in patients with CKD

Although there are various anthropometric and imaging measurement methods clinically available to assess adiposity (51, 80, 90), to fully clarify the pathophysiological condition in obesity, it is important to determine whether volumetric fat measurements can accurately characterize the heterogeneity of abdominal fat distribution between individuals (91). BMI and waist circumference are easy to examine and have been widely used to define obesity and abdominal obesity. Waist circumference has been used as an indicator closely associated with visceral fat (92). However, theoretically, both BMI and waist circumference cannot be used to distinguish between visceral and subcutaneous fat mass. Since VAT and SAT differ greatly in their functional significance and response to weight gain, anthropometric data alone is not sufficient for an accurate risk assessment of adiposity (80). Therefore, imaging methods need to be developed to identify individuals with excessive visceral adiposity

(51). Imaging measurement of adiposity can be performed by various methods, including computed tomography (CT), magnetic resonance imaging (MRI), dual-energy X-ray absorptiometry, and electrical bioimpedance (93). Among these, only CT accurately measures visceral fat area (VFA) (94). CT can be performed rapidly and interpreted to segment adipose tissue deposits and measure their area or volume (80). CT produces high-resolution images conveniently and with high repeatability (94), providing accurate localization data (95). Although CT is expensive and exposes the patient to radiation, quantitative CT is currently the technology of choice for the measurement and analysis of VFA (96). At present, though CT and MRI are ideal methods to assess adiposity in clinical research (51, 93, 97), studies using visceral fat assessment evaluated by these techniques are still lacking.

2.4 Attribute-based medicine for personalized medicine

In recent years, the concept of personalized medicine/precision medicine/tailored medicine has been developed alongside the concepts of patient-centered medicine (98, 99). In a clinical setting, personalized medicine can provide access to knowledge that either validates or alters a medical decision from one that is based on the evidence for the average patient to one that is based on the individual's unique characteristics/attributes (100). In personalized medicine, patients are treated individually according to their individual heterogeneous characteristics (101, 102), with the advantages of the disaggregation of data and analyses of differences within sub-cohorts having been reported (102, 103). Recently developed artificial intelligence/machine learning has the potential to bring about the ideal personalized medicine (100, 104, 105). However, there are still issues that need to be resolved to establish personalized medicine for patients with CKD (100, 104). Fröhlich et al. identified the following challenges in data science (artificial intelligence [AI]/machine learning) for personalized medicine (1): insufficient prediction performance for clinical practice (2), difficulties in interpretation, and (3) insufficient validation for clinical practice (105). Indeed, unlike in genetic diseases (106) where personalized medicine can be applied according only to genetic mutations (107–110), most patients with CKD are affected by multiple risk factors for disease progression (111, 112). In patients with CKD, the risk factors and pathophysiological conditions generally differ regarding patient attributes (113). In patients with obesity and CKD, multiple attributes, including age (114, 115), sex (116–119), race/ethnicity (17, 120), fat distribution (119, 121), the amount of VAT (119, 121, 122), and CKD stage (115), are known to more intricately influence the pathophysiology of fat accumulation and cardiorenal metabolic disease (51, 80). Such complex interactions of chronic diseases with obesity raise the difficulty of interpretation of pathophysiology, prognosis prediction, and validation in a clinical setting.

In such a multifactorial disease like CKD, we consider that attribute-based medicine (113, 123), supported by attributes/characteristics (100, 105) such as sex and age, is useful for the establishment of personalized medicine. That is, instead of jumping from traditional medicine in an entire cohort to personalized medicine in individuals, we interpose a step (attribute-based medicine) to bridge both approaches. Attribute-based medicine can help solve the challenges enumerated by Fröhlich et al. (105) by increasing the future accuracy of machine learning predictions, enabling patients and clinicians to interpret machine learning-generated predictions, and making it easier to validate in the clinical setting. Attribute-based medicine may provide a bridge between traditional statistical research and personalized medicine.

2.5 Attribute-based medicine for patients with obesity and CKD

Before devising personalized medicine approaches in patients with CKD, high-quality databases must be created and risk factors for the acceleration of the CKD progression must be identified (113, 115, 118, 119, 124–129), paying attention to attributes such as sex differences or ages (130, 131). Indeed, it has been reported that even data used in AI should be divided according to sex and attribute, which makes collecting data disaggregated by age and sex essential if AI is to fulfill its promise of improving outcomes for everyone (132–135). Therefore, from the standpoint of patient-centered medicine, women and the elderly should be treated based on research evidence from female (131, 136–139) and geriatric cohorts (140–142), respectively. In chronic diseases, sex and age are important modifiers of pathophysiology and disease development. However, data disaggregated by age, sex, or obesity are still scarcely available from prospective studies (132, 137). To establish research supporting precision/personalized medicine, it is necessary to conduct further large-scale studies which include the analyses of disaggregated data (143).

In cardiology, sex-specific CVD risk assessment using CT or MRI-based fat measures has already been validated (144, 145). In nephrology, human studies on visceral fat and kidney prognosis have established clear evidence for kidney prognosis, especially regarding sex-specific differences (51, 144, 146). Several indicators that reflect obesity, such as BMI, waist circumference, VFA, and the visceral-to-subcutaneous fat ratio (V/S ratio), seem to explain CKD progression. However, the rationale and merits of various indicators likely vary and are insufficient to establish strong evidence (119, 147–152). Therefore, to address the gaps in knowledge regarding the pathophysiology of obesity and its impact on kidney disease, it will be important to accurately assess volumetric fat measurements to clearly characterize the heterogeneity of abdominal fat distribution between individuals and the differences in fat distribution between sexes (91). In this regard, CT- or MRI-based measures should be more indicated to study the effect of VAT on kidney disease.

4 Discussion

4.1 Attribute-based medicine for patients with CKD and obesity: A consideration of the sex differences in visceral adiposity and CKD progression

Among the multiple attributes influencing the pathophysiology of fat accumulation, sex differences in visceral adiposity and CKD

progression are particularly important. Firstly, sex hormones have important roles in the accumulation and distribution of body fat (153). As a result, fat distribution significantly differs between the sexes, as men have relatively more visceral fat and women have relatively more subcutaneous fat (36, 154). Furthermore, men have higher levels of visceral fat (155) than premenopausal women, with the decline in estrogen levels upon menopause being associated with an increase in visceral fat in women (156). Post-menopause, the amount of estrogen secreted from the ovaries dramatically diminishes, resulting in a decrease in brain anorexigenic signaling through estrogen, evoking the storage of lipids in visceral fat, a major source of estrogen in postmenopausal women (157, 158).

Secondly, premenopausal women are generally protected from CVDs due to the activation of RAAS, with a previously established involvement of estrogen in this mechanism (159, 160). Although the angiotensin-converting enzyme/angiotensin II/angiotensin receptor 1 (ACE/Ang II/AT1R) axis plays a major role in the classic renin-angiotensin signaling pathway, namely in water and salt retention, vasoconstriction, and in proliferative, proinflammatory, and profibrotic processes (161), estrogen has been reported to reduce the activation of that axis (162, 163). Estrogen reduces ACE activity (164), AT1R expression (165, 166), and aldosterone production in animal models (167). Men and postmenopausal women have higher renin activity and levels (168, 169), as well as increased plasma aldosterone levels (170), than premenopausal women. These increases in RAAS activation and visceral fat in postmenopausal women can be avoided by estrogen replacement therapy (169, 171–173). Furthermore, estrogen shifts the balance toward the AT2R/ACE2/Ang- (1–7)/mitochondrial assembly receptor (MasR) axis [the protective/depressor renin-angiotensin signaling pathways (174)], which opposes the pressor actions of AT1R (160). Obesity is associated with the activation of the ACE/Ang II/AT1R axis (175, 176), with the overactivation of Ang II in obesity stimulating AT1R to promote hypertension, insulin resistance, and energy imbalance (176). However, the protective estrogen-RAAS interactions *via* AT2R/ACE2/Ang- (1–7)/MasR appear to be diminished by obesity (177) and aging (177), suggesting that the protective effect against CVD in women may be attenuated by an increase in visceral fat. Indeed, in human studies, the various vasoprotective effects of estrogen, including vasodilation, anti-inflammatory properties, and lipid profile decline, are nonexistent in hyperglycemic states and obesity (178–180). Features associated with obesity or metabolic syndrome in women generally emerge after menopause (181, 182), which may induce a concurrent progression of CKD (183–186).

Men with CKD generally have a worse prognosis than women, which leads to a substantially higher proportion of men with ESKD (187–189). Women seem to be protected against the development and progression of CKD (183, 190, 191), and the presence of estrogen further protects against kidney injury (192). Although the pathological mechanism underlying the sex-specific differences in CKD has not yet been completely elucidated, sex-specific differences in visceral fat accumulation (157, 158) are associated with sex-specific differences in CKD progression (20, 115, 119, 193, 194). For example, in a representative multicenter CKD study in Japan, using a ≥50% eGFR decline or ESKD as the endpoints, the sex-based Kaplan–Meier survival curves revealed that the kidney survival rate was significantly lower in men than in women among nonelderly patients (age <65 years) (113).

4.2 Attribute-based medicine for patients with CKD and obesity: A consideration of the cutoff of VFA 100 cm^2 in visceral adiposity

In Japan, the clustered number of metabolic syndrome components is greater than 1.0 for individuals with a VFA ≥100 cm^2 (13), with the best combination of sensitivity and specificity for determining patients with multiple risk factors identified for a VFA cutoff of 100 cm^2 (13). Furthermore, VFA ≥100 cm^2 is used as a diagnostic criterion for metabolic syndrome in Japan (12), with patients having VFA ≥100 cm^2 being at risk for cardiovascular (195, 196), coronary artery (197), and cerebral small vessel (198) diseases. Although, generally, there are sex differences in waist circumference criteria for metabolic syndrome (199), it has been reported that there is no sex difference in the metabolic significance of the amount of visceral fat (196, 200). The mean number of obesity-related cardiovascular risk factors exceeded 1.0 at 100 cm^2 of VFA both in men and women (196). These results indicate the significance of differentiating patients according to a 100 cm^2 VFA threshold (201), regardless of sex, as well as highlight the need for studies based on the 100 cm^2 threshold VFA value.

In kidney disease, the presence of metabolic syndrome (202) and a VFA ≥100 cm^2 (115) are associated with CKD progression. Interestingly, a VFA ≥100 cm^2 significantly interacted with the V/S ratio in terms of the renal prognosis (119). As metabolic complications are increased with a VFA ≥100 cm^2 (115), the significance of VFA or V/S ratio in patients with a VFA ≥100 cm^2 seems to become relatively less important. However, considering that many metabolic complications develop based on obesity, patients with a VFA ≥100 cm^2 need not only medical intervention for each metabolic disease but also a reduction of excessive visceral fat itself (200).

4.3 Attribute-based medicine for patients with CKD and obesity: A consideration of the aging in visceral adiposity and CKD progression

As menopause influences obesity among women, it is clinically important to consider the influence of aging itself. Indeed, for women aged <55 years, which includes both pre- and menopausal statuses, VFA is markedly lower (median value, 59.8 cm^2), with considerably fewer obesity-related cardiovascular risk factors, than for women ≥55 years of age or men (196). The average VFA increased with age in both men and women, above the 100 cm^2 threshold after the age of 40 years in men, and close to the 100 cm^2 threshold after the age of 60 years in women (196), with the mean number of obesity-related cardiovascular risk factors being >1.0 at ages 40 years in men and 60 years in women (196). Therefore, prevention of obesity-related diseases is required at an earlier stage for men than for women (200). On the other hand, though the incidence of CVDs in women lags behind men by 10 to 20 years (203), women generally live longer than men (160). Therefore, obesity management in postmenopausal women should not also be neglected.

Systemic renin and aldosterone levels decrease with age due to decreased renin production and release (204). It has been reported

that older individuals have lower plasma renin and aldosterone levels compared with younger controls (205, 206), with impaired responses to RAAS stimuli, such as sodium depletion, hyperkalemia, and upright posture (207, 208), in older individuals (especially in late-elderly individuals). Generally, the rate of CKD progression is slow in elderly individuals (209–212). Although the reason for this has not been elucidated in human clinical studies, we consider that decreased systemic RAAS activation/glomerular hyperfiltration/glomerular hypertrophy axis (73, 129, 204) may be a factor. Although, the presence of diabetes mellitus (DM) (209, 213) and an increased BMI (213) are associated with kidney disease progression in elderly individuals, when patients with CKD are analyzed using cross-classification approach in detail (113), interestingly, DM alone was not an aggravating factor for renal prognosis in non-obese patients with CKD aged ≥65 years. In patients with CKD aged ≥65 years, poor kidney prognosis was observed only when both DM and obesity were present (113). This implies a decrease in RAAS activation in patients with CKD aged ≥65 years, and simultaneouslysuggests that attention should be paid to the overlapping of obesity and DM even in the elderly. The age-based Kaplan–Meier survival curves revealed that the kidney survival rate was significantly lower in obese patients with DM and a BMI ≥25 kg/m^2 (4 years survival, 57.8%) than in non-obese patients with DM and a BMI <25 kg/m^2 (4 years survival, 70.7%) (113). As RAAS overactivation (43–47) is one of the important pathologies contributing to obesity/metabolic syndrome and DM, the effects of RAAS activation among elderly individuals with obesity should be examined more specifically in future studies.

4.4 Challenges for attribute-based medicine for obesity-related kidney disease

Currently, the biggest challenge in promoting attribute-based medicine for patients with CKD is the lack of evidence regarding visceral fat and kidney disease progression. We conducted a literature search in the PubMed database in December 2022 using the keywords “visceral fat,” “kidney,” and “outcome,” which yielded 130 relevant articles. Among these, only three studies from two cohorts reported statistically significant associations between obesity evaluated by visceral fat measured using CT or MRI and CKD progression (kidney function decline) over a >2-year longitudinal observation period (Table 1). One of these studies, from the cohort reported by Madero et al. (148), confirmed the association between VFA measured on CT and kidney function decline, defined as a decrease in eGFR of >30% during a median follow-up of 8.9 years; their recruited patients were limited to individuals aged 70–79 years (Table 1, upper line). The other two reports were from our cohort (115, 119). Manabe et al. (115) found that VFA was significantly associated with CKD progression in a cohort with a wide age range (mean age, 59.2 years). The hazard ratios of VFA regarding CKD progression were higher in patients with VFA <100 cm^2 than in patients with VFA ≥100 cm^2 but did not differ between sexes (Table 1, middle line). The study by Kataoka et al. (119) was the first to show that the V/S ratio was significantly associated with CKD progression, particularly in the sub-cohort of VFA <100 cm^2 compared with that of VFA ≥100 cm^2 (P-value for interaction <0.01). Additionally, the hazard ratios of the V/S ratio regarding CKD progression were higher in women than in men (Table 1, lower line). Therefore, in women and patients with low visceral adiposity, the V/S ratio appears to be an early indicator of CKD progression. In this manner, the studies on visceral fat measured by CT are suggestive of an association between visceral fat accumulation and CKD progression. However, sufficient evidence is not present to guide clinical decision-making; further studies with longer observation periods are necessary to detect unhealthy obesity. Furthermore, as patients with obesity or advanced CKD generally have many complications and risk factors (113), we expect that large-scale studies that appropriately manage confounding factors will be reported in the future.

5 Perspective

Attribute-based medical care and research are the first steps to developing personalized medicine. However, at present, attribute-based medical care is not widespread enough to provide individual medical care in a clinical setting. Although much has been elucidated about the pathophysiology of kidney injury in patients with obesity, data from human studies on visceral fat and kidney prognosis are

TABLE 1 Risks of visceral fat indicators for CKD progression.

Study	Variables	Patients	Study endpoint	Follow-up	OR/HR (CI 95%) in the entire cohort and sub-cohorts
Madero et al. (148)	VFA (the highest quartile)	2489 individuals aged 70–79 years	a ≥ 30% eGFR$_{cysC}$ decline	8.9 years	1.4 (1.0–1.9)
Manabe et al. (115)	VFA (10 cm^2 increase)	200 patients with CKD	a ≥ 50% eGFR$_{cre}$ decline or ESKD	12.3 years	1.1 (1.0–1.1) 1.1 (1.0–1.1) in women 1.1 (1.0–1.1) in men 1.3 (1.0–1.8) in VFA < 100 cm^2 1.1 (1.0–1.1) in VFA ≥ 100 cm^2
Kataoka et al. (119)	V/S ratio	200 patients with CKD	a ≥ 30% eGFR$_{cre}$ decline or ESKD	12.8 years	1.8 (1.0–2.9) 2.4 (1.0–4.6) in women 1.1 (0.6–2.1) in men 6.4 (2.4–17.3) in VFA < 100 cm^2 1.0 (0.5–2.0) in VFA ≥ 100 cm^2

CKD, chronic kidney disease; Follow-up, median follow-up duration; OR, odds ratio; HR, hazard ratio; CI, confidence interval; VFA, visceral fat area; eGFR$_{cysC}$, estimated glomerular filtration rate based on cystatin C; eGFR$_{cre}$, estimated glomerular filtration rate based on creatinine; ESKD, end-stage kidney disease requiring dialysis; V/S ratio, visceral-to-subcutaneous fat ratio evaluated using computed tomography.

insufficient to establish the necessary evidence for attribute-based medicine in obesity-related renal pathologies. The accumulation of larger and longer-term studies focusing on specific attributes is necessary to resolve the existing controversy, especially concerning sex-specific kidney disease prognosis.

Author contributions

HK performed the literature search and wrote the manuscript. KN and JH were involved in planning and supervising the work. All authors contributed to the article and approved the submitted version.

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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

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Incidence and risk factors of postoperative nausea and vomiting following laparoscopic sleeve gastrectomy and its relationship with Helicobacter pylori: A propensity score matching analysis

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Background: Postoperative nausea and vomiting (PONV) are common after laparoscopic sleeve gastrectomy (LSG), affecting patient satisfaction and postoperative recovery. The purpose of this study was to investigate the incidence and severity of PONV after LSG and the relationship between Helicobacter pylori (HP) and PONV.

Methods: Patients undergoing LSG in our center from June 1, 2018, to May 31, 2022, were divided into HP-positive and HP-negative groups for retrospective analysis. The independent risk factors of PONV were determined by univariate and binary logistic regression analysis using a 1:1 propensity score matching (PSM) method.

Results: A total of 656 patients was enrolled, and 193 pairs of HP-positive and negative groups were matched after PSM. Both groups of patients had similar clinical features and surgical procedures. PONV occurred in 232 patients (60.1%) after LSG, and the incidence of PONV in HP-positive patients was 61.10%. The incidence and severity of PONV were statistically similar in both groups (P=0.815). Multivariate analysis showed that the female sex (OR=1.644, P=0.042), postoperative pain (OR=2.203, P=0.001) and use of postoperative opioid (OR=2.229, P=0.000) were independent risk factors for PONV after LSG, whereas T2DM (OR=0.510, P=0.009) and OSAS (OR=0.545, P=0.008) independently reduced the incidence rate of PONV. There was no difference either in smoking (P=0.255) or alcohol drinking (P=0.801). HP infection did not affect PONV (P=0.678).

Conclusions: The incidence of PONV following LSG was relatively high. Female sex, postoperative pain and use of postoperative opioid predicted a higher incidence of PONV. Patients with T2DM and OSAS were less likely to have PONV. There was no clear association between HP infection and PONV after LSG.

KEYWORDS

nausea, vomiting, sleeve gastrectomy, bariatric surgery, pain, Helicobacter pylori

Introduction

Overweight and obesity are defined as an excess of body fat accumulation that threatens health. According to the updated data from the World Health Organization, in 2016, more than 1.9 billion adults were overweight globally. Of these, over 650 million were in obesity (1, 2). According to epidemiological studies, obesity can progressively cause and/or exacerbate a wide spectrum of chronic diseases, which include type 2 diabetes mellitus, chronic kidney disease (3), cardiovascular disease (3, 4), a range of musculoskeletal disorders (5, 6), and even certain types of cancer (7). Bariatric surgery becomes necessary for people with severe obesity who cannot sustain weight loss by non-surgical means (e.g., diet and exercise). Laparoscopic sleeve gastrectomy (LSG) has become the most common bariatric surgery because of its simple operation, fewer complications, and good effect in reducing weight and alleviating obesity metabolism-related complications (8–11). Of note, there are a variety of side effects and post-op risks related to bariatric surgery, including acid reflux, dilation of the esophagus, obstruction of the stomach, weight gain or failure to lose weight, infection, and postoperative nausea and vomiting (PONV) (12).

PONV, defined as nausea, vomiting, or retching occurring within 24 h following anesthesia, is the most common adverse reaction after LSG. Without preventive antiemetic treatment, its incidence can reach 80% (13). PONV will induce postoperative discomforts and cause serious complications, such as water-electrolyte disorder and aspiration pneumonia, resulting in prolonged hospitalization and increased medical expenses (14). Previous studies have identified the factors affecting the incidence of PONV came from three aspects: patient factors (e.g., female sex, anxiety, infection, metabolic disease, and gastrointestinal disease), medication/anesthesia factors (e.g., opioids, volatile agents, and nitrous oxide), and surgery factors (e.g., surgical time, procedure, and technique) (15, 16). In adults, the known risk factors for PONV include female sex, non-smoking status, use of postoperative opioids, younger age, and history of PONV or motion sickness (17). However, for obese patients, several factors may contribute to the high susceptibility to PONV. Because patients who undergo bariatric surgery are usually younger women and non-smokers, with laparoscopic or robotic surgery lasting more than one hour, and receive perioperative opioid analgesia, all these are risk factors for PONV. Besides, impaired splanchnic perfusion during pneumoperitoneum and gastric volume reduction (especially after LSG) may further lead to PONV (18–20).

Studies have shown that Helicobacter pylori (HP) infection is closely related to digestive tract diseases such as peptic ulcer, gastric cancer, gastric lymphoma, and chronic gastritis (21). There are many studies on the mechanism, prevention, and treatment, but few on the relationship between HP infection and gastrointestinal adverse reactions such as PONV. Several researches have shown that there is an association between HP and hyperemesis gravidarum, which indicates that HP can exacerbate nausea and vomiting during pregnancy (22–25). Thus, the aims of this retrospective study were to investigate the incidence and risk factors of PONV after LSG and to explore whether HP infection affects PONV in subjects receiving LSG using a propensity score matching (PSM) analysis.

Methods

Study population

This study was conducted at the Department of Metabolic and Bariatric Surgery in the First Affiliated Hospital of Jinan University. A preliminary assessment determined surgical qualifications by a multidisciplinary team including surgeons, endocrinologists, anesthesiologists, nutritionists, and nurses. This retrospective study included all patients with obesity who underwent LSG at our bariatric surgery center from June 1, 2018, to May 31, 2022. The exclusion criteria were: (1) age less than 18 years, (2) patients did not undergo HP examination before the operation, (3) patients who were transferred to the intensive care unit (ICU) immediately after the operation, (4) the revision surgery (a repeated surgery due to complications or unsatisfactory results after initial bariatric surgery), (5) patients received HP eradication treatment before the operation, (6) patients received antibiotic treatment within four weeks before the operation, (7) nausea or vomiting before anesthesia.

All Bariatric surgeries were performed by the same well-experienced surgical team. The surgical techniques of LSG and postoperative management were introduced previously (26). On the basis of PONV prophylaxis guidelines, we routinely gave palonosetron and dexamethasone at the end of the operation (13,

27). After surgery, we transferred the patients to post-anesthesia care unit (PACU) until complete recovery and monitored vital signs according to standard clinical practice. In the ward, we used a visual analogue scale (VAS) to evaluate nausea and vomiting or pain (least: 0–10: worst). Depending on the severity of PONV, we decided whether to use antiemetics. For the patients with PONV or cases were intolerable, we usually offered rescue antiemetic agent (including: 5 mg tropisetron, 10 mg metoclopramide or 4 mg ondansetron). On the basis of the level of pain, subjects with postoperative pain received analgesic management, such as flurbiprofen 50 mg, parecoxib 40 mg or tramadol 100 mg (26).

Since (1) we had informed all participants receiving LSG that the clinical data which were acquired during the perioperative period may be retrospectively analyzed and published; And (2) in our study, all data were collected as a regular part of surgical care, and none were designed to collect data specifically for the research, so there was no need for written informed consent. This study protocol was approved by the Ethical Committee of the First Affiliated Hospital of Jinan University (no. KY-2021-070).

Anesthesia protocol

All procedures were finished under general anesthesia following a standardized clinical routine. Routine monitoring of electrocardiogram, blood pressure, and pulse oximetry were carried out. General anesthesia was induced with propofol, remifentanil, and rocuronium, and the dosage of drugs depended on the body weight of the patient. The maintenance of anesthesia was implemented by the use of remifentanil and propofol, oxygen, and air (26). In accordance with the PONV prevention guidelines, we routinely provided dexamethasone and palonosetron at the end of surgery (13, 27).

Study outcomes

Nausea is defined as an unpleasant feeling associated with the urge to vomit. Vomiting is defined as successful or unsuccessful (retching) excretion of gastric contents (28). The risk factors and predictors for postoperative nausea and vomiting are generally considered to be almost identical (29). Consequently, nausea or vomiting is not considered as a separate outcome in our research (30). We focused our study on 6 h and 24 h after surgery.

In this study, the primary endpoint was the overall incidence of PONV within 24 h after surgery, with secondary outcomes being the severity of PONV, the type and use of rescue antiemetics, and the time for the first rescue antiemetic and analgesics. Based on the total VAS scores at 6 h and 24 h after operation and the use of rescue antiemetics, two groups were divided (PONV: total VAS score greater than 2 or use of rescue antiemetics; No PONV: total VAS score less than or equal to 2 and no use of rescue antiemetics). Depending on the total postoperative pain VAS (P-VAS) scores at 6 h and 24 h after surgery and the application of rescue analgesics, the definition of postoperative pain was the sum of P-VAS, which was higher than 2 points or applying rescue analgesics. At the same time, for further study, we respectively divided the PONV group and the pain group into three groups: mild (3-6 scores), moderate (7-12 scores) and severe (13-20 scores) (26).

Data collection

A professional researcher reviewed patients' electronic medical records and extracted the following data which contained demographic data and perioperative factors. The demographic variables included age, BMI, obesity-related comorbidity [type 2 diabetes mellitus (T2DM), hyperlipidemia (HLP), hypertension], and smoking status. Operational details were collected, mainly including duration of surgery, the use of prophylactic antiemetics and anesthesia methods. We used the C_{13} breath test to detect HP infection.

In our department, the same team performed one standardized questionnaire to all patients. By this way, we acquired the information including PONV score, pain level, alcohol consumption, and smoking status. PONV severity was assessed using the total VAS scores at 6h and 24h after the operation. A higher score indicated more severe nausea and vomiting (31). Pain status was scored with a VAS at 6h and 24h post-operation (32). The alcohol consumption level was quantified before operation using the Alcohol Use Disorders Identification Test (AUDIT) recommended by the World Health Organization. The AUDIT score could be classified into four risk levels: 0 point as a non-drinker; 1-7 points as low risk, 8-15 points as a moderate risk; 16-19 points as high risk; 20 and above as alcohol dependence (33). Smoking status was expressed by the Brinkman index (BI), which is the number of years of smoking multiplied by the number of cigarettes smoked per day. BI results could be divided into four sequential groups: non-smokers as 0; mild smokers as 1-200; moderate smokers as 200-400; and heavy smokers as > 400 (34).

Statistical analysis

To help overcome the selection bias from the confounding variables, we performed a PSM analysis in each group. The propensity score was calculated by logistic regression analysis. We applied the nearest-neighbor method to match the patients in a 1:1 ratio. As a result, A patient in the HP-positive group was matched with one patient in the HP-negative group. The caliper size was set 0.02 and bad matches were excluded from analysis.

Continuous variables of normal distribution were presented as means ± standard deviations (SD) and were analyzed using an independent t-test. Variables with a skewed distribution were presented as median (interquartile range) and were compared using the Mann-Whitney U-test. Categorical data were presented as percentages and compared using the χ^2 and Wilcoxon test. The risk factors of PONV post LSG were firstly analyzed by a univariate analysis. After screening the variables, the likelihood ratio stepwise forward method included the significantly related variables in the binary logistic regression analysis. The analysis indexes included the odds ratio (OR), 95% confidence interval (95% CI), and significance test results (P value).

All data were analyzed using SPSS 26.0 software (SPSS Inc., Chicago, IL). All *P* values were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

The study reviewed 822 patients (205 males and 617 females) who underwent LSG surgery in our hospital between June 1, 2018, and May 31, 2022. In those patients, 82 were younger than 18 years old, 25 were not examined for HP before the operation, 12 cases were transferred to ICU after the operation, 8 patients received revision surgery, 16 cases were treated for HP before the procedure, 10 cases were treated with antibiotics within four weeks before the operation, and 13 cases had nausea and vomiting before anesthesia. Finally, 656 patients were eligible to enter the study prior to the PSM, and we had 193 matched patients over 1:1 PSM, effectively balancing the preoperative confounding factors of the two groups. The research flow chart was shown in Figure 1. Demographic data and perioperative factors of all patients before and after PSM were shown in Table 1.

Occurrence and severity of PONV in HP-positive group and HP-negative group

Before PSM, there were 390 patients of PONV in 656 patients undergoing LSG, and the infection rate was 59.45%. There was no significant difference in the incidence of PONV between HP-positive and HP-negative patients (P=0.641) (Table 2).

Comparison of covariates before and after PSM in groups

Before PSM, there were 199 cases in the HP-positive group and 457 cases in the HP-negative group, respectively. There were significant differences between the two groups in terms of age (P=0.027) and hyperuricemia (P=0.018); After PSM, the infection of HP was taken as the dependent variable, and the above covariates were taken as the independent variables. After 1:1 matching of the data between the two groups, there were 193 cases in each of the two groups. The distribution of the above covariates between the groups reached equilibrium (all P > 0.05) (Table 1).

Comparison of occurrence and severity of PONV

Among the 193 patients in the HP-negative and HP-positive groups, 114 (59.1%) and 118 (61.1%) developed PONV within 24 h after the operation. Most PONV cases were mild. The incidence, severity (P=0.851), frequency of rescue antiemetics (P=0.615), and the earliest antiemetics use (P=0.359) in the two groups were not statistically significant (Table 3).

Univariate analysis

After PSM, 386 patients were finally included, including 100 males and 286 females. A total of 232 occurred PONV, with an incidence rate of 60.1%. According to PONV occurrence, those patients were divided into the PONV group and the no PONV group. The univariate analysis showed that females had a

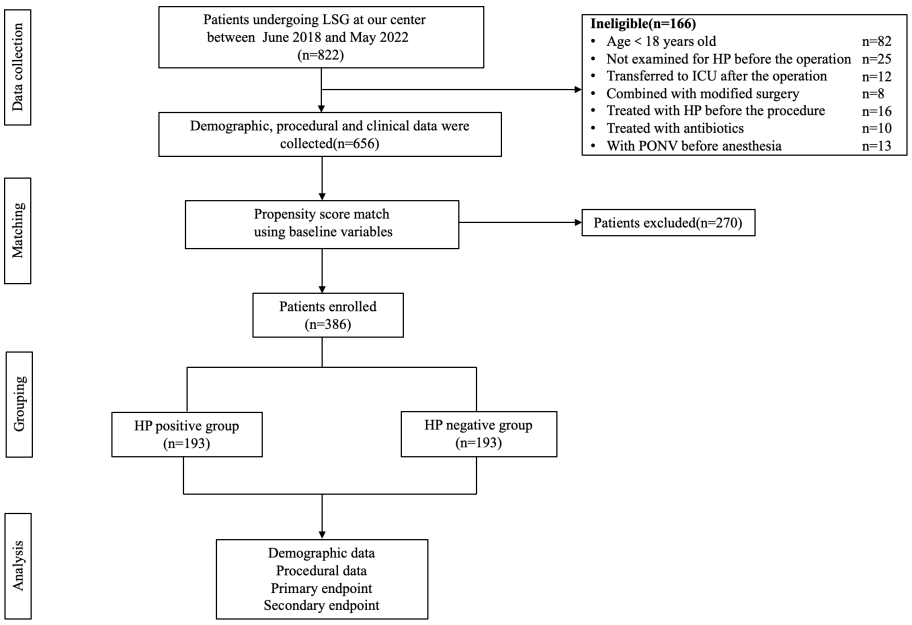

FIGURE 1
Flow diagram of this study.

TABLE 1 Demographical characteristics and clinical data of the patients (before and after PSM).

Variables	Before PSM			After PSM		
	HP-negative (n=457)	HP-positive (n=199)	P value	HP-negative (n=193)	HP-positive (n=193)	P value
Mean age (years)	30.87 ± 8.28	32.40 ± 7.69	**0.027**	30.70 ± 8.21	32.28 ± 7.68	0.052
Preoperative BMI (kg/m2)	37.28 ± 5.88	37.32 ± 5.49	0.937	37.61 ± 6.34	37.36 ± 5.51	0.683
Postoperative hospital stay (day)	4.04 ± 1.15	3.93 ± 0.94	0.237	4.08 ± 1.08	3.93 ± 0.95	0.146
Operation time (min)	130.65 ± 44.68	132.21 ± 47.56	0.687	131.22 ± 46.57	132.90 ± 47.96	0.728
Blood loss (mL)	13.52 ± 6.24	12.94 ± 6.42	0.276	12.28 ± 3.19	12.03 ± 6.38	0.384
Distance from incisal margin to pylorus (cm)	2.81 ± 0.39	2.76 ± 0.43	0.136	2.77 ± 0.42	2.78 ± 0.42	0.903
Female, n (%)	340 (74.4%)	151 (75.9%)	0.688	140 (72.5%)	146 (75.6%)	0.486
T2DM, n (%)	84 (18.4%)	49 (24.6%)	0.068	38 (19.7%)	45 (23.3%)	0.386
Hypertension, n (%)	94 (20.6%)	41 (20.6%)	0.992	33 (17.1%)	37 (19.2%)	0.597
Hyperlipidemia, n (%)	214 (46.8%)	97 (48.7%)	0.651	81 (42.0%)	95 (49.2%)	0.153
Hyperuricemia, n (%)	286 (62.6%)	105 (52.8%)	**0.018**	98 (50.8%)	105 (54.4%)	0.476
OSAS, n (%)	309 (67.6%)	137 (68.8%)	0.756	128 (66.3%)	131 (67.9%)	0.745
Esophagitis, n (%)	88 (19.3%)	30 (15.1%)	0.200	33 (17.1%)	30 (15.5%)	0.679
Alcohol consumption, n (%)	101 (22.1%)	53 (26.6%)	0.208	60 (31.1%)	50 (25.9%)	0.260
Smoking, n (%)	88 (19.3%)	36 (18.1%)	0.726	41 (21.2%)	35 (18.1%)	0.442
Postoperative pain, n (%)	213 (46.6%)	99 (49.7%)	0.459	88 (45.6%)	95 (49.2%)	0.476

Data are presented as Mean (M) ± Standard Deviation (SD), percentages (%) or median and interquartile range (IQR). Bold is used to highlight statistically significant p-values. PSM, propensity score matching; n, numbers; HP, helicobacter pylori; BMI, body mass index; T2DM, type 2 diabetes mellitus; OSAS, obstructive sleep apnea syndrome.

significantly higher risk of PONV than males after LSG (P=0.008). The incidence rate of PONV in patients with diabetes (P=0.003) and OSAS was lower than in those who had not those complications (P=0.007). The incidence of PONV was significantly higher in patients with postoperative pain (P=0.000) and use of postoperative opioid (P=0.001) than in patients without pain (Table 4).

Multivariate regression analysis

Significant and independent predictors of PONV incidence were determined by a multivariate logistic regression analysis. Variables that were statistically significant in the univariate analysis were included in the multivariate logistic regression analysis model. Results showed that female sex and postoperative pain were important independent predictors of the increase in the incidence rate of PONV. At the same time, type 2 diabetes (T2DM) and OSAS significantly and independently reduced the incidence rate of PONV after adjusting for confounding variables. The OR (95% CIs, P value) of PONV incidence after LSG was 1.644 (1.017-2.655, P = 0.042) in females and 2.203 (1.430-3.396, P = 0.001) in the pain group; The group of use of postoperative opioid was 2.229 (1.446-3.434, P = 0.000); T2DM group was 0.510 (0.306-0.848, P = 0.009) and OSAS group was 0.545 (0.349-0.853, P = 0.008) (Table 5).

Discussion

Incidence and severity of PONV

Previous studies have shown that postoperative PONV was the most common adverse effect of weight loss surgery, and its overall incidence exceeded 80% in some types of surgery (35). PONV following LSG was thought to be secondary to the sharp reduction of gastric volume and increased intragastric pressure (36). A retrospective chart review study showed that the incidence of

TABLE 2 Occurrence of PONV in 656 LSG patients with HP-positive and HP-negative.

	PONV group (n=390)	NoPONV group (n=266)	P value
HP-negative (n=457)	269 (58.9%)	188 (41.1%)	0.641
HP-positive (n=199)	121 (60.8%)	78 (39.2%)	

PONV, postoperative nausea and vomiting; LSG, laparoscopic sleeve gastrectomy; n, numbers; HP, helicobacter pylori.

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TABLE 3 Occurrence and severity of PONV and the Use of rescue antiemetics (n=193).

Variables	HP-negative (n=193)	HP-positive (n=193)		P value
Severity of PONV			Z=0.188	0.851
NO	79 (40.9%)	75 (38.9%)		
Mild	74 (38.4%)	87 (45.1%)		
Moderate	32 (16.6%)	24 (12.4%)		
Severe	8 (4.1%)	7 (3.6%)		
Times of rescue antiemetics			Z=0.503	0.615
NO	121 (62.7%)	113 (58.5%)		
1 time	38 (19.7%)	50 (25.9%)		
2 times	22 (11.4%)	20 (10.4%)		
≥ 3 times	12 (6.2%)	10 (5.2%)		
Earliest of having antiemetics			Z=0.918	0.359
No	106 (54.9%)	100 (51.8%)		
0-6 h after surgery	21 (10.9%)	18 (9.3%)		
6-12 h after surgery	30 (15.5%)	27 (14.0%)		
12-24 h after surgery	22 (11.4%)	34 (17.6%)		
> 24 h	14 (7.3%)	14 (7.3%)		

Data are presented as percentages (%). PONV, postoperative nausea and vomiting; HP, helicobacter pylori.

PONV in the LSG group (66.9%) was higher than that in the primary laparoscopic Roux-en-Y gastric bypass group (33.1%) (37). Another study pointed out that 65% of patients experience PONV within the first 24 h following LSG (18). Our study found that the incidence of PONV in Chinese patients at 0-24 h following LSG was 60.1%, lower than the above reported incidences of patients from other countries (38–40). This could be attributed to: in our center, tropisetron hydrochloride (a potent and selective 5-HT3 receptor antagonist) and metoclopramide (a dopamine antagonist) were routinely used during and right after the surgery to prevent PONV (41, 42).

Biological sex and PONV

It was identified that the female sex predicted a higher incidence of PONV following surgery (43–45). Halliday et al. Found that when two or even three preventive drugs were used, the incidence of PONV in female patients was still as high as 78% following weight loss surgery, which was three times than that of male patients during the same period (18). Another retrospective study showed that preventive antiemetic therapy did not have an ideal effect on preventing and treating PONV after weight loss surgery. After drug intervention, the incidence of PONV in female patients was still nearly 1/3 higher than that in male patients (60.4% vs. 42.9%), suggesting that the risk of PONV after bariatric surgery in female patients will not be significantly reduced with the use of preventive drugs (19). The incidence rate of PONV varies with the different phases of the menstrual cycle (19, 46, 47). However, this conclusion was contradicted by a randomized controlled trial study involving more than 5,000 patients in 2007, in which no association between the menstrual cycle stage or menopausal status and the incidence of PONV was identified (48). The molecular mechanism responsible for the correlation between the female sex and the incidence rate of PONV is still largely unknown.

Postoperative pain and PONV

Previous studies had shown that PONV was strongly associated with postoperative pain in LSG (26). Our study also demonstrated that postoperative pain was a risk factor for PONV after LSG. The possible reasons could be (1): in essence, high pain intensity was inclined to increase the risk of PONV, and (2) in our center, opioids, such as tramadol, were preferred for postoperative pain, which may increase the risk of PONV and constituted one of the major risk factors in the scoring system (49, 50). However, further study was warranted to confirm the impact of postoperative pain on PONV after LSG.

OSAS and PONV

A major finding of our research was that patients without OSAS had a higher risk of PONV than patients with OSAS. Obesity is

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TABLE 4 Univariate analysis of PONV after LSG (after PSM).

Variables	PONV Group (n=232)	NoPONV Group (n=154)	P value
Mean age (years)	31.41 ± 8.10	31.62 ± 7.83	0.803
Preoperative BMI (kg/m^2)	37.03 ± 5.76	38.18 ± 6.14	0.062
postoperative hospital stay (day)	4.16 ± 1.20	4.12 ± 0.97	0.727
Operation time (min)	128.67 ± 40.10	137.17 ± 56.02	0.105
Blood loss (mL)	11.68 ± 5.20	11.62 ± 5.27	0.916
Distance from incisal margin to pylorus (cm)	2.78 ± 0.42	2.77 ± 0.43	0.749
Gender, n(%)	183 (78.9%)	103 (66.9%)	**0.008**
T2DM, n(%)	38 (16.4%)	45 (29.2%)	**0.003**
Hypertension, n(%)	38 (16.4%)	21 (13.6%)	0.463
Hyperlipidemia, n(%)	105 (45.3%)	71 (46.1%)	0.870
Hyperuricemia, n(%)	117 (50.4%)	86 (55.8%)	0.297
OSAS, n(%)	128 (55.2%)	106 (68.8%)	**0.007**
Esophagitis, n(%)	34 (14.7%)	29 (18.8%)	0.277
HP-positive, n(%)	118 (50.9%)	75 (48.7%)	0.678
Alcohol consumption, n(%)			0.801
Non-drinker	166 (71.6%)	110 (71.4%)	–
Low-risk	26 (11.2%)	12 (7.8%)	–
Moderate-risk	13 (5.6%)	10 (6.5%)	–
High-risk	7 (3.0%)	5 (3.3%)	–
Alcohol dependence	20 (8.6%)	17 (11.0%)	–
Smoking habit, n(%)			0.255
Non-smoker	182 (78.4%)	128 (83.1%)	–
Light smoker	38 (16.4%)	20 (13.0%)	–
Moderate smoker	6 (2.6%)	4 (2.6%)	–
Heavy smoker	6 (2.6%)	2 (1.3%)	–
Postoperative pain, n(%)			**0.000**
No	105 (45.3%)	98 (63.6%)	–
Mild	95 (40.9%)	46 (29.9%)	–
Moderate	25 (10.8%)	8 (5.2%)	–
Severe	7 (3.0%)	2 (1.3%)	–
Use of postoperative opioid, n(%)			**0.001**
NO	105 (45.3%)	98 (63.6%)	-
1 time	90 (38.7%)	40 (26.0%)	-
2 times	25 (10.8%)	14 (9.1%)	-
≥ 3 times	12 (5.2%)	2 (1.3%)	-

Data are presented as Mean (M) ± Standard Deviation (SD), percentages (%) or median and interquartile range (IQR). Bold is used to highlight statistically significant p-values. PONV, postoperative nausea and vomiting; LSG, laparoscopic sleeve gastrectomy; PSM, propensity score matching; BMI, body mass index; T2DM, type 2 diabetes mellitus; OSAS, obstructive sleep apnea syndrome; HP, helicobacter pylori.

TABLE 5 Odds ratio (OR) and 95% confidence intervals (CI) of risk factors for PONV after LSG (after PSM).

	OR (95%CI)	P value
Female gender	1.644 (1.017-2.655)	**0.042**
T2DM	0.510 (0.306-0.848)	**0.009**
OSAS	0.545 (0.349-0.853)	**0.008**
Postoperative pain	2.203 (1.430-3.396)	**0.001**
Use of postoperative opioid	2.229 (1.446-3.434)	**0.000**

Bold is used to highlight statistically significant p-values. OR, odds ratio; CI, confidence intervals; T2DM, type 2 diabetes mellitus; OSAS, obstructive sleep apnea syndrome.

considered the main factor leading to OSAS, of which severity could be measured by the sleep apnea-hypopnea index (AHI). With the increase in BMI, the AHI of both males and females increases, and this trend is tendentious in males (51). Although OSAS did not affect the prognosis of bariatric surgery, it indeed affected the postoperative complication of cardiopulmonary function (52). Continuous positive airway pressure (CPAP) is currently the most effective method for treating moderate to severe OSAS, which improves the respiratory function of patients with morbid obesity and accelerates the reconstruction of preoperative pulmonary function (53). A previous study found that in subjects receiving Roux-en-Y gastric bypass, the no-CPAP group reported a higher incidence of oxygenation disturbance, but a slightly lower incidence, although not statistically significant, of PONV when compared with the CPAP group (54). Thus, a possible reason for the lower incidence of PONV in patients with OSAS in this study is the routine use of CPAP in the perioperative period of LSG. More substantial evidence and molecular pathway for this conclusion warrant further investigations.

Alcohol drinking, smoking, and PONV

A recent study reported decreased risks of PONV in alcoholics than non-drinkers and light-drinkers who underwent abdominal surgery (55). In addition, since chronic alcoholics have higher basal activity of cytochrome P450 2E1 (CYP2E1), which also accelerates the metabolic rate of volatile anesthetics, the main reason of PONV within the first two hours after surgery moderate- or heavy-drinkers (including alcohol dependence patients) may expect a reduced incidence of PONV post-LSG (56, 57). This is not consistent with what we demonstrated here. Previous studies have also built an association between the reduced incidence of PONV and cigarette smoking in Bariatric surgeries (58). However, we did not observe such correlations in our study.

HP and PONV

A previous study has demonstrated no association between HP infection and nausea after general anesthesia (59). Notash et al. Also found no relationship between HP infection and PONV who underwent general and urological surgery (60). The incidence of PONV in our research was similar in HP-positive and negative patients. Although HP may be related to severe pregnancy-related vomiting, it did not exacerbate LSG-related nausea. In bariatric surgery, to our best knowledge, this is the first report showing that HP infection did not affect the prevalence of PONV after LSG. However, since our research is a single center, more extensive cohort studies are needed for the validation of this conclusion.

Strengths and limitations

There are some limitations in this retrospective study: (1) The confirmation of a PONV event was determined by using rescue antiemetics or notating its manifestation in the medical records. This approach raises the possibility that the PONV frequencies were underestimated as some patients might experience untreated PONV; (2) Other potential factors, such as PONV history, migraine, and duration of anesthesia, were not considered, which may bias the results; (3) Since we only observed the PONV incidence within 24 h post-operation, a long-term follow-up study is needed to confirm and expand the conclusions; (4) Mechanistic research is required to investigate the molecular pathways leading to PONV after LSG and other types of bariatric surgery. However, as far as we know, this is the largest reported sample size in the study of PONV in LSG. Those confounding factors could be counterpoised after PSM. The relationship between HP and PONV in populations undergoing LSG was also interpreted. Further basic research is required to investigate the molecular mechanism leading to PONV after LSG and other types of bariatric surgery.

Conclusions

In conclusion, the incidence of PONV after LSG is relatively high. Female sex, postoperative pain and use of postoperative opioid predicted a higher incidence of PONV. Patients with T2DM and OSAS had less likelihood of a related PONV. There was no clear association between HP infection and PONV after LSG.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials. Further inquiries can be directed to the corresponding authors.

Ethics statement

As all participants receiving LSG were informed that the clinical data which were acquired during the perioperative period may be

retrospectively analyzed and published, and all data were collected as a standard part of surgical care, and none were designed to collect data specifically for the research, written informed consent was not required. This study protocol was approved by the Ethical Committee of the First Affiliated Hospital of Jinan University (no. KY-2021-070).

Author contributions

YS, JZ, and WY designed the study. YS collected patients' data. YS, JZ, and JX performed the analyses and wrote the paper. ZD and CW assisted with the study design and analysis. All authors contributed to the article and approved the submitted version.

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

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Efficacy of traditional Chinese exercise for obesity: A systematic review and meta-analysis

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Background: Obesity is considered one of the biggest public health problems, especially in the background of the coronavirus disease 2019 (COVID-19) lockdown. It is urgent to find interventions to control and improve it. We performed this systematic review and meta-analysis to summarize the effect of traditional Chinese exercise on obesity.

Methods: We searched PubMed, Embase, Cochrane Library, the China National Knowledge Infrastructure (CNKI), the Chinese Scientific Journal Database (VIP), the Chinese Biomedical Literature Database (CBM), and WanFang database for updated articles published from the inception of each database to June 2022. Randomized controlled trials (RCTs) on traditional Chinese exercise in weight reduction were included, and related data were extracted. The random-effects model was used to adjust for the heterogeneity of the included studies, and funnel plots were used to examine publication bias.

Results: A total of 701 participants were included in the 10 studies. Compared with the control group, the outcome of body weight [mean difference (MD) = –6.10; 95% CI = -8.79, -3.42], body mass index (MD = –2.03; 95% CI = -2.66, -1.41), body fat mass (MD = –3.12; 95% CI = -4.49, -1.75), waist circumference (MD = –3.46; 95% CI = -4.67, -2.24), hip circumference (MD = –2.94; 95% CI = -4.75, -1.30), and waist-to-hip ratio (MD = –0.04; 95% CI = -0.06, -0.03) in the intervention group had significant differences. Egger's test and funnel plots showed that the potential publication bias of the included studies was slight (p = 0.249).

Conclusion: Traditional Chinese exercise is an effective treatment for obesity; people under the COVID-19 lockdown could do these exercises to control weight. However, a precise and comprehensive conclusion calls for RCTs on a larger scale with more rigorous designs considering the inferior methodological quality and limited retrieved articles.

Systematic review registration: www.crd.york.ac.uk/PROSPERO/, identifier CRD42021270015.

KEYWORDS

obesity, traditional Chinese exercise, Tai Chi, Qigong, Baduanjin, Wuqinxi, COVID-19 lockdown, systematic review and meta-analysis

1 Introduction

In the past 50 years or so, the prevalence of obesity has increased worldwide, reaching an epidemic level (1). The prevalence of obesity has doubled worldwide since 1980 (2). Nearly a third of the global population has been determined to be obese or overweight (3). Since December 2019, when the coronavirus infection first emerged (4), this challenge faced by individuals has been more outstanding, with more than 50% of individuals with obesity reporting increased weight during the lockdown (5). The health problem of obesity ranges from psychological consequences to physical consequences, such as obstructive sleep apnea and arthritis, which severely affect the quality of life; the condition becomes even worse if other diseases occur together with it (6, 7). These diseases include hypertension (8), dyslipidemia, cardiovascular disease, type 2 diabetes mellitus, and dementia (9, 10). Therefore, weight reduction and weight loss maintenance are particularly important.

Studies have manifested that physical exercise is an effective way to achieve weight loss whether in women or in children (11–14). However, many kinds of physical exercise are intense or monotonous, making it difficult for people to maintain exercising. Moreover, patients with obesity cannot do too much intensive and strenuous exercise or it may induce severe exercise-induced muscle injury (15), thus the exercise intensity and duration for obese patients must be carefully selected (16). Traditional Chinese exercise (TCE), originating from traditional Chinese medicine tracing back to approximately 3,000 years ago, is used as a therapeutic and aerobic exercise, including tai chi, qigong, Baduanjin, Wuqinxi, Yijinjing, and other mind–body therapies (17, 18). This kind of exercise is part of low- to moderate-intensity aerobic exercises, which are different substantially from the high-intensity physical exercise (19).

Although meta-analyses have shown the effectiveness of TCE in knee osteoarthritis, stroke, and cognitive outcomes for older adults (20–22), whether TCE is more effective in weight reduction and weight loss maintenance than other exercise interventions or without treatment has not been reported yet. The main objective of this study was to identify whether TCE is effective in weight reduction and weight loss maintenance, to incorporate all available randomized controlled trials (RCTs), and to investigate relevant subgroups.

2 Materials and methods

2.1 Data source

We searched PubMed, Embase, Cochrane Library, the China National Knowledge Infrastructure (CNKI), the Chinese Scientific Journal Database (VIP), the Chinese Biomedical Literature Database (CBM), and WanFang Database for updated articles published from the inception of each database to 1 June 2022. At the same time, we searched for gray literature that met the inclusion criteria in Open Grey, Clinical Trials.gov, and WHO Clinical Trial Registration Center. When duplicate publications were identified, we chose the most complete and recent trial. Two investigators (YY and ZY) independently retrieved all related studies in the database and excluded duplicate publications. The search strategy for PubMed is presented in Table 1.

2.2 Inclusion and exclusion criteria

Our meta-analysis is reported in line with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (23) Statement and has been registered at the International Prospective Register of Systematic Reviews (number: CRD42021270015).

The inclusion criteria are as follows: 1) the studies must be RCTs; 2) age of participation >18 years; 3) diagnostic criteria for obesity in the study are employed according to the region and ethnic group (Asia-Pacific ≥25.0 kg/m^2 and America ≥30.0 kg/m^2 for adults); 4) the intervention group must do TCE, such as tai chi, qigong, Baduanjin, Yijinjing, or Wuqinxi, whether combined with other interventions like control group or not. The control group does casual exercise or only diet education, auricular plaster therapy, acupoint catgut-embedding therapy without exercise, etc.

Exclusion criteria are as follows: 1) the participation according to the region and ethnic group did not meet the body mass index (BMI) of obesity; 2) age of participation ≤18 years; 3) the intervention group did not do TCE or the control group also did TCE.

2.3 Study selection and data extraction

Two independent researchers (YY and ZY) read the title, abstract, and full text, screened the literature according to the inclusion and exclusion criteria, and cross-checked the results. If there is a disagreement, a third researcher (QX) will be consulted. Data extracted from the included literature included the following: first author, publication time, mean age or age range, sample size of the study, measures, intervention group and control group taken, duration time, and outcomes.

2.4 Quality assessment

The quality of the included studies was assessed by the revised Cochrane risk of bias tool (ROB 2.0) in the Cochrane Handbook for Systematic Reviews of Interventions. The following parameters were evaluated: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result. Each item was determined to be at high risk of bias, some concerns (unclear risk of bias), or low risk of bias. The combined evaluation of the above items resulted in overall bias.

TABLE 1 Search strategy in PubMed database.

Number	Search terms
#1	Obesity [MeSH Terms]
#2	Obese [Title/Abstract]
#3	#1 OR #2
#4	Traditional Chinese exercise [Title/Abstract]
#5	Tai chi [Title/Abstract]
#6	Tai ji [Title/Abstract]
#7	Qigong [Title/Abstract]
#8	Baduanjin [Title/Abstract]
#9	Liuzijue [Title/Abstract]
#10	Yijinjing [Title/Abstract]
#11	Wuqinxi [Title/Abstract]
#12	#4 OR #5-11
#13	randomized controlled trial [All field]
#14	randomly [All field]
#15	controlled clinical trial [All field]
#16	randomized [All field]
#17	random allocation [All field]
#18	placebo [all field]
#19	single-blind method [All field]
#20	double-blind method [All field]
#21	trials [All field]
#22	comparators
#23	allocation
#24	#13 OR #14-23
#25	#3 AND #12 AND #24

2.5 Statistical analysis

Review Manager software (Revman 5.4 Cochrane Collaboration) was used to meta-analyze the selected studies. The odds ratio (OR) was used as the effect size index for dichotomous variables, and the mean difference (MD) was used as the effect size index for continuous variables, with 95% confidence intervals (CIs) in forest plots. Sensitivity analysis and subgroup analysis will be used to analyze the source of heterogeneity. For the sensitivity analysis, it can help find the source of heterogeneity by reestimating the combined effect using the one-by-one elimination method. For subgroup analysis, the studies may be divided into different subgroups according to the duration time, different types of TCE, etc., and whether it could be shown that the subgroup factors were the source of heterogeneity. When high heterogeneity exists ($p < 0.1$ or $I^2 > 50\%$), a random-effects model was used for meta-analysis. Publication bias of major outcome indicators was analyzed by funnel plot in RevMan 5.4 software. When the funnel plot was symmetrical on both sides, the possibility of publication bias between studies was considered low.

2.6 Grading of evidence quality

The quality of evidence was graded according to the GRADE method and was classified as high grade, moderate grade, low grade, and very low grade. The assessment criteria used in the GRADE method included risk of bias, inconsistency, indirectness, imprecision, and publication bias. A grade would be given for each outcome.

3 Results

3.1 Search results

Our initial search yielded 196 articles in total, 28 of which were removed because of duplication. After screening the titles and abstracts, a further 38 items were taken away. A total of 130 articles were reviewed, among which 10 were included in this meta-analysis (24–33). No further study was identified by manual search. The flow diagram of study selection was shown in Figure 1 (guideline flow diagram).

3.2 Study characteristics

Ten studies with 701 participants were included for the meta-analysis. Outcomes of the studies are as follows: body weight (BW), BMI, body fat mass (BFM), waist circumference (WC), hip circumference (HC), and waist-to-hip ratio (WHR). The main characteristics of the 10 articles were summarized in Table 2.

3.3 Quality of the evidence

Results of risk of bias assessment for all included studies were summarized in Figure 2. For bias from the randomization process, eight RCTs were considered as low risk, one study as unclear risk, and one study as high risk. For bias from deviations from intended interventions, bias from missing outcome data, and bias from measurement of the outcome, all 10 RCTs were considered as low risk. For bias from selection of the reported result, six RCTs were rated as likely low risk, three studies were considered as unclear risk, and one study as high risk. For overall risk of bias, five RCTs were considered as low risk, four studies as unclear risk, and one study as high risk. The items that generated bias included the following: ① randomization was reported or but the process was not specifically described or the randomization method was incorrect; ② whether allocation concealment was implemented for the randomization protocol was not reported or unclear; ③ whether blinding was implemented was not clearly reported; ④ whether study protocol registration was not reported or the discussion of reported entries was unclear.

3.4 Outcome measures

3.4.1 Body weight

Seven studies (24, 25, 28–30, 32, 33) involving 487 participants reported the outcome of BW. The synthesized data indicated that the TCE group had effectively decreased weight as compared with that of the control group (MD = −6.10; 95% CI = -8.79, -3.42), with a high heterogeneity (I^2 = 70%, p = 0.0008).

Subgroup analysis showed that the heterogeneity in BW between the two groups disappeared when we excluded the studies whose duration time was less than 16 weeks [including three studies (24, 28, 33); MD = -3.95; 95% CI = -5.78, -2.13; I^2 = 0%] (Figure 3). However, whether the duration time was less than 16 weeks or more, there was a significant difference between the two groups in BW.

Subgroup analysis also showed that all kinds of TCE have a significant difference in BW (Figure 4), which was consistent with the results reported in every study. Heterogeneity analysis suggested that there was a high heterogeneity in the study of exercise styles of Baduanjin (I^2 = 81%, p = 0.02) and Yijinjing (I^2 = 89%, p = 0.0001) (Figure 4). It may be due to the existence of low-quality research or differences in the duration and time of the research.

3.4.2 Body mass index

All 10 included studies (24–33) totaling 701 participants were included in this outcome. The synthesized data indicated that the TCE group had a significant impact on decreasing BMI as compared with the control group (MD = −2.03; 95% CI = -2.66, -1.41), and there was a substantial heterogeneity for this synthesized outcome (I^2 = 64%, p = 0.0001). Therefore, these studies were combined using the random-effects model.

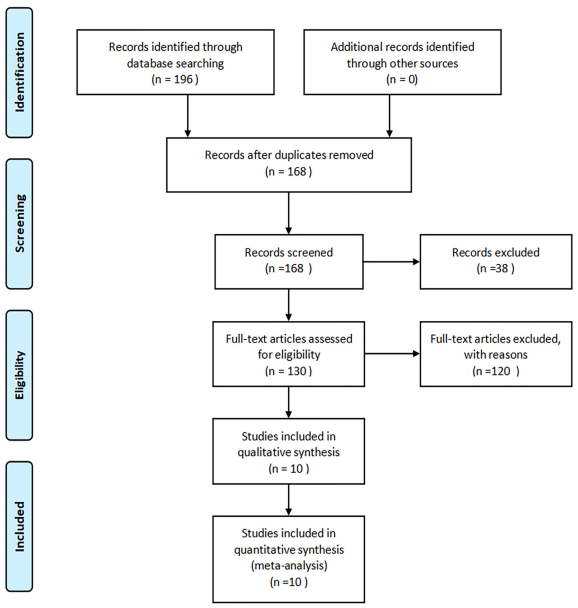

FIGURE 1
Flowchart of the study selection process.

Subgroup analysis showed that when excluding the studies whose duration time was less than 16 weeks, the heterogeneity in BMI between intervention groups and control groups decreased [including six studies (24, 26–28, 31, 33); MD = -1.73; 95% CI = -2.33, -1.13; I^2 = 40%; p = 0.12] (Figure 5).

3.4.3 Body fat mass

A total of five studies (25–27, 30, 33) involving 227 participants reported the effects of different interventions on the outcome of BFM. The TCE group had effectively decreased it as compared with that in the control group (MD = −3.12; 95% CI = -4.49, -1.75; I^2 = 36%; p < 0.00001) (Figure 6), and there was a low heterogeneity.

3.4.4 Waist circumference

A total of six studies (24, 25, 27–29, 32) reported this outcome. Heterogeneity analysis suggested that there was a high heterogeneity (I^2 = 86%, p = 0.003). The sensitivity analysis was carried out one by one, and it was found that when the research by Yu (32) was removed, the heterogeneity between the studies disappeared (I^2 = 0%, p = 0.52). The data analysis indicated that WC in the TCE group was lower than that in the control group (MD = −3.46; 95% CI = -4.67, -2.24) (Figure 7).

3.4.5 Hip circumference

A total of four studies (25, 27, 29, 32) involving 216 participants reported the effects of different interventions on the outcome of HC. There was a high heterogeneity (I^2 = 70%, p = 0.002). Sensitivity analysis suggested that after removing the research by Yu (32), the heterogeneity disappeared (I^2 = 0%, p = 0.68). The data indicated that the TCE group had an effect in decreasing HC as compared with that in the control group (MD = −2.94; 95% CI = -4.75, -1.30) (Figure 8).

3.4.6 Waist-to-hip ratio

Four studies (27, 29, 31, 32) involving 240 participants were included in this outcome. Compared with that in the control group, the TCE group had effectively decreased WHR (MD = −0.04; 95% CI = -0.06, -0.03), with no heterogeneity among the studies (I^2 = 0%, p < 0.00001) (Figure 9).

3.5 Evaluation of publication bias

The publication bias of outcomes was evaluated using funnel plots based on the 10 studies. It can be seen that the funnel plot was not symmetrical, which indicated that no significant publication bias existed (Figure 10).

3.6 Grading of Recommendations Assessment, Development and Evaluation (GRADE) for the main outcomes

GRADE assessment was conducted for the main outcomes, and the results showed that the evidence quality of BW, BMI, HC, BFM

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TABLE 2 Characteristics of the 10 studies included in the meta-analysis.

Study	Mean age or age range (AVG)	Sample size (IG/CG)	Intervention Group	Control Group	Duration time	Outcomes
Beebe2013 (25)	IG:60.4 ± 6.2 CG:62.6 ± 5.9	26(13/13)	Tai chi 45 min/week and diet education 45 min/week	diet education 45 min/week	16w	①②③④⑤
Song2015 (26)	IG:59.6 ± 4.72 CG:60.3 ± 4.50	30(15/15)	Tai chi 2×40 min/day and auricular plaster therapy 3-5×5-10 min/day	Auricular plaster therapy 3-5times/day	180d	②③
Fang2018 (27)	18-20	80(40/40)	Wuqinxi 3×45 min/week	Casual exercise 3×45 min/week	one semester	②③④⑤⑥
Li2015 (28)	60-70	60(30/30)	Wuqinxi 5-6×30 min/week	None	6m	①②④
Pan2013 (29)	IG:37.21 ± 8.81 CG:35.28 ± 8.87	64(32/32)	Baduanjin 14×30 min/week and acupoint catgut-embedding therapy twice/month	Acupoint catgut-embedding therapy twice/month	12w	①②④⑤⑥
Wang2015 (30)	19-26	51(23/28)	Yijinjing 3×60 min/week	None	10w	①②③
Yu2013 (31)	40-70	104(52/52)	Baduanjin 3-4times/week and Knowledge education twice a month	Knowledge education twice a month	12m	②⑥
Yu2017 (32)	18-23	46(23/23)	Baduanjin and qigong 5×60min/week	None	16w	①②④⑤⑥
Zhang2011 (33)	IGM:21.34 ± 1.21 IGW:21.55 ± 1.49 CGM:21.17 ± 1.52 CGW:20.86 ± 1.34	40(20/20)	Tai chi 5×30 min/week	None	20w	①②③
Zou2013 (24)	IG:57.42 ± 6.67 CG:57.52 ± 6.20	200(100/100)	Yijinjing 14×30 min/week and health education	Health education	6m	①②④

IG, intervention group; CG, control group; IGM, intervention group man; IGW, intervention group woman; CGM, control group man; CGW, control group woman; w, week; m, month; d, day; min, minute. ①BW, body weight; ②BMI, body mass index; ③BFM, body fat mass; ④WC, waist circumference; ⑤HC, hip circumference; ⑥WHR, waist-to-hip ratio.

and WHR was low; the evidence quality of WC was moderate (Table 3).

4 Discussion

Obesity has been a more outstanding issue during the coronavirus disease 2019 (COVID-19) lockdown, which should be paid more attention to and adopt measures to control. It is the cause of many cardiovascular and cerebrovascular diseases (34) and may be accompanied by an increase in the prevalence of diabetes, cancer, and other diseases (35). There is no doubt that patients with simple obesity can lose weight through exercise, thereby reducing diseases caused by obesity. However, studies reported that the excessive exercise load was not suitable for obese people. They cannot bear too much intensity training (11, 36). TCE is a therapeutic physical and mental aerobic exercise that has been used to improve physical and mental health in China for thousands of years (20, 37–39), which does not need too much space and could be taken even in the COVID-19 lockdown. This meta-analysis and systematic review provide a quantitative estimate of whether TCE is a significant effective strategy for obesity improvement.

We have shown that TCE works significantly in decreasing BW, BMI, BFM, WC, HC, and WHR, which means it plays a positive role in weight reduction and weight loss maintenance. These effects are consistent with the results of individually included RCTs. For different types of TCE, subgroup analyses indicated that there was

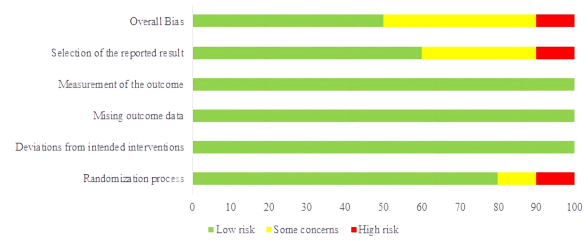

FIGURE 2
Risk of bias graph. (TCE) on body weight (BW).

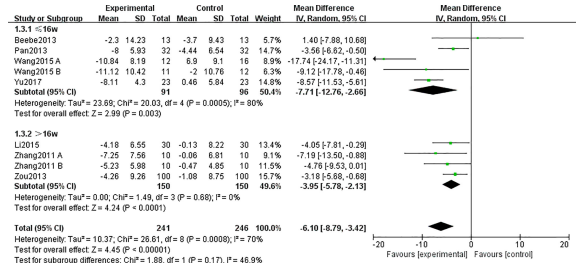

FIGURE 3
Forest plots and subgroup analysis of duration time for TCE on BW.

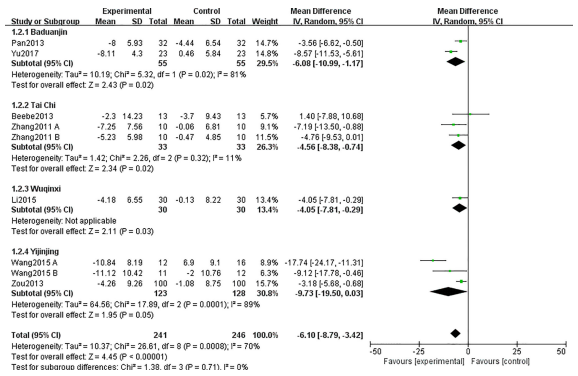

FIGURE 4
Forest plots and subgroup analysis of different exercise types for TCE on BW.

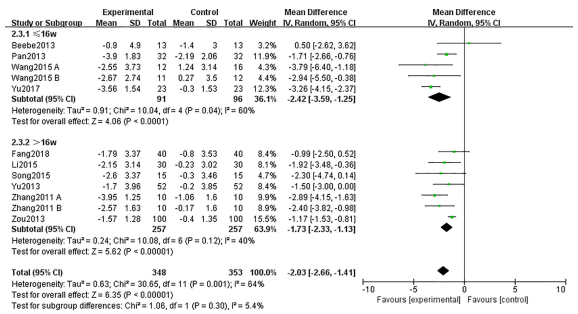

FIGURE 5
Forest plots and subgroup analysis for traditional Chinese exercise (TCE) on body mass index (BMI).

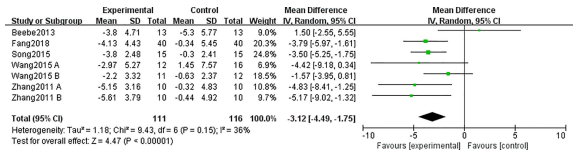

FIGURE 6
Forest plots for traditional Chinese exercise (TCE) on body fat mass (BFM).

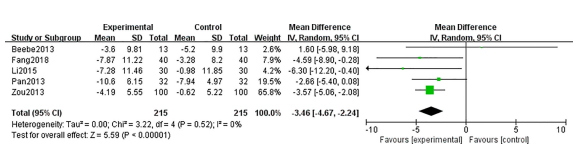

FIGURE 7
Forest plots for traditional Chinese exercise (TCE) on waist circumference (WC).

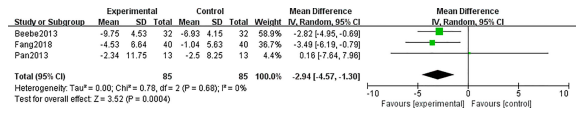

FIGURE 8
Forest plots for traditional Chinese exercise (TCE) on hip circumference (HC).

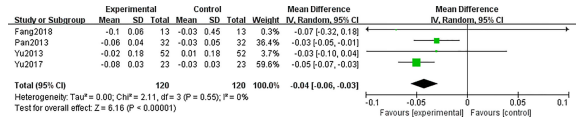

FIGURE 9
Forest plots for traditional Chinese exercise (TCE) on waist-to-hip ratio (WHR).

no significant difference among tai chi, Baduanjin, Yijinjing, and Wuqinxi in BW and BMI. And there was no significant difference among duration time, whether the time is more than 16 weeks or less; they both work. Many studies reported other outcomes, such as triglycerides, serum total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and blood pressure (34, 40). Obesity is often accompanied by lipoprotein atherogenicity, diabetes, hypertension, and hyperlipidemia (41), thus when controlling and treating obesity, TCE can reduce these indicators at the same time. (25, 26). Safety was not reported as an outcome because this outcome was not discussed in these studies. It should be recorded as an outcome in future research.

Our study has several strengths. Firstly, a detailed search strategy is employed by searching different kinds of databases and trial registries. Moreover, no language limitation is set to ensure inclusion of as much data as possible from appropriate studies. Additionally, we try to find heterogeneity between the studies included in our study and to decrease it, which make the results more convincing. Furthermore, our study is the first systematic review and meta-analysis to evaluate the efficacy of

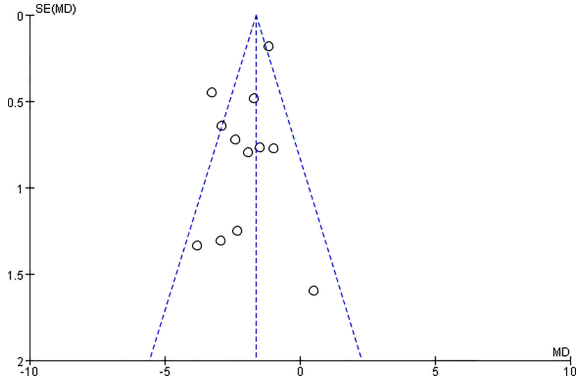

FIGURE 10
Funnel plots of the body mass index (BMI).

TABLE 3 Summary of GRADE.

Quality assessment							Number of patients		Effect	Quality	Importance
Outcomes	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	TCE group	Control group	Illustrative comparative risks (95% CI)		
BW	RCT	Serious[a]	Serious[b]	No serious	No serious[c]	Undetected	241	246	6.1 lower (8.79 to 3.42 lower)	⊕⊕⊖⊖ LOW[a,b,c]	IMPORTANT
BMI	RCT	Serious[a]	Serious[b]	No serious	No serious	Undetected	348	353	0 higher (1.91 to 1.36 lower)	⊕⊕⊖⊖ LOW[a,b]	IMPORTANT
BFM	RCT	Serious[a]	No serious	No serious	Serious[c]	Undetected	111	116	3.12 lower (4.49 to 1.75 lower)	⊕⊕⊖⊖ LOW[a,c]	IMPORTANT
WC	RCT	Serious[a]	No serious	No serious	No serious	Undetected	215	215	3.46 lower (4.67 to 2.24 lower)	⊕⊕⊕⊖ MODERATE[a]	IMPORTANT
HC	RCT	Serious[a]	No serious	No serious	Serious[c]	Undetected	85	85	2.94 lower (4.57 to 1.3 lower)	⊕⊕⊖⊖ LOW[a,c]	IMPORTANT
WHR	RCT	Serious[a]	No serious	No serious	Serious[c]	Undetected	120	120	0.04 lower (0.06 to 0.03 lower)	⊕⊕⊖⊖ LOW[a,c]	IMPORTANT

[a]Lacking blinding, randomization, or unclear allocation.
[b]Substantial heterogeneity.
[c]Small sample size.

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TCE on obesity; it could provide evidence for clinical workers that would help them make better clinical decisions. Moreover, we offer ways to control weight at home for those with obesity, which is very necessary in the background of the lockdown.

Our review has several limitations as well. First, the sample size of this meta-analysis was relatively small. As a result, the unknown risk of bias caused by incomplete data could constrain our results. Second, the research results are not restricted by the language of the published articles. Moreover, the intervention of the intervention group of the study is TCE, which is from China, thus eight of the studies are Chinese, which may be of relatively low quality. Third, the intervention protocol varied significantly in the aspect of exercise type (tai chi, Baduanjin, qigong, Yijinjing, Wuqinxi), duration time (from 10 weeks to 12 months), frequency (1–14 times per week), continued time of each exercise (from 30 to 60 min), and at the same time, the control group received different interventions as well; this may be the reason for the heterogeneity of the article.

Despite these limitations, this meta-analysis provides information on the association between TCE and obesity.

5 Conclusion

In summary, the results of our meta-analysis indicated that TCE may have statistically significant effects on decreasing participants' BW, BFM, BMI, HC, WC, and WHR, which indicated that it helps to control obesity. The findings presented in the meta-analysis may have important implications worldwide, especially in the COVID-19 lockdown. However, a precise and comprehensive conclusion calls for RCTs on a larger scale with more rigorous designs considering the inferior methodological quality and limited retrieved articles.

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 author.

Author contributions

Data curation: ZY, YY, QX, and KH; Formal analysis: ZY and QG; Investigation: ZY and XW; Methodology: ZY, YY, and QX; Project administration: ZY; Resources: YY, ZY, and XW; Software: ZY and XW; Supervision: ZY, KH, and QG; Validation: YY and KH; Visualization: ZY and YY; Writing – original draft: ZY, KH, and YY; Writing – review and editing: ZY and XW. All authors contributed to the article and approved the submitted version.

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/fendo.2023.1028708/full#supplementary-material

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Quality evaluation of metabolic and bariatric surgical guidelines

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Objective: To evaluate the quality of surgical guidelines on bariatric/metabolic surgery.

Methods: Four independent reviewers used the AGREE II (The Appraisal of Guidelines for Research and Evaluation II) tool to assess the methodological quality of the included guidelines and conducted a comparative analysis of the main recommendations for surgical methods of these guidelines.

Results: Nine surgical guidelines were included in this study. Five articles with AGREE II scores over 60% are worthy of clinical recommendation. The field of rigor of development was relatively low, with an average score of 50.82%. Among 15 key recommendations and the corresponding best evidence in the guidelines, only 4 key recommendations were grade A recommendations.

Conclusions: The quality of metabolic and bariatric guidelines is uneven, and there is much room for improvement.

KEYWORDS

bariatric surgery, metabolic surgery, guidelines, recommendations, bariatric

1 Introduction

In recent years, the global obesity rate has increased sharply, and the obesity crisis has become one of the biggest public health challenges in the 21st century. Additionally, with the increase in disease burden, the significant increase in medical costs and indirect loss of productivity also have a huge economic impact (1, 2). For example, most adults in the United States and the United Kingdom are considered overweight from a medical perspective (body mass index [BMI] of 25 – 29.9 kg/m^2) or obese (BMI of 30 kg/m^2 and more) (3). With the widespread prevalence of obesity, complications such as coronary heart disease, high blood pressure, stroke, certain types of cancer, non-insulin-dependent diabetes, gallbladder disease, dyslipidaemia, osteoarthritis, gout, and sleep apnoea occur

simultaneously (4, 5). Obesity and overweight can lead to shortened life expectancy and lower quality of life (6).

Although some new drugs (7) and diet and exercise programs (8) have been developed to counteract the continuous increase in the obesity rate, the above measures are still not ideal for controlling obesity. Bariatric surgery has been proven to be an effective method for the control of morbid obesity and metabolic syndrome (3) and has also received attention from international academic groups and experts on bariatric/metabolic surgery. Currently, metabolic and weight loss surgical guidelines have been developed (9–17). However, the methodological quality of these guidelines and the heterogeneity of the main recommendations have caused great confusion to the users of these guidelines. Therefore, the purpose of this study was to analyse the methodological quality of the guidelines for bariatric/metabolic surgery and the differences in recommendations between these guidelines and to provide the best evidence to help guideline users in choosing an appropriate guideline and to inform guideline developers when they update them.

2 Methods

2.1 Research design

In this study, the AGREE II tool was adopted to conduct methodological evaluation of clinical guidelines for metabolism and weight loss, and PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) principles were followed (18).

2.2 Retrieval strategy

In this study, metabolism and weight loss surgery-related guidelines were retrieved from PubMed, Ovid, Springer, Web of Science, CNKI, VIP database, Wanfang Database and other databases. This study examined relevant guidelines published from January 1, 2014, to January 1, 2021, that included supporting evidence for the main recommendations included in the guidelines and the impact of time span on evidence updates. This study also searched Google and Baidu Academics to obtain more guidelines. The language was limited to English and Chinese. Keywords included the Chinese and English keywords “bariatric surgery”, “metabolic surgery” and “guidelines”. Additionally, the reference lists of the included guidelines were manually searched.

2.3 Selection principle of guidelines

2.3.1 Inclusion criteria

(1) complete guideline text (2); the guidelines include information about metabolism and bariatric surgery (3); if the guidelines are updated, only the latest version will be included (4); guidelines published in English or Chinese.

2.3.2 Exclusion criteria

(1) duplicate guidelines, translated versions of the guidelines, secondary or multiple publications, and brief abstracts (2); the translated versions of the guidelines may have lost the information of the original versions, which may have affected the accuracy of the evaluation of this study (3); if multiple guidelines were issued by the same organisation, older versions of the guidelines were excluded.

According to the above inclusion and exclusion criteria, two reviewers (Xiao Wang and Yu-Lu Du) independently evaluated the obtained literature to determine whether to include or exclude the literature. Disagreements were resolved through negotiation until consensus or by consulting a third expert reviewer (Ya-Qi Zheng).

2.4 Quality assessment of the guidelines

2.4.1 AGREE II tools

This research used the latest version of the AGREE II tool (https://www.agreetrust.org/resource-centre/) to assess each bariatric/metabolic guideline that met the standards of this study. This tool consists of 6 domains and 23 items. (Table S1).

In this study, each bariatric/metabolic guideline was graded according to the AGREE II user manual by four independent reviewers (Zi-Han Qin, Xin Yang, Li-Ya An and Ting Yang). The reviewers were trained on the use of the AGREE II tool through a rigorous online training course on the AGREE website. Reviewers are guided and supervised by experts (Da-Li Sun, Yue-Ying Lin, Li-Ya An and Ting Yang) who have published a number of articles through the use of AGREEII. The team includes experienced specialists in bariatric/metabolic surgery (Da-Li Sun, Yue-Ying Lin, Li-Ya An and Ting Yang). The user manual defines each item and helps the user determine the guideline score for the item. Items are rated on a scale of 1 (completely inconsistent with the item) to 7 (completely consistent with the item). Domain scores are calculated by adding up the project scores for each domain for each reviewer, then normalizing them to the percentage of the highest score (19).

For each area of the AGREE II tool, “points earned” is calculated as the sum of all points scored by the grader for all items contained in that area. The “proportional domain score” is calculated as a standardized score using the following formula: (score obtained – lowest possible score)/(maximum possible score – lowest possible score). The maximum score for each area is obtained by multiplying the number of items in that area by the number of raters, multiplying by 7 (which corresponds to “strong agreement”). The minimum score is obtained by multiplying the number of items in the field by the number of raters and multiplying by 1 (which corresponds to “strong disagreement”).

2.5 Guidelines for extracting and regrading key recommendations and best evidence

In this study, the relatively high AGREE II scoring guidelines were used to extract and analyse important recommendations

related to metabolism and bariatric surgery, and a database search was conducted to further obtain the highest level of evidence supporting these recommendations. This study reclassified recommendations and evidence using the Oxford Centre for Evidence-based Medicine (OCEBM) grading system. (Table S2).

2.6 Statistical analysis

We used a descriptive statistical analysis method to calculate the standardized scores for each guideline, which were expressed as a percentage, and we also listed the median scores and the range of each domain. We adopted a two-way ANOVA to calculate the intra-class correlation coefficients (ICCs) to examine the agreement among the scores from the four reviewers. Consistency among raters was determined by ICCs and 95% confidence intervals (CIs). ICC is equal to the individual variation divided by the total variation, with a value between 0 and 1 for the 23 items identified in AGREE II. If the ICC is between 0.01 and 0.20, the degree of agreement is considered to be slight; if the ICC is between 0.21 and 0.40, the consistency is considered fair. If the ICC is between 0.41 and 0.60, the consistency is moderate. If the ICC is between 0.61-0.80, the degree of agreement is considered to be high; if the ICC is between 0.81-1.00, it is considered perfect. $P < 0.05$ indicates statistical significance. Statistical analysis was performed using IBM SPSS Version 19.0 (SPSS Inc., Chicago, IL, USA). (Table S3).

3 Results

3.1 Guideline features

A total of 264 records were obtained through database retrieval and other retrieval methods, which were evaluated by reading titles, abstracts and full texts. Finally, 9 guidelines for bariatric/metabolic surgery were included (Figure 1), 8 of which are original (9–14, 16, 17) and 1 of which was updated in 2020 (15). All guidelines were developed by local or national medical associations. Seven of the guidelines are international or from > 1 country (9–14, 16), one is from the EU (15), and one is from India (17). One of the nine guidelines is for children and adolescents (10), and the other eight are for adults (9, 11–17). The basic features of the included guidelines are listed in Table 1.

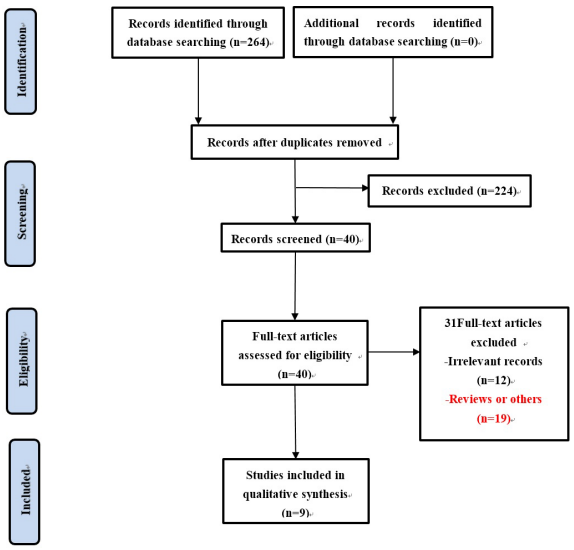

FIGURE 1
Flowchart of study selection.

3.2 Quality evaluation of the guidelines

The AGREE II standardized area scores for each metabolic and bariatric/metabolic surgery recommendation guideline and their overall recommendations are shown in Table 2. Domain scope and purpose and domain clarity and presentation methods had the highest median scores of 84.09% and 76.70%, respectively (range 77.78% to 91.67% and 62.50% to 91.67%, respectively). The median score for domain stakeholder involvement was 60.03% (range 47.22% to 86.11%). Only one guideline (11%) scored less than 50% (13). Editorial independence of the domain had the highest score range (0 to 100%). Two guidelines scored zero (9, 12), and three guidelines scored less than 50% (9, 10, 12). The median scores for applicability and rigor of development were 45.95% and 50.82% (ranging from 27.08% to 78.13% and 20.83% to 77.08%, respectively). According to the overall score of the guidelines, the five included guidelines scored well in all areas (10–12, 14, 15) and were classified as strongly recommended for clinical practice. Four guidelines were recommended for revisions (9, 13, 16, 17).

In this study, four evaluators participated in the evaluation of the guidelines, and the ICC value range of the metabolic and bariatric surgery guidelines was > 0.8 using AGREE II, indicating a high degree of internal item score consistency among reviewers.

3.3 Key recommendations in the guidelines and the best available evidence

To further analyse the reasons for the heterogeneity of recommendations for metabolism and bariatric surgery among different guidelines, this study referred to guidelines (15) with relatively high scores and relatively clear recommendation items, extracted the main recommendations in the guidelines, and sorted out the highest evidence supporting these main recommendations by searching the database. Additionally, recommendations (9–11, 14, 16) not included in the high quality guideline (15) are also sorted (Table 3). It mainly includes surgical indications, surgical methods, and preoperative and postoperative recommendations (Table 3).

4 Discussion

Compared to other disease-specific guidelines, the development of guidelines for metabolism and bariatric surgery can be a complex

TABLE 1 Characteristics of included guidelines.

Title	Authors	Organization	Short name	Country Applied	Grading system	Subjects	Version	Target population	Development Method
Reoperative surgery for nonresponders and complicated sleeve gastrectomy operations in patients with severe obesity. An international expert panel consensus statement to define best practice guideline	Kichler K, et al.,2018	ASMBS	Ki (9)	International	Not specified	Providing a clinical consensus guideline regarding standardization of indications, contraindications, surgical options, and surgical techniques when reoperating on patients who underwent a failed or complicated SG.	Original version	Adults	CB
ASMBS pediatric metabolic and bariatric surgery guidelines	Pratt JSA, et al.,2018	ASMBS	Pr (10)	International	Not specified	Removing the stigma against the surgical treatment of childhood obesity and educate paediatric physicians and providers about the need for early referral of patients suffering from severe obesity to a MBS program.	Original version	Children & Adolescents	EB
The first consensus statement on revisional bariatric surgery using a modified Delphi approach	Mahawar KK, et al.,2019	IFSO	Ma (11)	International	Not specified	Developing consensus amongst a group of international RBS experts on a range of practices and principles concerning this procedure following a Modified Delphi protocol.	Original version	Adults	CB
Bariatric surgery in class I obesity: a Position Statement from the International Federation for the Surgery of Obesity and Metabolic Disorders (IFSO)	Busetto L, et al.,2014	IFSO	Bu (12)	International	Not specified	Examining the use of bariatric surgery in the class I obesity range (BMI 30 - 35 kg/m^2).	Original version	Adults	EB & CB
Duodenal switch in revisional bariatric surgery: conclusions from an expert consensus panel	Merz AE, et al.,2019	ASMBS	Me (13)	International	Not specified	Generating expert consensus points on the appropriate use of BPD/DS in the revisional bariatric surgical setting	Original version	Adults	EB & CB
ASMBS Updated Position Statement on Bariatric Surgery in Class I Obesity (BMI 30-35 kg/m^2)	Aminian A, et al.,2018	ASMBS	Am (14)	International	Not specified	Assessing the evidence regarding the benefits and risks of bariatric surgery in patients with class I obesity (BMI of 30.0 – 34.9 kg/m^2), which accounts for more than 20% of the United States population	Original version	Adults	EB
Clinical practice guidelines of the European Association for Endoscopic Surgery (EAES) on bariatric surgery: update 2020 endorsed by IFSO-EC, EASO and ESPCOP	Lorenzo ND, et al.,2020	EAES	Lo (15)	Europe	GRADE	Aiming to increase health care knowledge among bariatric patients. Summarizing the latest evidence on bariatric surgery through state-of-the art guideline development, aiming to facilitate evidence-based clinical decisions	Updated version	Adults	EB & CB
IFSO (International Federation for Surgery of Obesity and Metabolic Disorders) Consensus Conference Statement on One-Anastomosis Gastric Bypass (OAGB-MGB): Results of a Modified Delphi Study	Ramos AC, et al.,2020	IFSO	Ra (16)	International	Not specified	Validating the results of the previous exercise as well as to expand into areas not previously covered.	original version	Adults	EB & CB
OSSI (Obesity and Metabolic Surgery Society of India) Guidelines for Patient and Procedure Selection for Bariatric and Metabolic Surgery	Bhasker AG, et al.,2020	OSSI	Bh (17)	India	Not specified	Enlisting the OSSI guidelines for patient and procedure selection for surgeons and allied health practitioners practising bariatric and metabolic surgery. Intending to guide Insurance Regulatory and Development Authority of India and multiple other stake-holders.	original version	Adults	EB & CB

EB, Guidelines based on evidence-based medicine; CB, Develop guidelines based on expert consensus; ASMBS, American Society for Metabolic and Bariatric Surgery; IFSO, International Federation for Surgery of Obesity and Metabolic Diseases; EAES, European Association of Endoscopic Surgery; EASO, European Association for the Study of Obesity; ESPCOP, European Society for the Peri-operative Care of the Obese Patient; OSSI, Obesity and Metabolic Surgery Society of India; SG, Sleeve gastrectomy; MBS, Metabolic and bariatric surgery; RBS, Revisional bariatric surgery; BMI, Body mass index; BPD/DS, Biliopancreatic diversion with duodenal switch; GRADE, Grade of recommendations assessment, development and evaluation.

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issue, as metabolism and bariatric surgery is a multidisciplinary, global issue. In this study, even within the same guidelines, the quality of metabolic and bariatric surgery guidelines was highly heterogeneous among different fields, and there were significant differences in the distribution of evidence level and recommendation strength between different categories of guidelines.

Analysing the included guidelines, the study identified several areas where improvement was needed in the development of the guidelines. In the development of guidelines, patient perceptions, expectations and preferences for medical care have become increasingly important. Stakeholder participation can well reflect the views of prospective users and patients. The implementation of the guidelines also requires multidisciplinary medical expertise. However, the guidelines included in this study did not provide details about the involvement of patients and their representatives.

Rigour of development is closely related to credibility in the implementation of the guidelines. This field assessment is used to locate and synthesize evidence and to develop and update recommendations (35). Unsystematic methods of retrieving evidence tend to lead to low-quality guidelines (11, 16, 17). The lack of clear evidence selection criteria and their strengths and limitations (9, 16) also lead to the low quality of the guidelines. Other causes include vague connection between recommendations and evidence, lack of external evaluation, failure to provide guideline update steps (9, 11, 16), and failure to consider side effects and risks when forming recommendations (17).

All nine guidelines included in the study scored > 60% for clarity and presentation. This shows that the recommendations in the guidelines are clear and easy to identify.

The overall score for the applicability of the guidelines was low in the included guidelines. This indicates that the hindrance and promotion factors in the application of the guidelines have not been fully understood in the formulation of the guidelines (16). No recommendations or tools are provided in the application to ensure its feasibility (9, 17). Additionally, the neglect of relevant resources (9, 11, 12, 14–17) that may be needed in the application of recommendations and the lack of monitoring and auditing standards (9, 11, 13, 14, 17) are also important reasons for the low score of the guidelines in the application field.

In the area of editorial independence of the guideline. The influence of sponsors' views on the guideline and the conflicts of interest between members of the organisations involved in the development of the guideline are rarely mentioned (9, 12). Therefore, conflicts of interest among members should be clearly recorded and publicized in the formulation of the guidelines to improve their independence.

There are significant differences in recommendations in the included guidelines. Therefore, this study further analysed the consistency and controversy between current recommendations and corresponding evidence of metabolic and bariatric surgery guidelines with reference to key recommendations from the guidelines for metabolic and bariatric surgery with relatively high scores.

4.1 Indications for bariatric/metabolic surgery

4.1.1 (1) Bariatric/metabolic surgery should be considered for patients with BMI≥ 35 kg/m^2 with associated comorbidities (recommendation strength: B; evidence level: 2b) (20)

For this recommendation, only 2 guidelines (15, 17) are relatively consistent in this recommendation, and the other 7 guidelines (9–14, 16) do not specify this recommendation. However, there is currently a lack high-quality empirical evidence, and the best evidence derives from a randomized controlled trial of 57 patients. The main conclusion is that surgery is very effective in the short term for patients with T2DM and obesity (20).

4.1.2 (2) Bariatric/metabolic surgery should be considered for patients with BMI≥ 30 kg/m^2 and type 2 diabetes with poor control despite optimal medical therapy (recommendation strength: A; evidence level: 1a) (21)

For this recommendation, four guidelines (14–17) are relatively consistent in this regard, while the other five guidelines (9–13) do not explicitly state this recommendation. The best evidence to date is a systematic review of 11 randomized controlled trials, with the main conclusion that bariatric/metabolic surgery is more effective than various medical/lifestyle interventions in reducing body weight, controlling blood glucose, alleviating T2DM, and improving other cardiovascular disease risk factors (21).

4.1.3 (3) Bariatric/metabolic surgery should be considered for patients with BMI≥ 30 kg/m^2 and obesity-related comorbidities that cannot lose enough weight through nonsurgical treatment (recommendation strength: B; evidence level: 2b) (22)

For this recommendation, two guidelines (12, 14) are relatively consistent in this regard, while the other seven guidelines (9–11, 13, 15–17) do not explicitly state this recommendation. There is a lack of high-quality evidence from a large sample. The best available evidence is a randomized controlled trial involving 80 patients who shows that surgical treatment was statistically significant compared with nonsurgical treatment in terms of weight loss, solving metabolic syndrome and improving quality of life for adults with mild to moderate obesity (body mass index, 30 kg/m^2 to 35 kg/m^2) (22).

4.2 Operative methods of bariatric/metabolic surgery

4.2.1 (1) SG should be preferred over AGB (recommendation strength: B; evidence level: 2b) (23)

Three guidelines [9, 10 and 15] agree on this recommendation. The other six guidelines (11–14, 16, 17) do not specify this

TABLE 2 AGREE II domain score and ICC of the included guidelines.

Guidelines	Scope and Purpose	Stakeholder Involvement	Rigour of Development	Clarity and Presentation	Applicability	Editorial independence	Overall assessment	ICC
Ki (9)	77.78%	50.00%	20.83%	79.17%	27.08%	0.00%	37.85%	0.947
Pr (10)	91.67%	86.11%	70.83%	77.78%	77.08%	47.92%	74.91%	0.881
Ma (11)	77.79%	52.78%	48.44%	77.78%	54.17%	93.75%	63.42%	0.892
Bu (12)	87.50%	52.78%	77.08%	66.67%	78.13%	0.00%	64.67%	0.905
Me (13)	83.33%	47.22%	56.25%	87.50%	46.88%	50.00%	59.29%	0.915
Am (14)	80.56%	79.17%	55.21%	84.72%	33.33%	100.00%	65.19%	0.837
Lo (15)	91.67%	69.44%	68.32%	91.67%	55.21%	50.00%	68.73%	0.913
Ra (16)	83.22%	51.39%	30.21%	62.50%	20.83%	50.00%	43.65%	0.897
Bh (17)	83.33%	51.39%	30.21%	62.50%	20.83%	50.00%	43.66%	0.922
Median score (range)	84.09% (77.78%-91.67%)	60.03% (47.22%-86.11%)	50.82% (20.83%-77.08%)	76.70% (62.5%-91.67%)	45.95% (27.08%-78.13%)	49.07% (0%-100%)	57.93% (37.85%-68.73%)	

ICC, Intraclass correlation coefficient.

recommendation. There is still a lack of high-quality research evidence with a large sample, and the best evidence at present is a cohort study of 71 patients, which shows that AGB surgery is inferior to SG surgery in weight loss (23).

4.2.2 (2) RYGB should be preferred over AGB (recommendation strength: B; evidence level: 2b) (24)

Two guidelines (10, 15) agree on this recommendation. The other seven guidelines (9, 11–14, 16, 17) do not explicitly state this recommendation. Currently, there is a lack of large-scale, high-quality randomized controlled studies, and the best evidence is based on a cohort study of 1295 patients in whom RYGB has a lower incidence of long-term complications than AGB (24).

4.2.3 (3) OAGB may offer greater short-term weight loss than SG (recommendation strength: B; evidence level: 2b) (25)

For this recommendation, two guidelines (15, 16) have agreed on this recommendation. The other seven guidelines (9–14, 17) do not specify this recommendation. Currently, there is a lack of high-quality research evidence, and the best evidence is a cohort study involving 123 patients. The main conclusion shows that the two surgery methods have excellent weight loss and maintenance effects in the short and medium term, and the results of T2D and HTN after OAGB are better (25).

4.2.4 (4) OAGB may offer greater short-term weight loss than RYGB (recommendation strength: A; evidence level: 1b) (26)

For this recommendation, two guidelines (15, 16) have agreed on this recommendation. The other seven guidelines (9–14, 17) do not specify this recommendation. The best evidence to date is a 253-patient randomized controlled trial that shows that the OAGB group has better short-term weight loss (26).

4.2.5 (5) RYGB is an acceptable revisional bariatric surgery option after AGB (recommendation strength: C; evidence level: 4) (27)

Two guidelines (10, 11) agree on this recommendation. There is currently a lack of related high-quality RCT studies, and the best evidence thus far comes from a case series analysis of 58 patients, which found that RYGB is a safe operation with good weight loss within 5 years. It can be regarded as a good revision operation after failure of AGB (27).

4.2.6 (6) BPD/DS and SADIs are acceptable revisional bariatric surgery options after SG (recommendation strength: C; evidence level: 4) (28, 29)

The best evidence to date comes from a case-analysis study of 96 patients, which found that BPD/DS is a safe and effective option after initial SG failure, especially in patients with severe obesity before SG. SADI-S results in a more significant reduction in overall weight than RYGB after failure of SG (28, 29).

4.2.7 (7) RYGB and BPD/DS are acceptable surgical options for patients with GERD after SG surgery, and BPD/DS is better than RYGB (recommendation strength: C; evidence level: 4) (28, 30)

Two guidelines (9, 13) agree on this recommendation, and the best evidence thus far comes from a case study of 10 patients. The main conclusion is that RYGB is an effective treatment for BE and reflux after SG, and RYGB alleviates BE and reflux in most cases (30).

TABLE 3 The key recommendations and the best evidence to support the recommendations at present.

	The key recommendations	The best evidence to support the recommendations at present	Strength of recommendation	Quality of evidence	Ki (9)	Pr (10)	Ma (11)	Bu (12)	Me (13)	Am (14)	Lo (15)	Ra (16)	Bh (17)
Indications of bariatric/metabolic surgery	Bariatric/metabolic surgery should be considered for patients with BMI≥ 35 kg/m2 with associated comorbidities	A RCT including 57 patients (20).	B	2b	1	1	1	1	1	1	3	1	3
	Bariatric/metabolic surgery should be considered for patients with BMI≥ 30 kg/m2 and type 2 diabetes with poor control despite optimal medical therapy	A systematic review of 11 RCTs (21)	A	1a	1	1	1	1	1	3	3	3	3
	Bariatric/metabolic surgery should be considered for patients with BMI≥ 30 kg/m2 and obesity-related comorbidities that cannot lose enough weight through nonsurgical treatment	A RCT including 80 patients (22).	B	2b	1	1	1	3	1	3	1	1	1
Operative methods of bariatric/metabolic surgery	SG should be preferred over AGB	A cohort study including 71 patients (23).	B	2b	3	2	1	1	1	1	2	1	1
	RYGB should be preferred over AGB	A cohort study including 1295 patients (24).	B	2b	1	2	1	1	1	1	3	1	1
	OAGB may offer greater short-term weight loss than SG	A cohort study including 123 patients (25).	B	2b	1	1	1	1	1	1	3	3	1
	OAGB may offer greater short-term weight loss than RYGB	A RCT including 253 patients (26).	A	1b	1	1	1	1	1	1	3	3	1
	RYGB is an acceptable revisional bariatric surgery option after AGB	A case series analysis including 58 patients (27).	C	4	1	2	3	1	1	1	1	1	1
	BPD/DS is an acceptable revisional bariatric surgery option after SG	A case series analysis including 33 patients (28).	C	4	3	1	3	1	3	1	1	1	1
	SADIs is an acceptable revisional bariatric surgery option after SG	A case series analysis including 63 patients (29).	C	4	3	1	3	1	3	1	1	1	1
	BPD/DS is a more acceptable revisional bariatric surgery option than RYGB after SG	A cohort study including 74 patients (28).	C	4	3	1	1	1	3	1	1	1	1
	RYGB is an acceptable surgery option for patients with gastroesophageal reflux disease after SG	A case series analysis including 10 patients(30)	C	4	3	1	1	1	3	1	1	1	1
Preoperative work-up	Preoperative nutritional assessment can be considered before bariatric/metabolic surgery	A RCT including 120 patients (31).	A	1b	1	2	3	1	1	1	3	1	1
	Psychological evaluation can be considered before bariatric/metabolic surgery	A cohort study including 2458 patients (32).	B	2b	1	1	3	1	1	1	2	1	1
Postoperative care	Micro and/or macronutrients supplementation is recommended after bariatric/metabolic surgery	A systematic review of 5 RCTs and 7 observational studies (33).	B	2a	1	2	1	1	1	3	3	1	1

(Continued)

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TABLE 3 Continued

	The key recommendations	The best evidence to support the recommendations at present	Strength of recommendation	Quality of evidence	Ki (9)	Pr (10)	Ma (11)	Bu (12)	Me (13)	Am (14)	Lo (15)	Ra (16)	Bh (17)
	Postoperative behavioural advice should be provided to patients undergoing bariatric/metabolic surgery	A RCT including 144 patients (34).	A	1b	1	2	1	1	1	1	3	1	1
	Pregnancy after bariatric/metabolic surgery should be delayed during the weight loss phase	Expert opinion (15).	D	5	1	2	1	1	1	1	3	1	3

BMI, Body mass index; RCT,Randomized controlled trial; SG, Sleeve gastrectomy; AGB, Adjustable gastric banding; RYGB,Roux-en-Y gastric bypass; OAGB, One anastomosis gastric bypass; BPD/DS, Biliopancreatic diversion with duodenal switch; SADIs, Single anastomosis duodeno-ileal bypass; indicates being recommended definitely (including position statements and agreement of consensus) = 3; indicates being mentioned or conditionally recommended = 2; indicates being not mentioned = 1.

4.3 Preoperative work-up

4.3.1 (1) Preoperative nutritional assessment can be considered before bariatric/metabolic surgery (recommendation strength: A; evidence level: 1b) (31)

Four guidelines (9–11, 15) have consistent opinions on this article. The other five guidelines (12–14, 16, 17) do not specify this recommendation. At present, the best evidence comes from a randomized controlled study involving 120 obese patients (31), and the results suggest that proper nutritional assessment and preoperative preparation of a balanced energy diet for morbidly obese patients can reduce the risk of surgery and improve efficacy.

4.3.2 (2) Psychological evaluation can be considered before bariatric/metabolic surgery (recommendation strength: B; evidence level: 2b) (32)

Two guidelines (11, 15) recommend psychological assessment. The other seven guidelines (9, 10, 12–14, 16, 17) do not explicitly state this recommendation. The best evidence to date comes from a cohort study of 2,458 patients, which showed that measures such as preoperative psychological evaluation can improve the prevalence of alcohol use disorder after bariatric/metabolic surgery (32).

4.4 Postoperative care

4.4.1 (1) Micro- and/or micronutrient supplementation is recommended after bariatric/metabolic surgery (recommended intensity: B; evidence level: 2a) (33)

This recommendation is relatively consistent in most guidelines (10, 13–15), while the other five guidelines (9, 11, 12, 16, 17) do not specify this recommendation. The best evidence to date includes a meta-analysis of 5 randomized controlled trials and 7 observational studies, showing that daily nutrient supplementation can effectively prevent postoperative complications (33).

4.4.2 (2) Postoperative behavioural advice should be provided to patients undergoing bariatric/metabolic surgery (recommendation strength: A; evidence level: 1b) (34)

For this proposal, two guidelines (10, 15) are consistent. The guidelines recommend providing motivation intervention for postoperative patients through a randomized controlled trial of 144 obese patients. The result indicates that participants accept behavioural intervention based on the scores on the Beck depression rating scale being significantly lower than those among standard treatment participants (34).

4.4.3 (3) Pregnancy after bariatric/metabolic surgery should be delayed during the weight loss phase (recommendation strength: D; evidence level: 5) (15)

Three guidelines (10, 15, 17) are consistent in this recommendation. At present, the best evidence comes from

expert opinions (15), with a low level of evidence and a lack of high-quality randomized controlled studies.

In summary, suggestions for improving the quality of metabolic and bariatric surgery guidelines are as follows: (1) Developers should set different groups (including patients and the public, etc.) clearly and fully consider the views and wishes of target groups when formulating clinical guidelines. (2) In the formulation, evidence standards should be clearly described, the link between recommendations and evidence should be shown clearly, and update steps should be provided. (3) Guideline developers should be familiar with guideline development standards, such as the AGREE II tool. (4) The guideline shall be externally reviewed by experts before publication. (5) Most of the key recommendations for metabolic and bariatric surgery are not supported by high-quality research evidence. It is recommended that international academic groups on metabolic and bariatric surgery organize and carry out multicentre high-quality research to provide high-quality evidence for the key recommendations on metabolic and bariatric surgery.

This study has some advantages and limitations.

The advantages of this study are as follows: (1) This study collated and analysed the key recommendations and relevant evidence in the recent guidelines for metabolic and bariatric surgery. This study identified issues of recommendations and evidence related to metabolic and bariatric surgery and suggests improvements that may help guideline makers and users identify gaps in practice and provide a reference for guideline users to select more reliable guidelines. (2) Most of the developers of the guidelines included in this study come from international organisations and from different backgrounds, including clinical experts and methodologists, who have rich experience in developing clinical guidelines, which improves the reliability of the results of this study.

The limitations of this study are as follows: (1) In this study, only the guidelines written in English were evaluated, and the guidelines published in other languages may be missed in this study, resulting in inadequate representation of some less developed countries. (2) AGREE II is a methodological tool that does not evaluate the content and clinical significance of guidelines and focuses mainly on the formulation of guidelines. Thus, even if the guidelines are based on low-quality evidence, but the methodology is developed in compliance with AGREE II standards, the guidelines may still be scored highly by the AGREE II tool. (3) We decided to include all populations without setting age limits, but only one remaining article deals with the population of children and adolescents. This may be due to the lack of literatures and guidelines.

5 Conclusion

The quality of metabolic and bariatric surgical guidelines varies visibly. High-quality guidelines require multidisciplinary collaboration. Using the AGREE II tool, this study found significant room for improvement in the guidelines for metabolic and bariatric surgery, especially in terms of rigor, stakeholders, adaptability, and independence of guideline development. Effectively addressing these issues has vital implications for developing high-quality recommendations for metabolic and bariatric surgery guidelines.

Author contributions

Z-HQ and XY were responsible for the information retrieval and the summary of characteristics of included guidelines; Y-LD and XW independently evaluated the obtained literature to determine whether to include or exclude the literature; Z-HQ, XY, L-YA, and TY were responsible for grading the included guidelines according to the AGREE II user manual; XY was responsible for writing, edit and revision of the body text; Z-HQ was responsible for the summary of key recommendations of included guidelines and corresponding best supportive evidences, and calculation of ICCs; Z-HQ, XY, and D-LS were drafted and revised the article; Z-HQ, XY, D-LS, and Y-YL were responsible for the theme, final editing, and preparation of the manuscript for submission; D-LS and Y-YL critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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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/fendo.2023.1118564/full#supplementary-material

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Clinical efficacy of weight loss herbal intervention therapy and lifestyle modifications on obesity and its association with distinct gut microbiome: A randomized double-blind phase 2 study

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Goals: To assess the efficacy and safety of Chinese Medicine Prescription "W-LHIT" in subjects with simple obesity, and to explore its potential mechanism of action.

Methods: Thirty-seven patients aged 18 to 60 from Wei-En hospital (Weifang City, Shandong, China), participated in a double blinded, placebo-controlled study. Subjects were randomly divided into 2 groups, 18 in treatment and 19 in placebo group. The treatment group took the "W-LHIT" capsules for two months, while the control group received placebo capsules. Both groups accepted healthy lifestyle education materials. After a 2-month treatment, the placebo group transferred to open-label treatment after unblinding.

Results: 72.22% participants in the treatment group lost more than 5% of their body weight, compared with 36.84% in the placebo group ($p < 0.001$). Body weight loss and body mass index reduction of the treatment group were also significantly higher than those of the placebo group ($p < 0.05$). These changes were accompanied by increased abundance of *Akkermansia muciniphila* and *Enterococcus faecium*, and decreased abundance of *Proteobacteria* in gut microbiota. Furthermore, the treatment group also showed improvement in obesity-related comorbidities such as hypertension and elevation of liver enzymes. No serious adverse reactions were found during the study period. Weight did not rebound at a follow-up visit 2 months after treatment.

Conclusion: W-LHIT significantly improved body weight and comorbid conditions without obvious adverse reaction or rebound weight gain. These effects were associated with increased abundance of probiotics in gut

microbiota. W-LHIT may have a potential for treating obesity in conjunction with healthy lifestyle modifications.

KEYWORDS

"W-LHIT" capsule, obesity, weight loss, gut microbiome, *Akkermansia muciniphila*, lifestyle modifications

Introduction

Obesity has been increasing in prevalence worldwide and is a serious threat to public health. Over the past 30 years, the prevalence of obesity among adults has exceeded 50% in some western developed countries (1). This increase is also observed in non-western and some developing countries, especially in China, where the number of overweight and obese people has been increasing at an alarming rate (2). A surge of related chronic diseases is strongly associated with the rising rate in obesity (3), such as type 2 diabetes, hypertension, atherosclerosis, cancers, and stroke, all of which have been major health threats in China for the past two decades (2). During the ongoing COVID-19 pandemic, obesity was found in early studies (4, 5) to be the second strongest predictor of hospitalization and need for mechanical ventilation in the elderly. In obese individuals, there is a reduced quality of life and decline in life expectancy (1). Due to increasing awareness of the risks of obesity, interest in weight loss management has grown significantly with calls for effective interventions.

Unhealthy eating styles and sedentary behavior and physical inactivity have been viewed as the key factors that contribute to obesity. Current guidelines recommend diet, exercise, and behavior modification as standard treatments for obesity. However, a study found that only 20% of adults who either tried to lose weight or to maintain their weight, could both eat fewer calories and complete at least 150 minutes of exercise per week (6). Simple exercise and diet are often challenging for patients attempting to maintain lasting results (7). Due to the risk of serious complications and the issues of nutritional management and treatment follow-up, bariatric surgery is only applicable to a specific subset of patients, and not widely applicable for clinical use (8). Pharmacological intervention can be an effective and widely accepted auxiliary method.

Weight loss herbal intervention therapy (W-LHIT) is a Chinese medicine (CM) prescription, consisting of 5 Chinese herbal medicines: *Ganoderma lucidum*, *Coptis chinensis*, *Astragalus membranaceus*, *Nelumbo nucifera gaertn* and *Fructus aurantii.* In our previous studies, we have showed that W-LHIT significantly and safely reduced the body weight of mice with high-fat diet, and normalized their glucose and cholesterol levels, without suppressing appetite (9). In addition, in our phase I clinical study with 14 patients with simple obesity, the W-LHIT capsules demonstrated clinically significant weight loss (Cao et al. Manuscript submitted).

Increasing evidence suggested gut microbiota played a significant role in the development of obesity. Many clinical or pre-clinical studies have shown that there are significant differences in the composition of gut microbiota between healthy versus obese individuals (10), with decreased diversity and richness in the gut microbiome of obese individuals (11). Also, causality between gut microbiota imbalance and obesity has been demonstrated in animal models. Gut microbiota dysbiosis can lead to an increase in lipopolysaccharide (LPS) in the host's circulatory system and induce chronic and low-level inflammation, thereby playing an important role in the occurrence and development of metabolic diseases such as obesity (12–14). Recently, multiple studies have confirmed that traditional Chinese medicine can improve obesity in animal models by regulating the composition of gut microbiota (13, 15). Chih-Jung Chang et al. reported that water extract of *Ganoderma lucidum* could reduce obesity and inflammation in high fat diet (HFD) fed mice, which was associated with reversing HFD-induced gut dysbiosis (as indicated by the reduction of *Firmicutes*-to-*Bacteroidetes* ratios and endotoxin bearing *Proteobacteria* levels) and maintaining intestinal barrier integrity (16). *Fructus Aurantii* extract also showed a similar anti-obesity effect by modulating the gut microbiota (17). Berberine, the principal component of *Coptis chinensis*, is known as a potent anti-obesity and lipid lowering agent. Moreover, its anti-obesity effect could be associated with the increased butyrate production in the gut and modulation of the gut microbiota (18).

In this study, we aimed to further evaluate the safety and weight loss efficacy of the W-LHIT capsules, in combination with a standardized lifestyle intervention. In addition, we used 16S pyrosequencing technology to evaluate the shift in the composition of the gut microbiota before and after W-LHIT capsule treatment to explore its potential role in understanding W-LHIT capsules in weight loss response.

Methods

Participants and W-LHIT capsules preparation

Participants who met simple obesity criteria were recruited from the Weifang community through WeChat Friends Circle,

advertisement, and local websites. Inclusion criteria were as follows: age 18-60 years with a body-mass index (BMI) of 28 - 48 kg/m^2, stable body weight within the past 3 months, ability to engage in physical activity and follow healthy diet guidelines, no cardiovascular, hepatic, or renal disease. Patients with disease-induced obesity (such as Cushing's syndrome or thyroid disease) were excluded. Exclusion criteria included the above-described diseases, psychiatric illness and psychological disorders, pregnancy and participants who cannot be clinically observed. Written informed consent was obtained from all subjects upon enrollment. The study was approved by the Medical Ethics Committee of Weifang Wei-En Hospital.

W-LHIT capsules were prepared in a GMP facility (Tian-jiang Pharmaceutical, Jiangsu, China). In our early research, we established HPLC fingerprints of individual herbal components, and monitored the quality of different batches of W-LHIT products by comparing the peak times and intensities of the identified compounds. Berberine was used as a key compound index (9). Nine capsules are equivalent to the daily crude herbal medicines dosage for 75 kg individual. The placebo capsule was filled with starch with the same weight and color as the formula.

Study design

Before enrollment, a CT scan of the chest was used to exclude lung disease and a color doppler ultrasound was used to exclude organic diseases of the heart, liver, and kidneys. Thyroid function, blood coagulation, and sex hormone lab tests were used to rule out obesity caused by endogenous diseases.

The design of the study was a randomized double-blind placebo-controlled trial. Fourty participants were randomly divided into treatment group (treated with W-LHIT capsules) and control group (treated with placebo) according to random number table with 20 cases in each group. All participants had a light diet and were advised to drink at least 1.5 L of water during the study. W-LHIT was dosed according to body weight, all subjects dosed 9 to 15 capsules daily (3 - 5 capsules a time, before meals, three times a day) for 2 months and then followed up for an additional 2 months. Other weight-loss drugs (including weight-loss dietary supplements) were discontinued during the treatment and follow up period. If a subject discontinued the study prematurely, the remainder of doses were recalled. Since researchers and subjects were both blinded, W-LHIT capsules and placebo were all distributed by a third-party designated distributor. After unblinding, the subjects in control group voluntarily participated in another open trial where they were given a 2-month supply of W-LHIT capsules for treatment, while the active group stopped W-LHIT. Both groups had their weight checked again 2 months later.

Primary outcomes

The primary core measurements included the changes in body weight (kg), BMI (kg/m^2), hip circumference (cm), waist circumference (cm), blood pressure (Seat systolic blood pressure, SSBP; seat diastolic blood pressure, SDBP, mmHg) and heart rate (sub/min), and they were assessed at the beginning (0 M, one day before treatment), mid-term (1 M, 30 days after treatment), and endpoint (2 M, 60 days after treatment) of the weight loss intervention (please see the supporting information for detailed methods). The proportion of individuals losing more than 5% of baseline weight was also assessed at the end of the intervention, including the open trial.

Secondary outcomes

The Secondary measurements included assessments of fasting blood glucose, high-sensitivity C-reactive protein (hs-CRP), liver function including alanine aminotransferase (ALT), aspartic aminotransferase (AST), glutamate transferase (GGT), total bilirubin (TBIL), total protein (TP) and albumin (A), renal function including urea nitrogen (BUN), and creatinine (CRE), highly sensitive C - reactive protein (CRP), fasting lipids including cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL-C) and high-density lipoprotein cholesterol (HDL-C), oral glucose tolerance test (OGTT; 75-g glucose, glucose, insulin, and C-peptide concentration changes from 0 to 120 min), and analysis of human fat composition, bone mineral density, bilateral knee joint, and lumbar vertebrae X-ray.

Healthy lifestyle interventions

Healthy lifestyle interventions were carried out throughout treatment. All subjects were provided personalized healthy diet guidance according to their living habits, physical condition, and work characteristics. They were also encouraged to adhere to or increase physical exercise based on their physical examination results and their physical condition. However, there was not an obligatory requirement to engage in a strict diet or exercise program. All subjects regularly attended classes on the risk of obesity and how to develop a healthy lifestyle. A WeChat group was established to supervise all enrolled subjects. A contracted nurse reminded all the subjects to take the medication every day, to follow diet and exercise recommendations, and to post recipes for meals. Subjects were able to report their diet and exercise every day and share their weight loss symptoms and experience in this group forum.

Safety assessment

Safety assessment included adverse events and standard laboratory tests (hematological and biochemical tests). Adverse events were recorded by the nurse through the WeChat group. A physical examination and an exercise cardiopulmonary function test (pulmonary function, electrocardiogram, and finger oxygen %) were performed at enrollment and on the 60th day.

16S Pacbio sequencing of fecal sample DNA

Fresh fecal samples were collected before and after the treatment for the gut microbial analysis. DNA was extracted from the subjects' feces using a Power Soil® DNA Isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions. The bacterial 16S rDNA was amplified by PCR using universal primers (27F 5′ -AGRGTTTGATYNTGGCTCAG-3′, 1492R 5′ -TASG GHTACCTTGTTASGACTT-3′). 16S Pacbio sequencing of the PCR products was performed on an Illumina MiSeq platform at Biomarker Technologies Co, Ltd. (Beijing, China).

After using the SMRT Link tool (version 8.0, provided by Pacbio), then Lima v1.7.0 software to obtain the Barcode-CCS sequence data, UCHIME v8.1 software to remove the chimera sequence, the optimization-CCS sequence was obtained for bioinformatics analysis. Trimmed sequences from each sample were clustered into operational taxonomic units (OTUs) based on a 97% sequence similarity using USEARCH v10.0 method. The taxonomical analysis was performed by alignment with the Bacterial Silva database (Release132, http://www.arb-silva.de). Alpha diversity indexes were evaluated based on the richness (Chao 1, Ace) and diversity (Shannon index, Simpson index) of these OTUs using Mothur v.1.30 (http://www.mothur.org/). A PLS-DA analysis (Partial Least Squares Discriminant Analysis) was performed according to the supervised matrix of distance. The linear discriminant analysis effect size (LEfSe) was conducted for the quantitative analysis of biomarkers (LDA threshold > 4) among each group. A Metastats analysis was used to identify the most differently abundant taxa between the placebo and treatment groups. A predicted KEGG pathway was also performed using Picrust software to obtain significant differences in gene function of the flora in the placebo and treatment groups.

Statistical analysis

Data were exhibited as the mean with [95% confidence interval] (CI),95(n=18 or 19). All statistical analyses were performed using one-way ANOVA followed by Bonferroni *post hoc* by Prism 9 software (GraphPad Software, Inc, La Jolla, CA). P value smaller than 0.05 considered statistically significant.

Results

Weight loss

14 women and 26 men who met the screening criteria were recruited. 2 participants withdrew due to personal issues, 1 participant withdrew due to treatment compliance issues, and 37 participants (active n=18 vs placebo n=19) completed the trial. As shown in Table 1, baseline characteristics at randomization were compared between the treatment group and placebo group.

Weight loss during the study is shown in Figure 1A. From randomization to the 60th day (2 months), both groups showed sustained weight loss, but mean weight loss and BMI reduction of W-LHIT capsule-treated subjects were significantly greater than that of placebo group, with mean weight reduction of -7.05 [-9.10, -4.99] kg (- 7.37%, p <0.001) in treatment group, compared to -4.39 [-5.68, -3.09] kg (- 4.78%, p <0.001) in placebo group (p <0.05, Figure 1A). Similar results were obtained for BMI, with significant reduction in both groups. The mean BMI in the treatment group decreased by -2.39 [-3.00, -1.77] kg/m^2 (- 7.40%, p <0.001), significantly higher than the reduction of -1.61 [-2.09, -1.12] kg/m^2 (- 4.73%, p <0.001) in the control group (p <0.05, Figure 1B). Both groups showed a reduced hip circumference (-7.61cm verse -4.32 cm, p = 0.067, Figure 1C) and waist

TABLE 1 Baseline data.

	Treatment (n=18)	Control (n=19)	P value
Sex (F/M)	6/12	7/12	0.93
Age (y)	39 ± 6.4	39 ± 4.6	0.92
Body Weight (kg)	96 ± 7.5	95 ± 8.7	0.92
BMI	32.25 ± 1.4	34.04 ± 2.5	0.21
Hip Circumference (cm)	115 ± 2.5	116 ± 4.8	0.51
Waist Circumference (cm)	107 ± 4.8	108 ± 6.0	0.78
SDBP (mmHg)	91 ± 5.9	90 ± 5.2	0.98
SSBP (mmHg)	135 ± 9.5	133 ± 8.7	0.76
HR	77 ± 4.6	78 ± 4.0	0.68
HS-CRP (mg/L)	4.5 ± 1.4	4.9 ± 1.5	0.87
Total cholesterol (mmol/l)	5.4 ± 0.5	5.2 ± 0.9	0.80
Triglycerides (mmol/l)	2.0 ± 0.6	3.0 ± 1.1	0.16
HDL cholesterol (mmol/l)	1.1 ± 0.08	1.2 ± 0.15	0.24
LDL cholesterol (mmol/l)	3.3 ± 0.3	3.1 ± 0.4	0.68

F, female; M, male; y, year; BMI, body mass index; SDBP, sitting diastolic blood pressure; SSBP, sitting systolic blood pressure; HR, heart rate, beats per minute; HDL, high-density lipoprotein; LDL, low-density lipoprotein. Data shown as mean with (±) 95% CI.

circumference (-7.44 cm verse -6.79 cm, p = 0.71, Figure 1D), but without statistically significant difference between the groups. During the study, 72.22% of the subjects in the treatment group lost more than 5% of their body weight and 77.78% of the subjects lost at least 5% of their BMI, much higher than the control group (36.84% both in body weight and BMI), as shown in Table S1. Encouragingly, the number of participants in the treatment group whose BMI decreased from obesity to overweight (5 verse 3) and from severe obesity to obesity (5 verse 3) was much higher than that in the control group.

The results of the analysis of human fat components were consistent with the results of the above weight loss. Body fat rate, fat weight, fat-free body weight in both groups were significantly reduced. However, the percentage of skeletal muscle was only significantly increased in the treatment group. Moreover, the reduction of body fat rate (- 14.8% vs - 4.56%, $p < 0.01$), body fat (- 10.0% vs - 3.6%, $p < 0.01$) and fat-to-muscle ratio (- 12.0% vs - 3.33%, $p < 0.01$) in treatment group was statistically significant compared with that of the placebo group (Table S2).

Changes in blood pressure, blood sugar, and blood lipid

As shown in Figures 2A, B, the blood pressure of both groups decreased significantly. The seated diastolic blood pressure (SDBP) decreased by -8.67 [-12.59, -4.64] mmHg (- 9.52%, p <0.001) and -3.21 [-6.47, -0.083] mmHg (- 3.59%, p <0.05), respectively, with a significant difference between the two groups (p <0.05, Figure 2A). The seated systolic blood pressure (SSBP) decreased by -7.99 [-13.29, -2.598] mmHg (- 5.88%, p <0.05) and -7.16 [-13.67, -0.6621] mmHg (- 5.33%, p <0.05), in the treatment and placebo groups, respectively. Among the subjects, there were 13 people with hypertension, 6 people in the treatment group (5 people at Stage 1 hypertension, 1 person at Stage 2), and 7 people in the placebo group (6 people at Stage 1, 1 person at Stage 2). There were 5 subjects in the treatment group and 2 subjects in the placebo group whose blood pressure returned to normal after 2 months of treatment. There was no noticeable change in heart rate for all subjects (Figure 2C).

The fasting blood sugar level of subjects in the treatment group also showed a significant reduction of -0.28 [-0.46, -0.096] mmol/L, $p < 0.01$), while that of subjects in the placebo group only decreased slightly (- 0.1 [-0.17, 0.095] mmol/L, shown in Figure 2D). All subjects in the treatment group whose blood sugar level exceeded the threshold (6.1 mmol/L) had varying degrees of blood glucose reduction. The blood sugar of the 4 subjects with blood glucose levels between 6.1-6.7 mmol/L fell below the threshold. In addition, all the concentration of glucose tolerance, C-peptide, and insulin in the treatment group significantly decreased after W-LHIT treatment, and their mean area under the curve (AUC) decreased

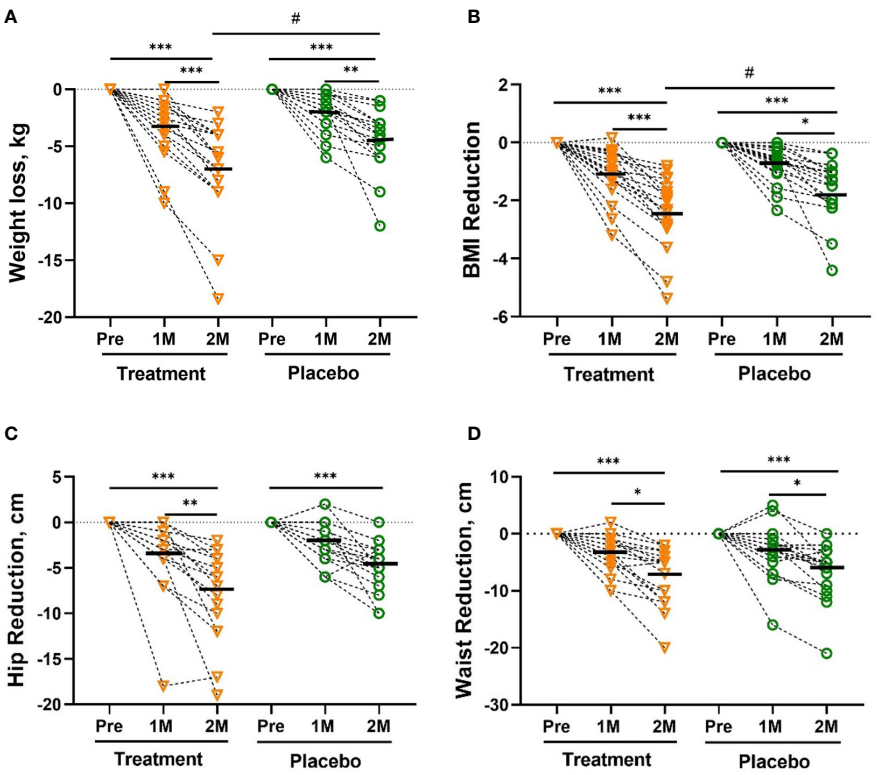

FIGURE 1
The Reduction in body weight **(A)**, BMI **(B)**, hip circumference **(C)**, waist circumference **(D)**. The reduction in body weight was significantly greater in the treatment group than in the placebo group after 2 months (2M). Bars were shown as mean of each group. All intra-group analysis were performed by using one-way ANOVA followed by bonferroni *post hoc*, and all inter-group analysis for baseline and treatment effects were performed by using t test followed by Mann-Whitney (# represents $p < 0.05$) by Prism 9 software (*, ** and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$). Body mass index, BMI.

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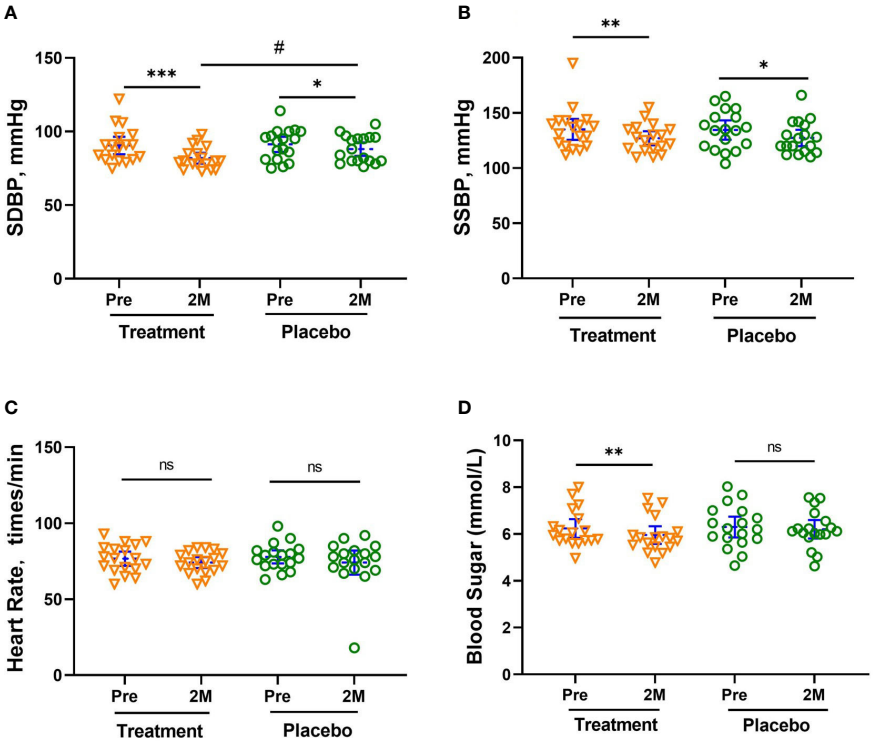

FIGURE 2
Mean Changes in SDBP **(A)**, SSBP **(B)**, HR **(C)** and Blood sugar levels **(D)**. The reduction in SDBP was significantly greater in the treatment group than in the placebo group after 2 months (2M). Bars (blue) were shown as mean of each group with 95% CI. All intra-group analyses were performed using one-way ANOVA followed by Bonferroni *post hoc*, and inter-group was performed by using t test followed by Mann-Whitney by Prism 9 software, (*, ** and *** represent $p < 0.05$, $p < 0.01$ and $p < 0.001$; # represents $p < 0.05$, ns represents no significant difference). Seated systolic blood pressure, SSBP, seated diastolic blood pressure, SDBP, mmHg.

by 5.87%, 10.54% and 32.99% respectively. While the fasting blood glucose and the above indicators in placebo group declined at individual time points, there was no significant pattern of change at most time points (Table 2).

Additionally, W-LHIT significantly reduced total cholesterol (TC, Figure 3A, $p < 0.01$), blood triglycerides (TG, Figure 3B, $p < 0.01$) and low-density lipoprotein cholesterol (LDL-C, Figure 3C, $p < 0.001$). The concentrations of TC, TG and LDL-Chol in the treatment group were decreased by 3.87%, 19.2% and 12.0%, respectively. There were no noticeable changes in TC, TG, HLD-C and for the placebo group (Figure 3).

Hs-CRP is positively correlated with atherosclerosis. Subjects in both treatment and control groups also showed a significant reduction in their Hs-CRP level, (-73.73% [-60.71%, -83.24%], p <0.001 vs -46.77% [-14.04%, - 79.51%], p <0.01, Figure 4).

Effect on body weight 2 months off double blind placebo-controlled trial and open trial

Two months after the end of treatment, the body weight of all subjects in the treatment group maintained at the same level after treatment or slightly decreased ($p = 0.45$, Figure 5) for the following two months after completion of therapy.

After unblinding, 12 of the 19 subjects in the placebo group subsequently completed an additional two months of W-LHIT capsules treatment. As shown in Figure S1, in the absence of healthy lifestyle interventions, only one-third of subjects lost nearly 5% of their body weight, with a mean weight and BMI loss of 3.13 [4.23, 2.03] kg (-3.60%) and 1.13 [1.57, 0.70] kg/m^2 (-3.51%). The reductions in systolic and diastolic blood pressure were consistent with those in the treatment group, by 2.49% and 9.80%, respectively ($p < 0.001$).

Laboratory safety profile

Most subjects at enrollment had mild to moderate fatty liver, and 14 subjects had moderate fatty liver. During the treatment, 6 subjects (5 in the treatment group) with moderate fatty liver improved to mild fatty liver, and 4 subjects with mild fatty liver in the treatment group returned to normal. Among them in the treatment group whose glutamyl transferase (GGT, liver function index, Figure S2) levels was above the threshold, their GGT levels decreased significantly after treatment, while that of subjects in the placebo group showed no significant changes.

There was no statistically significant difference between the treatment group and the placebo group in the levels of other liver and kidney function indices, including alanine aminotransferase

TABLE 2 Results of GGT, CRT and ICT in the two group before and after treatment.

Characteristic		Normal values	Treatment (n=18)		Placebo (n=19)	
			Baseline	Post-treatment	Baseline	Post-treatment
Glucose (mmol/L)	Fasting	3.90-6.10	5.67 ± 0.8	5.50 ± 0.68	5.58 ± 0.81	5.53 ± 0.77
	30 min.	7.78-8.89	9.55 ±1.29	8.98 ± 1.13***	8.82 ± 1.47	8.58 ± 1.45*
	60 min.	7.78-8.89	9.47 ± 2.87	8.96 ± 2.53*	8.32 ± 2.99	8.55 ± 2.91
	120 min.	3.90-7.80	7.09 ± 1.59	6.60 ± 1.9**	7.18 ± 2.21	7.03 ± 2.03
	180 min.	3.90-6.10	5.2 ± 1.73	4.89 ± 1.38*	5.00 ± 1.17	5.38 ± 1.35
	AUC		1379 ± 135.8	1298 ± 130.6	1317 ± 151.7	1324 ± 145.2
C-peptide (ng/mL)	Fasting	0.52–4.38	3.72 ± 1.27	3.22 ± 0.86*	3.96 ± 1.37	3.86 ± 1.29
	30 min.	3.58–13.2	11.16 ± 4.48	9.89 ± 3.68**	9.5 ± 3.37	9.86 ± 3.7
	60 min.	3.58–13.2	12.49 ± 4.05	11.13 ± 2.48*	10.02 ± 2.74	11.24 ± 2.76
	120 min.	1.2–11.3	10.66 ± 3.63	9.46 ± 2.68**	10.24 ± 3.13	10.22 ± 2.54
	180 min.	0.38–6.56	6.34 ± 2.76	6.05 ± 2.28*	6.08 ± 2.6	6.75 ± 2.76
	AUC		1783 ± 248.6	1595 ± 188.4	1676 ± 188.2	1592 ± 199.4
Insulin (μU/mL)	Fasting	2.3–26.0	21.44 ± 11.01	17.57 ± 7.689*	24.09 ± 14.19	21.99 ± 12.26
	30 min.	10.5–61.8	170.33 ± 125.32	126.39 ± 72.06**	120.6 ± 84.15	108.66 ± 74.98*
	60 min.	10.5–61.8	155.62 ± 95.62	92.31 ± 55.29***	104.76 ± 57.01	122.09 ± 62.14
	120 min.	1.02– 41.09	112.34 ± 74.74	71.06 ± 39.55**	104.08 ± 63.73	88.59 ± 43.48
	180 min.	0.25– 11.53	41.55 ± 33.24	32.03 ± 23.13*	34.98 ± 35.88	40.85 ± 40.40
	AUC		20422 ± 5487	13684 ± 3112	15999 ± 3583	15863 ± 4092

Data are shown as mean ± SD. Intra-group analyses were performed by using t test followed by Wilcoxon by Prism 9 software, p value, **< 0.01, ***< 0.001. GTT, Glucose tolerance test; CPRT, C-peptide release test; IRT, Insulin release test; F.B.S, Fasting blood sugar.

(ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), total protein, and albumin. The electrolytes, routine urine, blood coagulation, and routine blood of all subjects were within the normal range, and there was no significant change (Table S3).

Clinical adverse reactions

Seven subjects reported slight gastrointestinal (GI) reactions (including 5 subjects in the open trail, Table S4), no subjects reported obvious adverse symptoms during the whole W-LHIT treatment. The GI side effects included decreased hyper appetite, mild nausea, and increased frequency of stools. All subjects alleviated these side effects quickly by taking W-LHIT after meals or reducing the dose.

Characteristics of 16S Pacbio sequencing results

The effects of W-LHIT on the intestinal microbiota composition were assessed by sequencing the bacterial 16S rRNA. A total of 417,913 optimization-CCS sequences and 352 OTUs (97% similarity) were obtained from the 74 samples through a single molecules real-time sequencing analysis, with an average of 5,647 reads and 82 OTUs per sample (Table S5). These reads/OTUs were assigned to 11 different phyla (15 class, 27 order, 52 family, 112 genus, and 219 species, and the principal bacterial phyla of all groups were *Firmicutes*, *Proteobacteria*, and *Bacteroides*. Main changes in microbial diversity observed before and after treatment included the enrichment of *Proteobacteria* (a slight increase (29.8% vs 32.8%) in the placebo group versus a slight decrease (32.3% vs 29.8%) in the treatment group), *Verrucomicrobiota* (a decrease (1.73% vs 0.7%) in the placebo group but an obvious increase (4.47% vs 10.52%) in the treatment group), and *Bacteroidetes* (a parallel decrease in both groups, 24.04% vs 20.86% in the placebo group and 22.75% vs 18.31% in the treatment group) (Table S6 and Figure 6B) showed details of the composition on Genus in both group.

The alpha diversity indexes, including Ace and Chao indices, rarefaction, Shannon-index (Table S4), and rank abundance curve (Figure 6A), indicated that there was similar richness and sufficient sequence coverage in all samples. However, the placebo group had a significant decrease in reads, OUTs, and the classification of microorganisms (family, genus, species), while the treatment group had a slight increase in OUTs and the classification of microorganisms (phylum, class, order, family, genus, species).

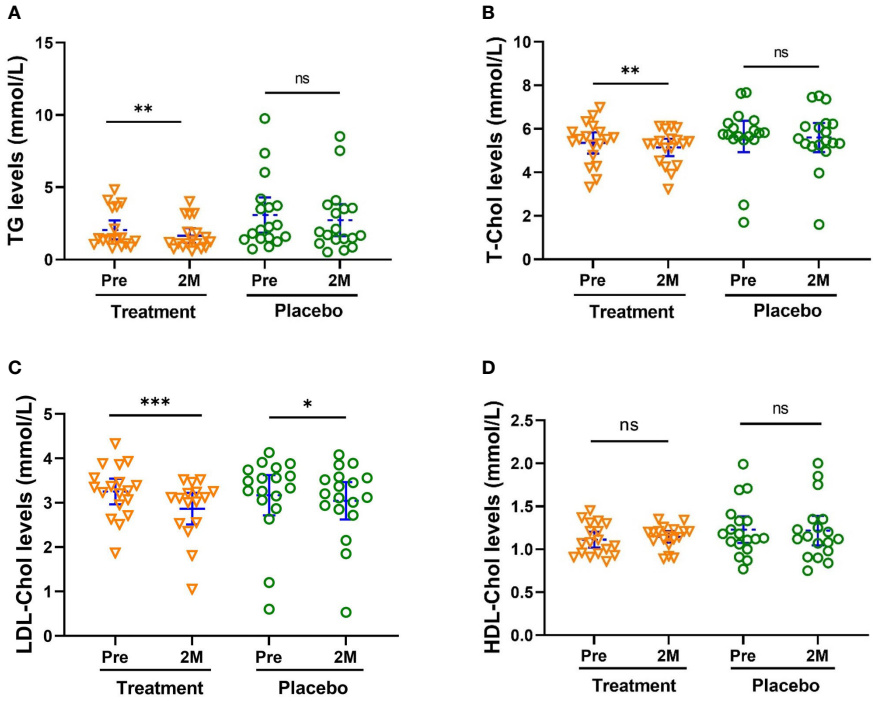

FIGURE 3
W-LHIT significantly reduced total cholesterol (TC), blood triglycerides (TG) and low-density lipoprotein cholesterol (LDL-C). **(A)** TC; **(B)** TG; **(C)** LDL-C; **(D)** HDL-C. Bars were shown as mean of each group with 95% CI. All intra-group analyses were performed using one-way ANOVA followed by Bonferroni *post hoc*, and inter-group was performed by using t test followed by Mann-Whitney by Prism 9 software (*, ** and *** represent $p < 0.05$, $p < 0.01$, $p < 0.001$, ns represents no significant difference). TC, Total cholesterol; TG, triglycerides; LDL-C, low-density lipoprotein; and HDL-C, high-density lipoprotein cholesterol.

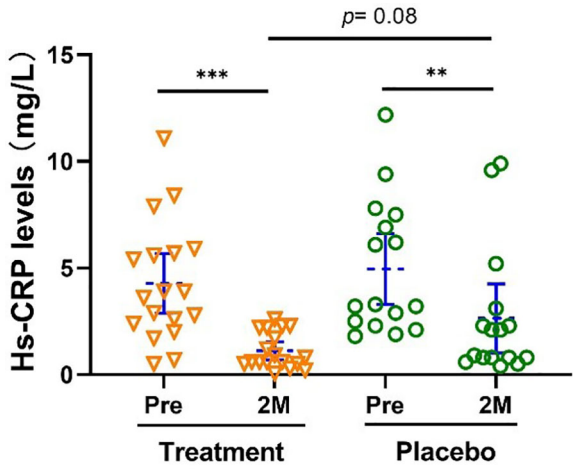

FIGURE 4
Hs-CRP level was significantly reduced after the treatment. Bars were shown as mean of each group with 95% CI. Intra-group analyses were performed using one-way ANOVA followed by Bonferroni *post hoc*, inter-group analyses were performed by using t test followed by Mann-Whitney by Prism 9 software (** and *** represent $p < 0.01$ and $p < 0.001$). Hs-CRP, High-sensitivity C-reactive protein.

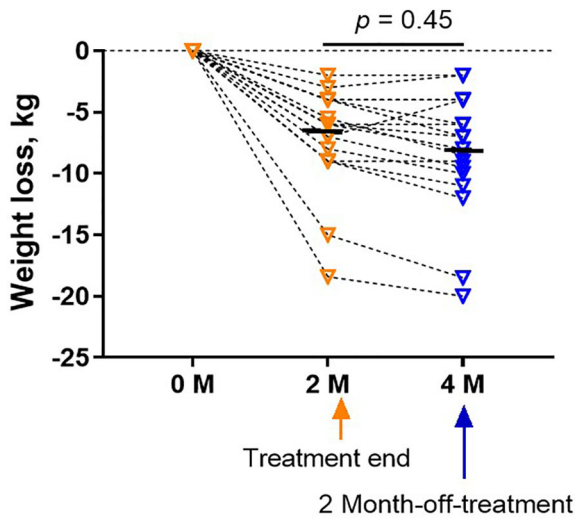

FIGURE 5
Weight did not rebound in the treatment group at a follow-up visit 2 months after the treatment. Bars were shown as mean of each group. The analyses were performed using one-way ANOVA followed by Bonferroni *post hoc* by Prism 9 software.

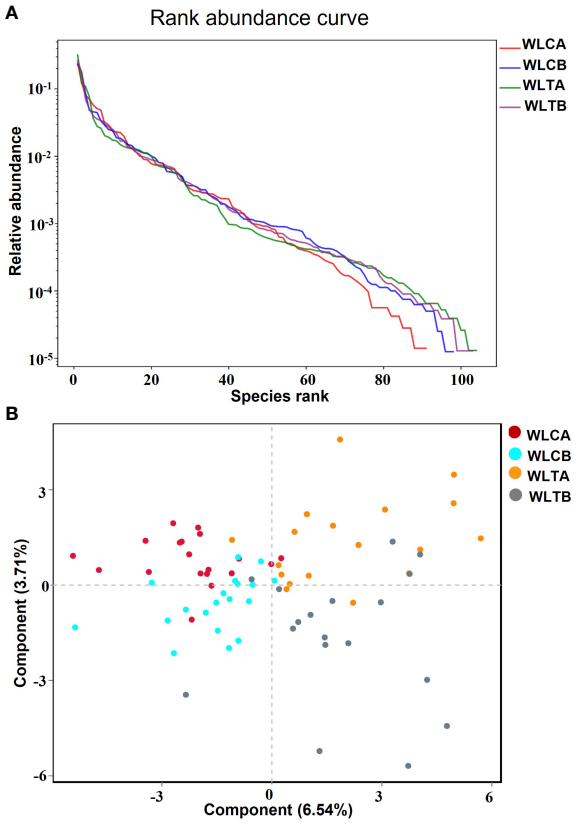

FIGURE 6
(A) Rank abundance curve. Each curve represents a group; placebo group (WLCB (baseline), WLCA (post treatment)) and treatment group (WLTB (baseline) and WLTA (post treatment)). **(B)** PLS-DA plots based on unweighted unifrac metrics. Each symbol represents a sample from the placebo group or treatment group, respectively.

Differences in microbial structure between the W-LHIT treatment group and the placebo group

To identify whether W-LHIT-mediated weight loss is associated with changes in the gut microbiota, we then profiled the overall microbial structure of the placebo and W-LHIT treatment groups. The PLS-DA results (Figure 6B) based on the weighted Unifrac distance matrix revealed that the overall structure of the bacteria did not change significantly in either group, and only a small part of the microbial structure between the two groups changed significantly (Figures 7A, B and Tables S5, 6). A LEfSe (Line Discriminant Analysis (LDA) Effect Size) analysis was used to discover the key biomarkers (LDA score > 4) of the gut microbiota under W-LHIT and placebo treatment, as shown in Figures 7C, D. The enriched phylotypes in the treatment group were the genera *Akkermansia* (p = 0.013, metastats) within the family *Akkermansiaceae* (p= 0.005, metastats) (within the order *Verrucomicrobiota* (p = 0.04, metastats), the species *Enterococcus_faecium* (p = 0.037, metastats) within the genera *Enterococcus* (p = 0.039, metastats). The enriched phylotypes in the placebo group were the species *Haemophilus_parainfluenzae* (p = 0.014, metastats) within the genera *Haemophilus* (p = 0.019, metastats), the species *Faecalibacterium_prausnitzii* (p = 0.006, metastats) and *Eubacterium_rectale* (p = 0.006, metastats) within the class *Clostridia* (p = 0.007, metastats). Furthermore, the analysis of the KEGG metabolic pathway found that only glycan biosynthesis and metabolism (Figure 7E) was statistically different between the treatment group and the placebo group.

Discussion

In recent years, China has ranked first in the world for obesity and type 2 diabetes (19). Obesity related complications such as type 2 diabetes and hypertension have posed a serious threat to people's health. There has been a growing consensus worldwide on the importance of obesity treatment not merely to achieve weight loss, but also to ameliorate adiposity-based complications (20, 21). Along with lifestyle intervention, W-LHIT capsule treatment resulted in 72.22% of subjects losing more than 5% of their body weight within 2 months, and 78.22% of the subjects to reduce more than 5% of their BMI. In addition, W-LHIT capsule can also reduce blood pressure, blood glucose and blood lipid of the subjects. In addition, W-LHIT capsule treatment also resulted in a greater reduction of blood pressure, blood sugar and blood fat. In this study, most of the subjects had impaired glucose tolerance, and about one third of the subjects were diabetic. In the treatment group, except for one diabetic subject whose blood glucose and blood lipid levels did not improve during the treatment period, other diabetic subjects showed significantly reduced levels of cholesterol, low-density lipoprotein, glucose tolerance, C-peptide release, and insulin release. In the placebo group, diabetic subjects showed little improvement in blood glucose and lipid levels. The results of subjects' liver and kidney function, and other biochemical indicators indicate W-LHIT's favorable safety and tolerance profile. These promising results indicate a potential role for W-LHIT for addressing weight control & management problems of simple obesity patients.

Unhealthy dietary habits and sedentary lifestyle are key contributing factors leading to obesity. Therefore, healthy lifestyle interventions (including healthy diet guidance and physical activity) can play a profound role in the treatment process. During the treatment, the average weight reduction of the placebo group was 4.58 kg (- 4.9%). For comparison, no healthy lifestyle intervention was performed in the open trial. The results showed that the body weight was only reduced by 3.6% in the open treatment trial, which was significantly lower than the average weight loss of the treatment group with lifestyle interventions (-7.05kg, -7.2%). Despite the insufficient sample size during open-treatment trials, we can still see the importance of healthy lifestyle interventions for obesity treatment. There was a limitation that the daily food intake calories and physical activity for each subject were not quantified during the treatment, and the healthy lifestyle intervention was tailored for each subject under the guidance of the physician. So, the difference in weight loss of subjects could have possibly been affected differently. The quality-of-life scale score did not measure changes of psychological and physiological functions before and after treatment. Improvement of the quality-of-life score could further

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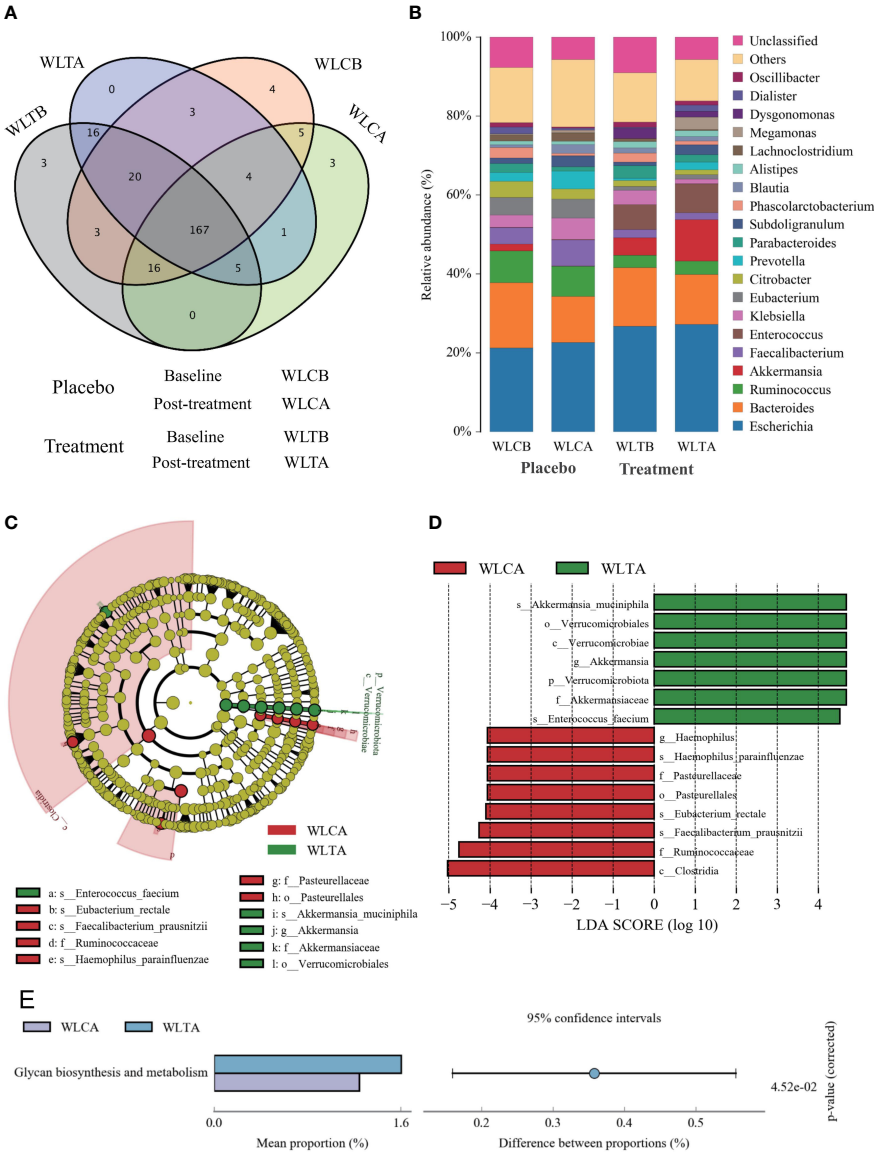

FIGURE 7
The structures and compositions of the gut microbiota before and after treatment in the two groups. **(A)** The Venn diagram of OTUs (species). **(B)** The composition of relative abundance on genus. **(C)** Phylogenetic cladogram of microbial lineage in fecal samples of treatment group and placebo group, with colors representing the most abundant differences in composition. **(D)** Key phylotypes of the gut microbiota responding to W-LHIT treatment. The histogram shows the lineage with LDA value of 4 or higher determined by LEFSe. **(E)** Key KEGG metabolic pathway responding to W-LHIT treatment.

contribute to enhancing weight loss (22). In subsequent research, further enhancement of the quality-of-life scale would be included.

As a marker of obesity, the level of hs-CRP is significantly elevated in the obese individual, and positively correlated with BMI obesity (23). Hs-CRP is synthesized by the liver in response to the stimulation of interleukin-6 and tumor necrosis factor-α, indicating a state of inflammation (24). In our study, the hs-CRP level decreased by 72.59% after W-LHIT treatment, which confirmed that managing obesity can help reduce the risk of cardiovascular disease and comorbidities by inhibiting the inflammatory mechanism (25).

Although there has been no consensus on how changes in the composition of gut microbiota can contribute to obesity, cumulative evidences have demonstrated that the occurrence of obesity is strongly associated with gut microbiota dysbiosis, which can result in chronic, persistent low-grade inflammatory reactions and abnormal lipid metabolism (12, 26, 27). In a review on the profile of the gut microbiota in obese adults, the consistent conclusion was that obese individuals (in comparison to leaner individuals) have a greater *Firmicutes/Bacteroidetes* ratio, *Fusobacteria*, *Proteobacteria*, *Mollicutes*, *Lactobacillus*, and reduced *Verrucomicrobia* (*Akkermansia muciniphila*), *Faecalibacterium* (*Prausnitzii*), *Bacteroides*, *Methanobrevibacter smithii*, *Lactobacillus plantarum* (27). Our results support the trend that there is a negative correlation between Firmicutes/Bacteroidetes ratio and BMI. After W-LHIT treatment, the BMI of both groups decreased, but the *Firmicutes/Bacteroidetes*

ratio both increased, with the mean ratio increased from 1.95 to 2.53 in the placebo group and from 1.54 to 1.78 in the treatment group. Compared with normal-weight individuals, obese individuals have a significant increase in *Proteobacteria* and a significant decrease in *Verrucomicrobia*. Shin et al. considered that an increased prevalence of *Proteobacteria* may be an active feature of metabolic disorders (28, 29). We found that the composition of *Proteobacteria* in 72.9% of the obese subjects exceeded 20% before treatment. The average composition of *Proteobacteria* in the treatment group decreased by about 2.6%, possibly indicating movement toward normalization of the gut microbiota, but in the placebo, group increased by about 3%. One of the new generation of probiotic candidates, *Verrucomicrobia* (*Akkermansia muciniphila*), can degrade mucin, and is closely related to host health (30). It has been found to enhance the intestinal barrier function and the effects of immunotherapy (31), enhance glucose tolerance and reduce insulin resistance (32), and moderate inflammatory responses (33, 34), exhibiting beneficial therapeutic roles in obesity, type 2 diabetes, atherosclerosis, tumors, and inflammatory bowel disease (IBD)- related gastrointestinal disturbances (30). Another exciting result in our study was that the abundance of *Verrucomicrobia* (*Akkermansia muciniphila*) in the W-LHIT treatment group significantly increased from 4.4% to 10.5%. Increasing evidence indicates that berberine (key compound index) target the gut microbiota and reversely modulate the structure and diversity under pathological conditions, thus exerting poly-pharmacological effects (18, 35) such as anti-obesity (36), anti-hyperlipidemia (37), anti-diabetes (38).

Coptis chinensis is the sovereign medicine in W-LHIT prescription. However, 2 subjects in the treatment group occasionally experienced mild gastrointestinal adverse events due to the large oral dose (9-15 capsules) of W-LHIT and the bitter taste of its main components (*Coptis chinensis*). *Coptis chinensis* has been used for thousand years in China safely at its therapeutic dose to treat various inflammatory disorders and related diseases, such as diarrhea, vomiting, abdominal distention, high fever coma, toothache, diabetes and eczema (39), and it is highly safe at its therapeutic dose. Linn et al. found 20 patients administered with *Coptis chinensis* at a daily dose of 3 g for 1055 patient-days without any organ toxicity or electrolyte imbalance (40). Our follow-up study will refine the ingredients further and enteric coating should be another good way to address this issue. *Coptis chinensis* is cold in nature (39), so, patients with weak spleen and stomach should use it with caution.

This study still has limitations. Firstly, no lean mass subjects were enrolled in this trial, the concern should be addressed in our follow-up study. Secondly, weight loss is a long-term and arduous work for subjects with sever obesity. So, only two months treatment is insufficient, and subjects with severe obesity need to receive longer intervention duration. Thirdly, this is a single-center study, limited by the small sample size, and some efficacy indicators between the treatment and control groups are not statistically significant, including the hip circumference (p =0.09) and waist circumference (p = 0.7). Due to the small sample size, several results of the two groups failed the normality test (alpha=0.05), so, the data processed by the 2-way ANOVA method shows that there is no statistical difference between them in weight loss, SDBP reduction and LDL-Chol reduction. The data processed by the unpaired t test followed by Mann-Whitney method have significant differences. In the follow-up study, we will further expand the sample size to obtain more meaningful results. Despite these limitations, we found that WLHT may be of great significance in managing weight loss of patients by ameliorating microbiome dysbiosis.

In conclusion, our current study assessed the efficacy and safety of W-LHIT capsules in 37 Chinese patients with simple obesity. We found that W-LHIT significantly reduced the weight of subjects with simple obesity, by greater than 5% of body weight in 72.6% of participants, which was significantly higher than in only 36.6% of the placebo group. In addition to weight loss, subjects in the treatment group also had significant improvements in blood pressure, blood glucose, and blood lipids. During the 2-month treatment, 7 subjects reported slight-mild gastrointestinal adverse reactions. However, with taking the medicine after meal or reducing the dosage, all the adverse reactions were gradually relieved. In addition, the results of 16S gut microbiota showed that W-LHIT significantly increased the abundance of *akkermansia muciniphila* and the *Firmicutes/Bacteroidetes* ratio, and decreased the abundance of *Proteobacteria*, facilitating the normalization of gut microbial ecosystem.

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 Wei-En hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

C-HW, M-CW, and Y-MS were significantly involved in conducting experiments. M-ZC, YS, KS, and NY were involved in data analysis. M-ZC was significantly involved in manuscript preparation. X-ML, DC, and M-SM were significantly involved in study design, data interpretation, and manuscript revision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

This study shared the US Patent No: US20160296573A1 Weight loss formulations, methods, and compositions based on Traditional Chinese Medicine by X-ML, DC, and NY. Author DC was employed by Healthy Freedom LLC. Authors NY and K.S are members of General Nutraceutical Technology LLC.

The remaining 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/fendo.2023.1054674/full#supplementary-material

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Association of family income to poverty ratio and vibration-controlled transient elastography quantified degree of hepatic steatosis in U.S. adolescents

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Introduction: Inequality in socioeconomic status plays an important role in the prevalence of metabolic diseases in adolescents. The purpose of this study was to explore the association between family income and the degree of hepatic steatosis quantified by vibration-controlled transient elastography (VCTE) among U.S. adolescents.

Methods: This cross-sectional study included two cycles of the National Health and Nutrition Examination Survey (NHANES) 2017-2020. Multivariate linear regression and smoothing curve fitting were used to investigate the linear and nonlinear relationship between PIR and hepatic steatosis, respectively. Subgroup analysis and interaction tests were used to test whether this relationship was stable across groups.

Results: Of the 1,574 adolescent participants, 456 lived in poor households and 307 lived in wealthy households. After adjusting for all covariates, PIR (Ratio of family income to poverty) was significantly negatively associated with the degree of hepatic steatosis [-4.78 (-7.39, -2.17)], and this remained stable after converting PIR to a categorical variable. In addition, this significant negative association was more pronounced in women [-7.62 (-11.38, -3.87)], non-Hispanic blacks [-7.19 (-14.43, 0.06)], Mexican Americans [-6.80 (-13.63, 0.03)], and participants with BMI >30 cm^2 [-10.83 (-19.70, -1.96)].

Conclusions: PIR was significantly and negatively associated with the degree of hepatic steatosis in US adolescents. Additional prospective studies are needed to confirm our findings.

KEYWORDS

ratio of family income to poverty, hepatic steatosis, NAFLD, NHANES, socioeconomic status

1 Introduction

Over the past three decades, non-alcoholic fatty liver disease (NAFLD) has developed into the most common cause of chronic liver disease worldwide (1, 2), with an alarming 36.1% prevalence of NAFLD in children and adolescents in the context of obesity (3). Worryingly, there is epidemiological evidence that this number will continue to rise in the future (4). The persistence of NAFLD in childhood into adulthood may be a cause of serious liver and metabolic disease and is critical for early risk factor detection and screening (5).

Many biomarkers have been shown in epidemiological studies to be strongly associated with NAFLD in the past (6–8). However, non-negligible sociological factors are also receiving increasing attention in the liver metabolism of children and adolescents (9). Metabolic disorders are now increasingly common in young adults, exhibit gender and racial differences, and are attributed to many interrelated factors such as genetic, environmental, and social factors. Socioeconomic disadvantage is common among U.S. adolescents, so studying the impact of family SES on the emergence of metabolic disease in early adolescence may help to prevent and manage the social actions and policies of health and economic burden throughout the life course (10, 11). A scoping review that included seven studies from different countries and regions showed a significant increase in obesity rates among adolescents with low socioeconomic status (SES) (12, 13). In addition, parental income status has been shown to be negatively associated with the prevalence of metabolic syndrome in adolescents (14, 15). Unequal socioeconomic status can affect normal organ metabolism in adolescents in terms of nutritional intake (16, 17), lifestyle habits (18), and metal exposure (19). However, there is no evidence to suggest whether adolescent household income is associated with the degree of hepatic steatosis as opposed to the obvious physical characteristics of appearance.

Therefore, we performed a cross-sectional study based on National Health and Nutrition Examination Survey (NHANES) 2017-2020 to investigate the relationship between family income to poverty ratio and the degree of hepatic steatosis quantified by vibration-controlled transient elastography (VCTE) among US adolescents.

2 Methods

2.1 Study population

The National Center for Health Statistics at the Centers for Disease Control and Prevention gathered data for the NHANES 2017-2020, which we examined. For proper coverage of the noninstitutionalized civilian population of the country, this cross-sectional survey employs stratified multistage probability cluster sampling (20–22). The National Center for Health Statistics (NCHS) Research Ethics Review Board authorized the study protocol. At the time of recruiting, all subjects provided written consent. We excluded 1287 participants without PIR data, 5862 participants without CAP data, 113 participants with Hepatitis B or C, and 6724 age more than 20 years. The study eventually included 1574 adolescent participants (Figure 1).

2.2 Study variables

We used the poverty-to-income ratio (PIR), an index of income related to household needs, by calculating annual changes in household size and cost of living and tracking the consumer price index from household income and federally determined poverty thresholds (23, 24). We divide the PIR into three levels, low-income level (PIR < 1), middle-income level (PIR 1-4), and high-income level (PIR > 4) (25).

The degree of hepatic steatosis was assessed using controlled attenuation data obtained by VCTE (26). Measurements were obtained by a professional operator from each participant for at least 10 measurements, and the device calculated a median controlled attenuation parameter (CAP) with a CAP value ranging from 100-400 dB/m, with higher values indicating higher liver fat content. A recent study describes the cut-off values for the grade of steatosis (27).

Age, gender, race, diabetes status, BMI, waist circumference, physical activities, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were all covariates in this study. The interpretation, measurement and calculation of all variables can be found on the official NHANES website (https://www.cdc.gov/nchs/nhanes/).

2.3 Statistical analysis

All analyses were performed with R (version 4.2) and Empowerstats (version 4.1). The chi-square test and t-test were used to assess the demographic characteristics of the participants by PIR classifications. Multivariate linear regression analyses were used to investigate the associations between PIR and CAP. The nonlinear association between PIR and CAP was explored by weighted generalized additive model and smoothed curve fitting. The parallel mediation model uses individual indicators as mediators. Subgroup analysis and interaction tests were used to investigate the relationship between PIR and CAP in different groups. A two-tailed P value < 0.05 was considered statistically significant.

3 Results

3.1 Baseline characteristics

The weighted characteristics were separated into three categories (low income, middle income, and high income) based

Abbreviations: VCTE, vibration-controlled transient elastography; NHANES, National Health and Nutrition Examination Survey; NCHS, National Center for Health Statistics; NAFLD, non-alcoholic fatty liver disease; SES, socioeconomic status; PIR, poverty income ratio; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CAP, controlled attenuation parameter; NCDs, non-communicable diseases.

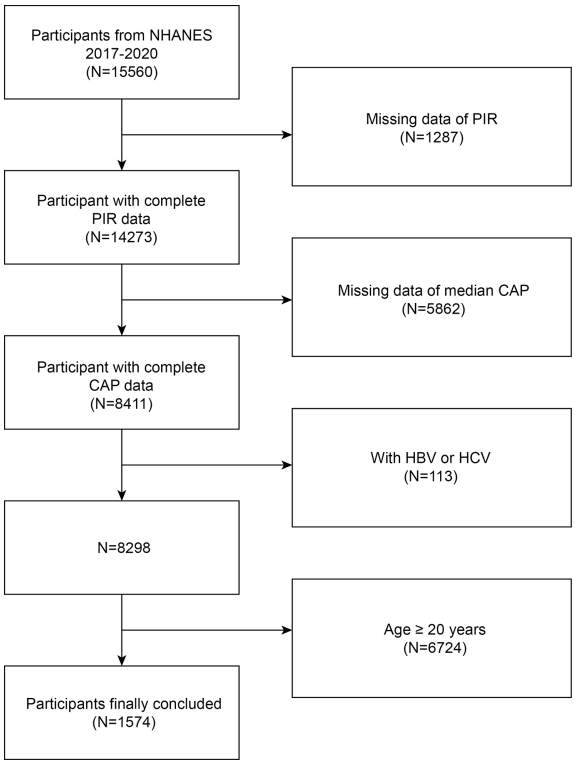

FIGURE 1
Flow chart of participants selection. NHANES, National Health and Nutrition Examination Survey; CAP, Controlled Attenuation Parameter; HBV, Hepatitis B; HCV, Hepatitis C; Ratio of family income to poverty, PIR.

on PIR. In total, 822 male and 752 female adolescents were involved, the mean PIR among all participants was 2.17. Notably, more than 28.9% of adolescents live in households with incomes below the poverty line (PIR<1), compared to 19.5% of adolescents living in wealthy households. Adolescents living below the poverty line are more likely to be of a race other than non-Hispanic white and have higher BMI, waist circumference and HDL-C than adolescents living in wealthy households (Table 1).

3.2 Association between PIR and hepatic steatosis

Table 2 shows the results of multivariate linear regression analyses for three models. There was a significant negative linear association between PIR and CAP in unadjusted model [-3.51 (-5.17, -1.84)]. The negative correlation between PIR and CAP remains significant even after adjusting for all covariates [-4.78 (-7.39, -2.17)]. We further investigated the association between different PIR levels and CAP after transforming PIR into categorical variables. In the fully adjusted model, the significant negative association between different levels of PIR and CAP persisted. Using the PIR of low-income participants as the reference group, the median CAP significantly decreased by 7.73 dB/m for every 1-score increase in PIR of participants in the middle-income group [-7.73 (-17.18, 1.73)] and by 21.85 dB/m for every 1-score increase in PIR of participants in the high-income group [-21.85 (-33.94, -9.76)]. In addition, we further performed generalized model smoothed curve fitting to confirm the non-linear relationship between PIR and CAP. The results validated a negative non-linear negative relationship between PIR and CAP (Figure 2).

3.3 Subgroup analyses

In subgroup analyses stratified by sex, race, age, and BMI, we found inconsistent associations between PIR and CAP (Table 3). Although the association between PIR and CAP remained negative in all subgroups, this linear negative association remained significant only in women, in participants aged 12-15 years, and in those with a BMI greater than or equal to 30. More importantly, the results of the interaction test showed that sex modified the association between PIR and CAP among adolescents, and no significant dependence of race, age, and BMI on this negative correlation.

4 Discussion

In this study, we assessed the association between PIR and degree of hepatic steatosis in US adolescents and found that low PIR was significantly associated with higher degree of hepatic steatosis, and this association was more prominent in females, non-Hispanic black, Mexican American, and BMI >30 kg/m^2 participants. To our knowledge, this is the first epidemiological study to investigate PIR and degree of hepatic steatosis in an adolescent population.

TABLE 1 Basic characteristics of participants by family PIR.

Characteristics	Low income (PIR < 1, N=456)	Middle income (PIR 1-4, N=811)	High income (PIR ≥ 4, N=307)	*P*-value
Age (years)	15.60 ± 2.34	15.15 ± 2.20	15.50 ± 2.15	0.001
Sex, n (%)				0.089
Male	231 (50.66%)	444 (54.75%)	147 (47.88%)	
Female	225 (49.34%)	367 (45.25%)	160 (52.12%)	
Race/ethnicity, n (%)				<0.001
Non-Hispanic White	113 (24.78%)	269 (33.17%)	147 (47.88%)	
Non-Hispanic Black	154 (33.77%)	198 (24.41%)	33 (10.75%)	
Mexican American	68 (14.91%)	141 (17.39%)	28 (9.12%)	
Other race/multiracial	121 (26.54%)	203 (25.03%)	99 (32.25%)	
Diabetes, n (%)				0.409
Yes	2 (0.44%)	7 (0.86%)	0 (0.00%)	
No	448 (98.25%)	796 (98.15%)	306 (99.67%)	
Borderline	6 (1.32%)	8 (0.99%)	1 (0.33%)	
Days of physical activities, n (%)				0.060
0-3	157 (34.45%)	288 (30.53%)	89 (28.87%)	
4-7	299 (65.55%)	523 (69.47%)	218 (71.13%)	
BMI (kg/m^2)	25.49 ± 7.13	25.05 ± 7.14	23.48 ± 5.38	<0.001
< 25 25-30 >30	259 (57.43%) 94 (20.84%) 98 (21.73%)	487 (60.42%) 154 (19.11%) 165 (20.47%)	212 (69.51%) 62 (20.33%) 31 (10.16%)	
Triglycerides (mg./L) HDL-C (mg/L) LDL-C (mg/L)	72.04 ± 43.60 50.37 ± 11.27 89.46 ± 26.94	68.28 ± 39.86 51.88 ± 12.39 85.87 ± 24.57	69.88 ± 30.51 52.56 ± 11.45 89.22 ± 25.85	0.595 0.040 0.237
Waist circumference (cm)	84.49 ± 17.31	83.88 ± 16.71	80.38 ± 13.06	0.002
Median CAP (dB/m)	227.27 ± 56.45	223.58 ± 54.16	210.83 ± 48.88	<0.001

Mean ± SD for continuous variables: the P value was calculated by the weighted linear regression model.
(%) for categorical variables: the P value was calculated by the weighted chi-square test.
PIR, Ratio of family income to poverty; BMI, body mass index; HDL-C, High-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CAP, Controlled Attenuation Parameter.

TABLE 2 The associations between family PIR and hepatic steatosis.

Exposure	Model 1 [β (95% CI)]	Model 2 [β (95% CI)]	Model 3 [β (95% CI)]
Ratio of family income to poverty	-3.51 (-5.17, -1.84)	-3.65 (-5.37, -1.93)	-4.78 (-7.39, -2.17)
PIR classification			
Low income (PIR < 1)	ref	ref	ref
Middle income (PIR 1-4)	-3.69 (-9.87, 2.49)	-4.40 (-10.63, 1.83)	-7.73 (-17.18, 1.73)
High income (PIR ≥ 4)	-16.44 (-24.23, -8.64)	-16.89 (-24.85, -8.93)	-21.85 (-33.94, -9.76)

Model 1: no covariates were adjusted. Model 2: age, gender, and race were adjusted. Model 3: age, gender, race, BMI, physical activities, diabetes status, Triglycerides, HDL-C and LDL-C were adjusted.
PIR, Ratio of family income to poverty; BMI, body mass index; HDL-C, High-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CAP, Controlled Attenuation Parameter.

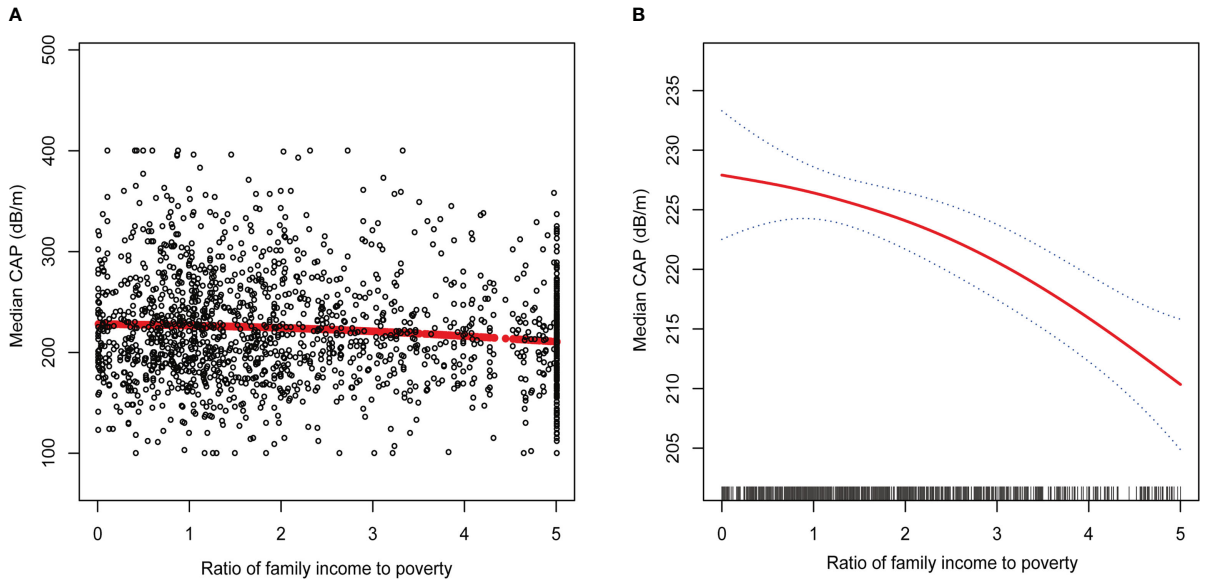

FIGURE 2
The association between PIR and degree of hepatic steatosis. **(A)** Each black point represents a sample. **(B)** The solid red line represents the smooth curve fit between variables. Blue bands represent the 95% of confidence interval from the fit. Controlled Attenuation Parameter; Ratio of family income to poverty, PIR.

Previous epidemiological studies have highlighted that SES inequality is closely related to metabolic syndrome and that this association varies across regions and countries (28, 29). A cohort study from Sweden used parental physical labor at age 16 as a criterion for low SES and showed that disadvantaged socioeconomic status in childhood or adolescence specifically increased the risk of metabolic syndrome in women (30). In addition, two studies, also from NHANES 1999-2002, demonstrated that the higher the PIR and education level, the lower the probability of obesity, hypertension and hyperglycemia in adolescents (31, 32). Our results are consistent with the above findings, demonstrating that low PIR is significantly associated with higher BMI and waist circumference, and in addition, gender differences are not negligible in this association.

TABLE 3 Subgroup analysis of the association between family PIR and median CAP.

Subgroup	OR (95%CI)	P for interaction
Sex		0.020
Male	-1.92 (-5.61, 1.78)	
Female	-7.62 (-11.38, -3.87)	
Age		0.820
12-15	-6.28 (-9.85, -2.70)	
16-19	-3.36 (-6.98, 0.27)	
Race/ethnicity		0.867
Non-Hispanic White	-1.96 (-5.93, 2.02)	
Non-Hispanic Black	-7.19 (-14.43, 0.06)	
Mexican American	-6.80 (-13.63, 0.03)	
Other race/multiracial	-2.17 (-5.19, 0.86)	
BMI		0.065
<24.9	-1.03 (-3.58, 1.53)	
25-29.9	-2.35 (-7.50, 2.80)	
≥30	-10.83 (-19.70, -1.96)	

Age, gender, race, BMI, physical activities, diabetes status, Triglycerides, HDL-C and LDL-C were adjusted. In the subgroup analyses, the model is not adjusted for the stratification variable itself.
PIR, Ratio of family income to poverty; BMI, body mass index; CAP, Controlled Attenuation Parameter.

Evidence from the Western Australian Pregnancy Cohort Study, which included pregnancy-related characteristics of parents of 1170 17-year-old adolescents, demonstrated that lower family income at birth was significantly associated with NAFLD in male offspring when not coterminous with obesity (33). Although there is epidemiological evidence confirming a negative association between PIR and NAFLD in adolescents, these studies tend to diagnose NAFLD by liver enzyme and index calculations (34). Two main problems with such a diagnostic approach are that it does not accurately measure the severity of hepatic steatosis in participants and does not allow for a further description of the association between PIR and NAFLD (35). In addition, several studies have shown that the degree of hepatic steatosis calculated by liver enzymes or indices may be statistically biased and may significantly underestimate the number of participants with NAFLD in epidemiological studies (36). VCTE, as recommended by the American Gastroenterological Association for risk stratification and management of patients with NAFLD, is highly accurate in diagnosing the degree of hepatic steatosis and the degree of fibrosis (37, 38). Therefore, we used VCTE data to avoid diagnostic inaccuracies and the inability to quantify the degree of hepatic steatosis in the current study.

Explaining the PIR differences observed in adolescent hepatic steatosis is expected to be as complex as defining population differences in the metabolic syndrome because of the intricate interactions between the metabolic syndrome and multiple known disease factors, environmental factors, cultural factors, climatic factors, and genetic factors (39). However, through a review of studies describing the comorbidity of NAFLD in adolescents, understanding the prevalence and characteristics of hepatic steatosis in the adolescent population may provide potential evidence to help improve strategies to prevent NAFLD and related liver diseases in the adolescent population. Epidemiological evidence suggests that groups with higher household incomes adopt healthy but higher cost-of-living lifestyles early in life, while groups of children and adolescents in low-income households live with an increased prevalence of risky behaviors (40, 41). Based on observed forms including inability to afford healthy food, exposure to harmful environments, and lack of access to quality health services (42). In addition, ethnic and cultural differences in minority groups in high-income countries are also associated with a significantly higher risk of obesity and metabolic disease than other groups (43, 44). More importantly, the exposure of parents with low SES to non-communicable diseases (NCDs) during fetal life and infancy increases the risk of developing childhood NCDs (45). For example, offspring of diabetic parents exhibit earlier and more pronounced insulin resistance features in early adolescence, providing plausible evidence for biological factors (46). Recent epidemiological evidence also provides a novel explanation for the mechanism behind the negative association between PIR and hepatic steatosis: this negative association may derive mainly from mediating factors arising from PIR, such as diet quality and physical activity, with further effects of these variables on liver metabolism (47).

Our study has some limitations. First, due to the design of the cross-sectional study, we were unable to determine the causal relationship between PIR and degree of hepatic steatosis (48, 49). In addition, because the PIR is linked to a large number of variables, we were unable to include all covariates that had a potential impact on, which may lead to incomplete accuracy of the results. Despite these shortcomings, our study has several advantages. This study includes data from a large and representative cross-sectional survey. More importantly, this study confirms the association between PIR and hepatic steatosis and quantifies for the first time the effect of PIR on the degree of hepatic steatosis.

5 Conclusion

Our results suggest that PIR negatively correlated with degree of hepatic steatosis among U.S. adolescents. Differences of PIR in the population should be considered in the diagnosis and treatment of NAFLD.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found here: https://www.cdc.gov/nchs/nhanes/.

Ethics statement

The studies involving human participants were reviewed and approved by The National Center for Health Statistics (NCHS) Research Ethics Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

MT and RX designed the research. MT, ML, and YZ collected, analyzed the data, and drafted the manuscript. YZ and RX revised the manuscript. All authors contributed to the article and approved the submitted version.

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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

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Relationship between bone mineral density and hyperuricemia in obesity: A cross-sectional study

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Background: Obesity is an increasingly severe global public health issue. This study aims to estimate the cross-sectional association between bone mineral density (BMD) and hyperuricemia (HU) in obesity.

Method: A total of 275 obese subjects (126 men and 149 women) participated in this cross-sectional study. Obesity was diagnosed as body mass index (BMI) ≥28 kg/m^2, whereas HU was defined as the blood uric acid level of 416 μmol/L in men and 360 μmol/L in women. The BMD of the lumbar spine and right hip was measured by dual-energy X-ray absorptiometry (DXA). The multivariable logistic regressions were employed to examine the relationship between BMD and HU in obesity, with the adjustment of gender, age, fasting blood glucose, fasting insulin, homeostasis model assessment of insulin resistance (HOMA-IR), cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein, creatinine, blood urea nitrogen, high-sensitivity C-reactive protein (hs-CRP), cigarette smoking, and alcohol drinking status.

Result: The overall prevalence of HU was 66.9% in this obese population. The mean age and BMI of this population were 27.9 ± 9.9 years and 35.2 ± 5.2 kg/m^2, respectively. The multivariable-adjusted OR (the highest *vs.* lowest BMD quartile) demonstrated a negative relationship between BMD and HU in total (OR = 0.415, 95%CI: 0.182–0.946; p = 0.036), L1 (OR = 0.305, 95%CI: 0.127–0.730; p = 0.008), L2 (OR = 0.405, 95%CI: 0.177–0.925; p = 0.032), and L3 (OR = 0.368, 95%CI: 0.159–0.851; p = 0.020) lumbar vertebrae. In the subgroup analysis for the male population, the BMD was also negatively associated with HU in total (OR = 0.077, 95%CI: 0.014–0.427; p = 0.003), L1 (OR = 0.019, 95%CI: 0.002–0.206; p = 0.001), L2 (OR = 0.161, 95%CI: 0.034–0.767; p = 0.022), L3 (OR = 0.186, 95%CI: 0.041–0.858; p = 0.031),

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and L4 (OR = 0.231, 95%CI: 0.056–0.948; p = 0.042) lumbar vertebrae. However, such findings did not exist in women. In addition, there was no significant relationship between hip BMD and HU in obesity.

Conclusion: Our results showed that the lumbar BMD was negatively associated with HU in obesity. However, such findings only existed in men, rather than women. In addition, no significant relationship between hip BMD and HU existed in obesity. Due to the limited sample size and nature of the cross-sectional design, further large prospective studies are still needed to clarify the issues.

KEYWORDS

bone mineral density, hyperuricemia, obesity, cross-sectional, uric acid

Introduction

Obesity is an increasingly severe clinical and public health issue. Unfortunately, more than 100 million patients suffer from obesity worldwide (1). More importantly, obesity has become a major public health concern in China. It was estimated that more than half of Chinese adults are either overweight or obese in a recent national survey (2, 3). Emerging evidence indicated that obesity is associated with a higher risk of hyperuricemia (HU) (4). Uric acid is an end product of purine metabolism in the human body, and HU is always recognized as the precursor of gout (5). In addition, HU is also considered to be related to metabolic syndromes/indices (3, 6), renal injury (7), inflammation, and endothelial dysfunction (8) in obesity. Therefore, the identification of modifiable factors for HU appears to be an important step in the clinical management of obesity.

Generally speaking, bone mineral density (BMD) is an important and common clinical indicator for the diagnosis of osteoporosis (9). As far as we know, a large number of studies have examined the association between HU and BMD. However, no final conclusion can be obtained (9–20). The potential reasons for such discrepancy may be attributed to the variety of population characteristics, including genetic background, age, body fat proportion or confounders adjusted, and different lifestyle factors (14). Therefore, it is significant and necessary to consider the issue in different sub-populations. Obesity is a health issue with special clinical characteristics. Importantly, a causal relationship between obesity and lower BMD was confirmed in a two-sample Mendelian randomization study recently (21). Indeed, increased marrow adiposity is attributed to the shift from osteogenic to adipogenic differentiation of bone marrow mesenchymal stem cells (22). However, obesity is also positively associated with HU (23, 24). Experimental evidence did suggest that purine catabolism in adipose tissue could be enhanced in obesity (25). It is interesting and necessary to reveal how BMD is involved in HU in the context of obesity. Therefore, this study was performed to investigate the relationship between BMD and HU in the obese population.

Materials and methods

Study population

This cross-sectional study was reviewed and approved by the Medical Ethics Committee of Xiangya Hospital, Central South University (202103043). A total of 275 obese patients (126 men and 149 women) were recruited from Xiangya Hospital (from August 2019 to December 2021). The inclusion criteria were as follows: 1) BMI ≥ 28 kg/m^2, 2) available completed clinical data, and 3) patients agreeing to participate in the study. The exclusion criteria were as follows: 1) postmenopausal women; 2) bone disease (new fractures and malignancies), severe hepatic and renal insufficiency, thyroid and parathyroid disorders, and some other diseases involved in bone metabolism; 3) long-term drug users with affected bone or uric acid metabolism (e.g., glucocorticoids, anticoagulants, thyroid hormones, proton pump inhibitors, antiepileptics, allopurinol, and benzbromarone); 4) those who have undergone iodine, barium, or nuclear medicine isotope tests in the past week (bone scan, kidney scan, PET-CT, enhanced CT, etc.); 5) subjects with implanted materials that affect BMD assessment (bone cement, surgical nails, steel stents, metal implants, pacemaker lead wires, etc.).

Blood biochemistry

The blood biochemical indices of subjects who fasted for 8–12 h were obtained. The fasting venous blood was collected in the morning. Blood uric acid, fasting blood glucose, fasting insulin, cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein, serum creatinine, blood urea nitrogen, and high-sensitivity C-reactive protein (hs-CRP) were collected and recorded. Moreover, the homeostasis model assessment of insulin resistance (HOMA-IR) was also calculated. The blood biochemical indices were tested using the automatic biochemical analyzer. Uric acid was determined by the uricase–peroxidase method. Fasting

blood glucose and insulin were determined by hexokinase and chemiluminescence methods. Cholesterol was determined by the enzyme method, triglyceride was determined by the GPO-POD method, and low-density lipoprotein and high-density lipoprotein were determined by a direct method. Moreover, creatinine was determined by the basic picric acid method, urea nitrogen was determined by the glutamate dehydrogenase method, and hs-CRP was determined by the immunoturbidimetric method. HOMA-IR was calculated by the following formula: fasting blood glucose (mmol/L) * fasting insulin (mIU/L)/22.5. HU was defined as a blood uric acid level of 416 μmol/L in men and 360 μmol/L in women.

Measurement of bone mineral density

The BMD of the lumbar spine and right hip was measured by dual-energy X-ray absorptiometry (DXA) (U.S. Lunar), which is commonly used in clinical and scientific research due to its small repeatability and diagnostic error. The BMD was measured by the same experienced senior doctor, and quality control testing was carried out properly. Lumbar spine BMD measurements included the first, second, third, and fourth lumbar vertebrae (L1 to L4) and total lumbar vertebra, whereas right hip BMD measurements included the femoral neck, trochanter, Ward's triangle, and total hip (Figure 1). Anatomically,. Ward's triangle is a fragile triangular area formed by the cross of the femoral neck between the medial pressure bone trabecula, lateral tension bone trabecula, and intertrochanteric line of the femoral neck (a common site for fracture). The BMD levels were expressed as BMD values (g/cm^2) directly.

General information collection

The gender, age, height, and weight of the included subjects were collected and recorded. The body mass index (BMI) was calculated by the following formula: BMI (kg/m^2) = weight (kg)/height (m)^2. Both cigarette smoking and alcohol drinking status were evaluated by the questions "Do you smoke/drink? Yes/No".

Statistical analysis

The SPSS 23.0 software was utilized for the analysis. The normal distribution data are expressed by means and standard deviation, whereas the skewness distribution data are expressed by median and interquartile spacing. The classified data are expressed as percentage. The t-test was employed for normal distribution data, whereas the chi-square test was used for classified data. The rank sum test was used for skewed distribution data. Moreover, multiple logistic regression was used for correlation analysis. The differences in general data, blood biochemical indicators, and BMD between obese men and women were also compared. The BMD and HU served as exposure and outcome, respectively. The BMD was divided into quartiles from low to high, with the lowest quartile as the reference. The following covariates were adjusted: gender, age, fasting blood glucose, fasting insulin, HOMA-IR, cholesterol, triglycerides,

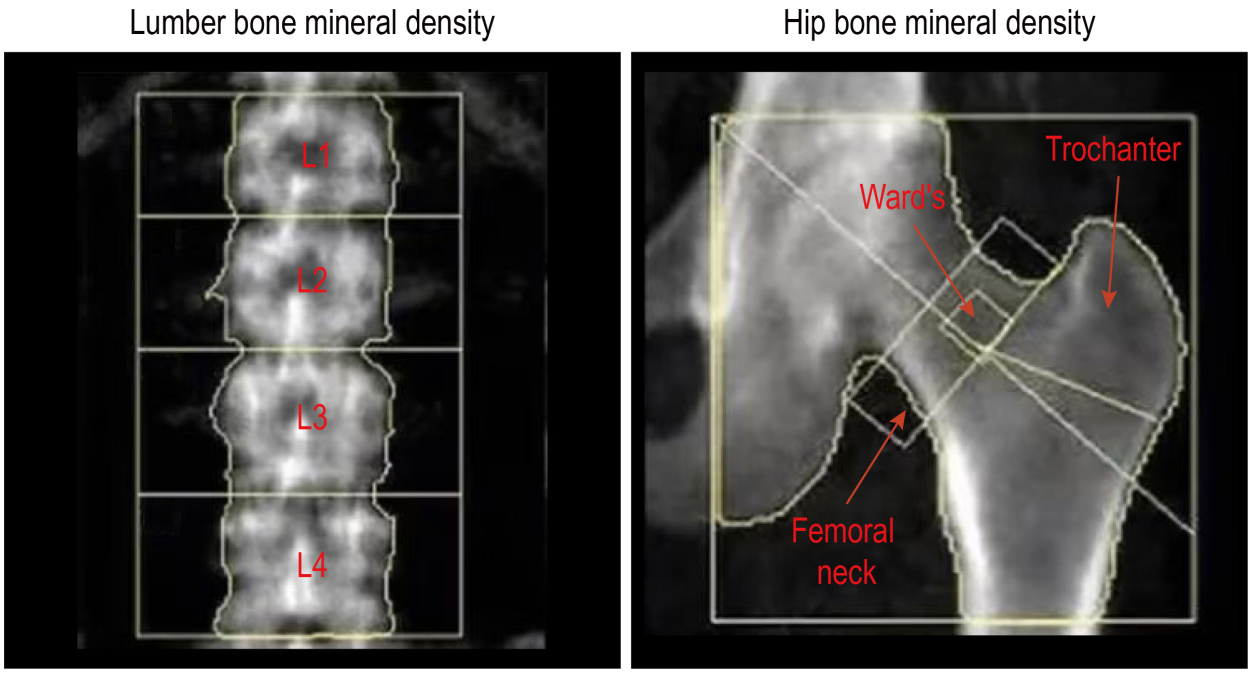

FIGURE 1
The assessment of bone mineral density in the lumbar spine and hip.

low-density lipoprotein, high-density lipoprotein, creatinine, blood urea nitrogen, hs-CRP, cigarette smoking, and alcohol drinking status. $p < 0.05$ was considered statistically significant.

Results

The main characteristics of the obese population

The main characteristics of the obese population are presented in Table 1. A total of 275 subjects were included in our study: 126 and 149 were men and women, respectively. The mean age and BMI of this population were 27.9 ± 9.9 years and 35.2 ± 5.2 kg/m^2, respectively. The BMI, uric acid, triglyceride, HDL cholesterol, creatinine, cigarette smoking, alcohol drinking, total hip BMD, femoral neck BMD, and trochanter BMD were all significantly different between men and women.

The relationship between lumbar BMD and hyperuricemia in obesity

The results of the relationship between lumbar BMD and HU in obesity are presented in Table 2. The multivariable-adjusted OR (the highest *vs.* lowest BMD quartile) demonstrated a negative

TABLE 1 The main characteristics of the obese population.

Characteristics	All	Male	Female	p-Value
Participants (n)	275	126	149	–
Age (years)	27.9 ± 9.9	28.1 ± 10.6	27.7 ± 9.2	0.726
BMI (kg/m^2)	35.2 ± 5.2	36.9 ± 5.5	33.7 ± 4.4	**<0.001**
Uric acid (μmol/L)	444.2 ± 118.6	500.5 ± 121.0	396.6 ± 93.2	**<0.001**
Hyperuricemia (yes, %)	66.9	65.9	67.8	0.737
FBG (mmol/L)	5.41 (1.10)	5.37 (1.10)	5.43 (1.00)	0.736
FINS (mIU/L)	20.9 (15.7)	20.8 (15.6)	20.9 (15.7)	0.211
HOMA-IR	5.14 (4.33)	5.39 (4.68)	5.05 (4.30)	0.319
Triglyceride (mmol/L)	1.71 (1.36)	2.06 (1.40)	1.50 (1.11)	**<0.001**
Cholesterol (mmol/L)	5.07 ± 1.02	5.13 ± 1.14	5.02 ± 0.91	0.383
LDL cholesterol (mmol/L)	3.29 ± 0.72	3.30 ± 0.77	3.28 ± 0.68	0.748
HDL cholesterol (mmol/L)	1.11 ± 0.24	1.04 ± 0.22	1.16 ± 0.24	**<0.001**
Creatinine (μmol/L)	75.1 ± 19.5	85.7 ± 23.2	66.0 ± 8.4	**<0.001**
BUN (mmol/L)	4.44 ± 2.11	4.55 ± 1.32	4.34 ± 2.60	0.403
hs-CRP (mg/L)	3.24 (3.62)	2.90 (3.68)	3.44 (3.47)	0.227
Cigarette smoking (yes, %)	23.3	40.5	8.7	**<0.001**
Alcohol drinking (yes, %)	14.5	26.2	4.7	**<0.001**
Total hip BMD (g/cm^2)	1.033 ± 0.139	1.073 ± 0.133	1.001 ± 0.136	**<0.001**
Femoral neck BMD (g/cm^2)	0.901 ± 0.121	0.940 ± 0.124	0.870 ± 0.110	**<0.001**
Trochanter BMD (g/cm^2)	0.762 ± 0.103	0.783 ± 0.106	0.745 ± 0.098	**0.009**
Ward's triangle BMD (g/cm^2)	0.853 ± 0.179	0.870 ± 0.186	0.839 ± 0.174	0.229
Total lumbar BMD (g/cm^2)	1.041 ± 0.120	1.033 ± 0.127	1.047 ± 0.113	0.304
L1 BMD (g/cm^2)	0.990 ± 0.123	0.991 ± 0.122	0.990 ± 0.124	0.947
L2 BMD (g/cm^2)	1.044 ± 0.124	1.038 ± 0.129	1.048 ± 0.121	0.510
L3 BMD (g/cm^2)	1.073 ± 0.134	1.056 ± 0.139	1.087 ± 0.129	0.056
L4 BMD (g/cm^2)	1.047 ± 0.130	1.036 ± 0.144	1.056 ± 0.118	0.197

The p-value is derived from comparison between men and women.
BMI, body mass index; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoprotein cholesterol; BUN, blood urea nitrogen; hs-CRP, high-sensitivity C-reactive protein; BMD, bone mineral density; L1, first lumbar vertebra; L2, second lumbar vertebra; L3, third lumbar vertebra; L4, fourth lumbar vertebra. The bold values indicate the datawith a significant P value (P<0.05).

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TABLE 2 Multivariable-adjusted ORs of hyperuricemia according to lumbar bone mineral density level in obese subjects.

	Total		Male		Female	
	Multivariable-adjusted OR	p-Value	Multivariable-adjusted OR	p-Value	Multivariable-adjusted OR	p-Value
Total lumbar						
Quartile 1	1	/	1	/	1	/
Quartile 2	1.064 (0.437–2.593)	0.891	0.146 (0.022–0.953)	**0.044**	2.510 (0.734–8.586)	0.143
Quartile 3	0.645 (0.279–1.492)	0.305	0.099 (0.017–0.569)	**0.010**	1.277 (0.403–4.047)	0.678
Quartile 4	0.415 (0.182–0.946)	**0.036**	0.077 (0.014–0.427)	**0.003**	0.984 (0.320–3.026)	0.978
L1						
Quartile 1	1	/	1	/	1	/
Quartile 2	0.588 (0.243–1.424)	0.239	0.062 (0.006–0.643)	**0.020**	0.788 (0.248–2.502)	0.686
Quartile 3	0.464 (0.187–1.152)	0.098	0.036 (0.003–0.400)	**0.007**	0.798 (0.245–2.600)	0.709
Quartile 4	0.305 (0.127–0.730)	**0.008**	0.019 (0.002–0.206)	**0.001**	0.854 (0.264–2.767)	0.793
L2						
Quartile 1	1	/	1	/	1	/
Quartile 2	0.916 (0.387–2.169)	0.842	0.250 (0.049–1.278)	0.096	1.240 (0.382–4.026)	0.720
Quartile 3	0.667 (0.288–1.542)	0.343	0.160 (0.031–0.818)	**0.028**	1.081 (0.347–3.366)	0.893
Quartile 4	0.405 (0.177–0.925)	**0.032**	0.161 (0.034–0.767)	**0.022**	0.690 (0.221–2.157)	0.524
L3						
Quartile 1	1	/	1	/	1	/
Quartile 2	0.634 (0.263–1.526)	0.309	0.321 (0.064–1.612)	0.168	2.013 (0.625–6.481)	0.241
Quartile 3	0.532 (0.221–1.278)	0.158	0.206 (0.044–0.962)	**0.045**	0.916 (0.288–2.912)	0.881
Quartile 4	0.368 (0.159–0.851)	**0.020**	0.186 (0.041–0.858)	**0.031**	1.461 (0.463–4.617)	0.518
L4						
Quartile 1	1	/	1	/	1	/
Quartile 2	1.392 (0.581–3.336)	0.459	0.767 (0.160–3.675)	0.740	4.752 (1.340–16.855)	**0.016**
Quartile 3	0.966 (0.417–2.238)	0.936	0.377 (0.086–1.649)	0.195	1.525 (0.453–5.132)	0.496
Quartile 4	0.538 (0.238–1.220)	0.138	0.231 (0.056–0.948)	**0.042**	1.400 (0.456–4.299)	0.557

The multivariable model was adjusted for age (continuous data), BMI (continuous data), gender (male, female), FBG (continuous data), FINS (continuous data), HOMA-IR (continuous data), triglyceride (continuous data), cholesterol (continuous data), LDL cholesterol (continuous data), HDL cholesterol (continuous data), creatinine (continuous data), BUN (continuous data), hs-CRP (continuous data), cigarette smoking (yes or no), and alcohol drinking (yes or no).
BMI, body mass index; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoprotein cholesterol; BUN, blood urea nitrogen; hs-CRP, high-sensitivity C-reactive protein; L1, first lumbar vertebra; L2, second lumbar vertebra; L3, third lumbar vertebra; L4, fourth lumbar vertebra. The bold values indicate the data with a significant P value (P<0.05).

relationship between BMD and HU in total (OR = 0.415, 95%CI: 0.182–0.946; p = 0.036), L1 (OR = 0.305, 95%CI: 0.127–0.730; p = 0.008), L2 (OR = 0.405, 95%CI: 0.177–0.925; p = 0.032), and L3 (OR = 0.368, 95%CI: 0.159–0.851; p = 0.020) lumbar vertebrae. In the subgroup analysis for the men population, the BMD was also negatively associated with HU in total (OR = 0.077, 95%CI: 0.014–0.427; p = 0.003), L1 (OR = 0.019, 95%CI: 0.002–0.206; p = 0.001), L2 (OR = 0.161, 95%CI: 0.034–0.767; p = 0.022), L3 (OR = 0.186, 95%CI: 0.041–0.858; p = 0.031), and L4 (OR = 0.231, 95%CI: 0.056–0.948; p = 0.042) lumbar vertebrae. On the contrary, no significant relationship between lumbar BMD and HU was obtained in women.

The relationship between hip BMD and hyperuricemia in obesity

The results of the relationship between hip BMD and HU in obesity are presented in Table 3. The multivariable-adjusted OR

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TABLE 3 Multivariable-adjusted ORs of hyperuricemia according to hip bone mineral density level in obese subjects.

	Total		Male		Female	
	Multivariable-adjusted OR	p-Value	Multivariable-adjusted OR	p-Value	Multivariable-adjusted OR	p-Value
Total hip						
Quartile 1	1	/	1	/	1	/
Quartile 2	1.774 (0.701–4.489)	0.226	3.457 (0.453–26.405)	0.232	2.828 (0.740–10.799)	0.128
Quartile 3	2.095 (0.804–5.455)	0.130	0.425 (0.071–2.547)	0.349	1.758 (0.452–6.840)	0.416
Quartile 4	0.996 (0.365–2.718)	0.994	0.523 (0.076–3.614)	0.511	2.002 (0.521–7.688)	0.312
Femoral neck						
Quartile 1	1	/	1	/	1	/
Quartile 2	1.079 (0.437–2.669)	0.869	0.366 (0.056–2.416)	0.297	1.570 (0.426–5.784)	0.498
Quartile 3	0.852 (0.326–2.229)	0.745	0.286 (0.042–1.927)	0.198	1.690 (0.448–6.378)	0.439
Quartile 4	1.086 (0.377–3.129)	0.879	0.126 (0.015–1.056)	0.056	1.660 (0.403–6.845)	0.483
Trochanter						
Quartile 1	1	/	1	/	1	/
Quartile 2	0.954 (0.390–2.332)	0.918	0.119 (0.016–0.883)	**0.037**	1.382 (0.393–4.864)	0.614
Quartile 3	1.376 (0.524–3.615)	0.517	0.359 (0.072–1.790)	0.212	1.491 (0.403–5.514)	0.549
Quartile 4	0.986 (0.375–2.590)	0.976	0.325 (0.058–1.828)	0.202	0.908 (0.241–3.426)	0.887
Ward's						
Quartile 1	1	/	1	/	1	/
Quartile 2	1.551 (0.610–3.945)	0.357	1.031 (0.153–6.921)	0.975	1.912 (0.538–6.797)	0.316
Quartile 3	1.531 (0.581–4.031)	0.389	0.974 (0.166–5.715)	0.976	2.352 (0.576–9.600)	0.233
Quartile 4	1.965 (0.695–5.556)	0.203	0.920 (0.134–6.319)	0.933	1.973 (0.439–8.868)	0.375

The multivariable model was adjusted for age (continuous data), BMI (continuous data), gender (male, female), FBG (continuous data), FINS (continuous data), HOMA-IR (continuous data), Triglyceride (continuous data), cholesterol (continuous data), LDL cholesterol (continuous data), HDL cholesterol (continuous data), Creatinine (continuous data), BUN (continuous data), hs-CRP (continuous data), cigarette smoking (yes or no), and alcohol drinking (yes or no).
BMI, body mass index; FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; LDL cholesterol, low-density lipoprotein cholesterol; HDL cholesterol, high-density lipoprotein cholesterol; BUN, blood urea nitrogen; hs-CRP, high-sensitivity C-reactive protein. The bold values indicate the data with a significant P value (P<0.05).

(the highest *vs.* lowest BMD quartile) demonstrated no significant relationship between BMD and HU in the total hip (OR = 0.996, 95%CI: 0.365–2.718; p = 0.994), femoral neck (OR = 1.086, 95%CI: 0.377–3.129; p = 0.879), trochanter (OR = 0.986, 95%CI: 0.375–2.590; p = 0.976), and Ward's triangle (OR = 1.965, 95%CI: 0.695–5.556; p = 0.203). In the subgroup analysis, no significant relationship between BMD and HU was obtained in the male and female populations.

Discussions

Our results showed that the lumbar BMD was negatively associated with HU in obesity. However, such findings only existed in men, rather than women. On the contrary, no significant relationship between hip BMD and HU was obtained.

Generally speaking, the relationship between BMD and HU is conflicting. Several studies found a positive association between BMD and HU in postmenopausal and older women (16, 26–31). Furthermore, a positive association between lumbar spine BMD and serum uric acid (SUA) was confirmed in postmenopausal women, rather than men (32). On the contrary, no significant relationship between lumbar spine BMD and SUA was obtained in US participants over 30 years (33). Therefore, the relationship between BMD and HU may vary depending on the population. In consideration of the special characteristics of obesity, it is necessary to be concerned about such issues in the context of obesity. It was reported that obesity was associated with a lower level of BMD (21) and a higher prevalence of HU (2), which might partly contribute to the inverse relationship between BMD and HU in obesity (especially the BMI in this population is extremely high: 35.2 ± 5.2). Interestingly, such findings were not found in women, which was

in contrast to the previous evidence regarding postmenopausal and older women. It should be noted that the women in our study are relatively young (27.7 ± 9.2 years old). It is reported that estrogen is associated with a higher level of BMD and a lower level of serum uric acid. In this condition, a higher level of estrogen in our women population may contribute to a potential negative relationship between BMD and HU. The aforementioned positive relationship between BMD and HU regarding postmenopausal and older women may be neutralized in the context of young women. Therefore, a final combined null relationship was obtained in our results. Moreover, the BMI in men is significantly higher than that in women (36.9 *vs.* 33.7). Thus, the potential obesity-derived negative relationship between BMD and HU may be much stronger in men, which may partly contribute to the sex difference results in our study. However, our results should be carefully interpreted due to the limited sample size and potential residual confounding with unmeasured variables. Furthermore, the results regarding the lumbar and hip were totally different. Moreover, the L1 lumbar BMD seems to contribute mostly. Therefore, the location for analysis may be involved in the relationship between BMD and HU. Moreover, the lumbar BMD seems to be more sensitive to reflect the issue of obesity. Nevertheless, our results may be restricted by the nature of the cross-sectional design and limited sample size, and more large prospective studies are still needed.

The underlying mechanism behind the inverse relationship between BMD and HU can be listed as follows. The hydrophobic lipid layer of the cell membrane may interfere with the antioxidant properties of uric acid (it mainly acts in human plasma) (34, 35). The intracellular free oxygen radicals are generated during uric degradation, which further enhances intracellular superoxide generation by interacting with NADPH oxidase. It inhibits osteoblast bone formation and stimulates osteoclast bone resorption (36–38). In addition, uric acid might exert adverse effects on bone health by affecting 25-OH-D (1,25D) and parathyroid hormone (PTH) levels. An inverse correlation between 1,25D concentrations and uric acid has been revealed in the HU model and chronic kidney disorder subjects (39–41). However, this association could be reversed by allopurinol, a drug that lowers uric acid levels (41). Similarly, serum uric acid was positively correlated with PTH levels (39, 42). Furthermore, the proinflammatory cytokines causing acute gout attack, including tumor necrosis factor-α, interleukin-1, interleukin-6, and interleukin-8, promote osteoclast differentiation and increases bone resorption (36). However, the issue of obesity has not been considered in current experimental evidence, which should be addressed by further study.

Our study has several advantages. To begin with, this is the first study on the association between BMD and HU in obesity so far. In addition, our study has specified that the relationship between BMD and HU may be varied depending on the bone location. Finally, our results may appeal to the public to pay more attention to bone health in the obese population: an increased level of BMD may be beneficial to the clinical management of obesity. However, the limitations of our study should also be acknowledged. First, the cross-sectional design precludes causal relationships, and further prospective studies are still needed to clarify the concerns. Second, the questionnaire surveys are used to collect some clinical data (smoking and alcohol drinking status, etc.). The potential recall bias cannot be excluded. Third, the sample size of the obese subjects is relatively small, which may inevitably influence the reliability of our results. Fourth, the included subjects are relatively young. Therefore, our results may not reflect the issue at all ages. Finally, some residual or unmeasured confounder remains possible.

In conclusion, our results showed that the lumbar BMD was negatively associated with HU in obesity. However, such findings only existed in men, rather than women. In addition, no significant relationship between hip BMD and HU was obtained. Further large well-designed prospective studies are still needed.

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 Ethics Committee of Xiangya Hospital, Central South University (202103043). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MW and YiZ decided and conceptualized this article and revised the draft. YiZ and MT wrote the manuscript. MT and JW collected and analyzed the data. YiZ and JW prepared the figures and tables. MW and JW were the guarantors of the overall content. All authors contributed to the article and approved the submitted version.

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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

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Asthma prevalence is increased in patients with high metabolism scores for visceral fat: study reports from the US

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Objective: Data from NHANES 2001-2018 were used to examine the relationship between metabolism score for visceral fat (METS-VF) and asthma prevalence.

Methods: We assessed the association between METS-VF and asthma disease using multiple logistic regression analysis from the National Health and Nutrition Examination Survey (NHANES), 2001-2018, followed by subgroup analysis for sensitive populations. To determine whether METS-VF and asthma disease had a non-linear relationship, smooth curve fitting was used, and threshold effect analysis was used to verify the relationship.

Results: Among the 36,876 participants, 4,919 self-reported having asthma. When all confounders were controlled for, a positive association was found between METS-VF and asthma prevalence (OR = 1.27, 95% CI: 1.22,1.32), and this positive association was stronger with elevated METS-VF (P for trend = 0.01). According to the smooth curve fitting analysis, METS-VF and asthma prevalence do not have a linear relationship. The double-segmented threshold effect analysis suggested a negative correlation but no statistically significant difference between METS-VF less than 5.24 and asthma prevalence (OR = 0.60, 95% CI: 0.33, 0.91). Besides, other METS-VF showed positive associations with asthma prevalence before and after the effective inflection point. According to subgroup analysis, METS-VF is associated with asthma prevalence among participants aged 40 – 59, male, Mexican American, with hypertension and diabetes, and without asthma history.

Conclusion: A positive correlation between METS-VF and asthma was observed and this positive correlation was non-linear, and participants with METS-VF above 5.24 should be cautious about the high risk of asthma. The relationship should be given more attention to participants who are aged 40-59 years old, male, Mexican American, have hypertension, diabetes, and who do not have a family history of asthma.

KEYWORDS

asthma, visceral obesity, METS-VF, NHANES, cross-sectional study

1 Introduction

Experiencing and repeating exacerbations of asthma will reduce the patient's lung function and quality of life (1, 2). Asthma is a chronic multifactorial airway inflammation disease. Asthma prevalence has generally increased worldwide since the end of the last century. However, because of numerous risk factors, the incidence of asthma may vary from region to region (3). Consequently, researchers are interested in other factors other than allergies and heredity that may influence asthma. As the global economy develops, the diet of the global population becomes more westernized, which leads to obesity becoming a public health problem (4). Based on knowledge of asthma risk factors, previous studies had identified obesity as a major risk factor for asthma in children and adults (5)., Previous research found that obesity ranked fifth among the 10 treatable traits that could influence the development of asthma (6). In a cross-sectional study, compared to the lean and obese groups, the odds ratio for asthma in adults was 1.5 (7). These studies suggested that control of obesity is effective in improving the onset of asthma.

In the current study exploring the association between obesity and asthma, body mass index (BMI) was used to measure whether participants developed obesity (8, 9). There is, however, some evidence that suggests that body mass index Z-scores may not be reliable (5). According to the World Health Organization, obesity affects adults with a body mass index (BMI) of 30kg/m^2. However, this body mass index may indicate different physiological or metabolic characteristics (10, 11). Currently, BMI is used mainly for assessing peripheral obesity and is considered only to be a rough indicator of obesity response (12). There is a relationship between visceral fat distribution and asthma development (13) and impaired lung function in adolescents and adults, whereas peripheral obesity is not associated with these outcomes. In addition, previous study also found that abnormal fat accumulation even in normal weight people also had a negative impact on lung function (14). This might be related to the fact that central obesity is more likely to activate the inflammatory state of the body. Previous studies demonstrated that obese individuals have higher levels of chronic inflammatory biomarkers such as C-reactive protein, interleukin 6, tumor necrosis factor-α, fibrinogen activator inhibitor 1, eosinophil chemotactic factor, and vascular endothelial growth factor (15). Compared to peripheral obesity, central obesity, which responds to the degree of visceral adiposity, was more likely to have an activated inflammatory state (16). Since magnetic resonance imaging (MRI) scans are the gold standard for assessing visceral adipose tissue (VAT), their cost limits their widespread use and it is difficult to repeat the procedure in the near future (17). As a result, there are still few studies linking visceral fat distribution to asthma.

Abbreviations: NHANES, National Health and Nutrition Examination Survey; BMI, body mass index; PIR, ratio of family income to poverty; NCHS, National Center for Health Statistics; CI, confidence interval; OR, odds ratio; MetS, metabolic syndrome; TG, Triglyceride; TC, Cholesterol; FPG, fasting plasma glucose.

In order to assess VAT, the concept of the metabolism score for visceral fat (METS-VF) was proposed (18). This concept has been shown to offer a better advantage to the (18) than BMI for disease development in multiple systems. However, the relationship between METS-VF and asthma has not been clearly demonstrated. The purpose of this study is to examine the association between METS-VF and asthma prevalence using data from the US Survey of Disease and Nutrition Examination (NHANES).

2 Materials and methods

2.1 Data source

The NHANES database was used for this study. NCHS is an agency of the Centers for Disease Control and Prevention of the Centers for Disease Control and Prevention that conducts NHANES annually to assess health, nutrition, and health behaviors among unstructured populations in the US. To obtain representative data, NHANES uses a multistage probability sampling design. As part of the implementation of the NHANES protocols, the NCHS Research Ethics Review Board reviewed and standardized all procedures in accordance with the Human Research Subject Protection Policy of the US Department of Health and Human Services (HHS). Each individual takes part in the survey. The National Health and Nutrition Examination Survey (NHANES) released all data for this study without additional authorization or ethics review.

2.2 Study population

A total of nine NHANES survey cycles (2001-2018) were selected for cross-sectional studies in this study. In the survey, 91,352 people participated and only adults were surveyed (n = 41,150), so minors aged 20 and under were excluded (n = 91,352). We also excluded participants with the following missing information, which included METS-VF (n=12514), education (n=39), marital (n=14), hypertension (n=130), diabetes (n=24), smoking (n=17), activity (n=18), asthma (n=30), coronary heart disease (n=135), cancer (n=32), and serum uric acid (n=1). The final studies included 36,876 participants, including 4,919 participants with self-reported asthma. As shown in Figure 1, the exclusion criteria apply.

2.3 Data collection and definition

Metabolic Score Visceral Fat (METS-VF) is a measure of exposure to adipose tissue. METS-VF is a visceral fat metabolism score combining insulin resistance index (METS-IR), waist to height ratio (WHtR), age and sex.WHtR=WC(cm)/HT(cm), METS-IR= $\mathrm{Ln}((2\times \mathrm{GLU})+\mathrm{TG})\times \mathrm{BMI})/(\mathrm{Ln}(\mathrm{HDL\text{-}C}))$.METS-VF=4.466 + $0.011[(\mathrm{Ln}(\mathrm{METS\text{-}IR}))^3]+3.329[(\mathrm{Ln}(\mathrm{WHtR}))^3]+0.319$ (sex)+0.594(Ln(age))(where GLU is expressed in mg/dL, TG in mg/dL, BMI in kg/m^2, HDL-C in mg/dL, Age in years, and sex was a

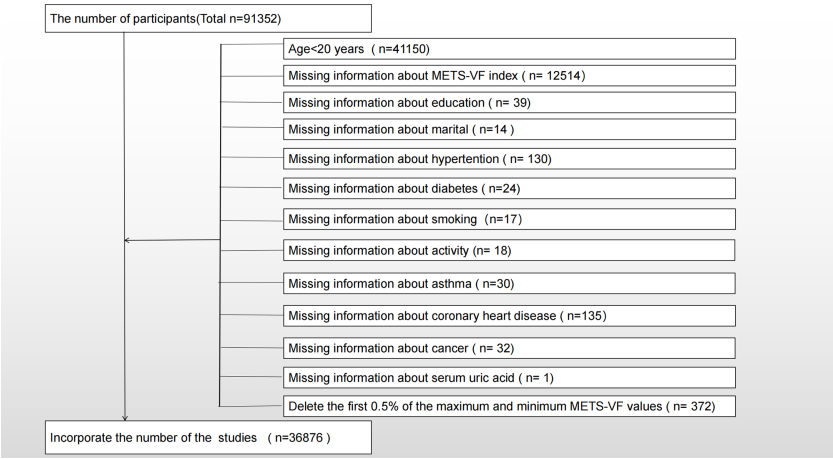

FIGURE 1
Flow chart for participants.

binary response variable (men=1, women=0)).The concentrations of triglycerides and fasting blood glucose were determined enzymatically by using an automated biochemical analyzers. Chemical analyzers Roche Cobas 6000 and Modular P were used to measure serum triglyceride concentrations. According to the questionnaire, they were asked "Ever been told you have asthma?" to determine if they had asthma. The occurrence of asthma was designed as the outcome variable.

Multivariate-adjusted models summarize potential covariates that might confound the METS-VF index's association with asthma (19). Covariates in our study included gender (male/female), age (years), ethnicity, education level, poverty income ratio (PIR), marital status (married or living with a partner/single), alcohol consumption (drinking alcohol or not), physical activity (vigorous/moderate/below moderate), cholesterol level (mg/dl), uric acid level (mg/dl), smoking status (smoking or not), hypertension (whether or not), diabetes mellitus (whether or not), coronary heart disease (whether or not), cancer (whether or not), and dietary intake factors. Including energy intake, fat intake, sugar intake, and water intake. Besides 2001-2002, all participants had two 24-hour dietary recalls during the remaining years. Our analysis will factor in the average consumption rate for these two recalls. A detailed description of measurement procedures for the study variables can be found at www.cdc.gov/nchs/nhanes/.

2.4 Statistical methods

A multistage sampling design employed in selecting a representative non-institutionalized US population was illustrated through the application of NHANES sampling weights, stratifications, and clustering in all statistical analyses. Using the weights provided by the dataset, the "survey design" R package in the R language was developed to explain NHANES' complex multistage stratified sampling technique. Categorical variables were presented as weighted survey means and 95% CIs, while continuous variables were presented as weighted survey means and 95% CIs. An analysis of continuous variables was conducted using survey-weighted linear regression, and an analysis of categorical variables was carried out with a survey-weighted chi-square test. Based on the guidelines (20), multiple logistic regression models were used to compare the prevalence of METS-VF index, different three-tier arrays of METS-VF index, and asthma between the three models. Covariates were not adjusted in Model 1. Ethnicity, marital status, and educational level were adjusted in Model 2. Except for age and sex (whose values are calculated into the METS-VF index, they are not adjusted in Model 3), all variables were adjusted. To further evaluate the relationship between the METS-IR index and asthma prevalence, smoothing curve fitting (penalized spline method) and generalized additive model (GAM) regression were used. When nonlinear relationships are detected, inflection points(the most significant difference in effect before and after a specific METS-VF value) are determined by the like natural ratio test. Next a multiple regression analysis was performed stratified by sex, age, race, hypertension, diabetes and whether relatives had asthma. A $p < 0.05$ was considered statistically significant. A combination of Empower software, available at www.empowerstats.com (X&Y Solutions, Inc., Boston, MA) and R version 4.0.2 was used to conduct analyses (http://www.r-project.org, Theon).

3 Results

3.1 Participant characteristics

Ultimately, 36876 participants participated in this study, including 4919 participants who self-reported asthma. The median age of asthmatics was lower and a higher proportion of participants were female. Asthmatic participants had a higher METS-VF compared to non-asthmatic participants. Results of participant baseline characteristics are shown in Table 1.

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TABLE 1 Baselines characteristics of participants, weighted.

Characteristic	Non-asthma formers (n=31957)	Asthma formers (n=4919)	P-value
Age(years)	47.04 (46.63,47.44)	44.51 (43.86,45.15)	<0.0001
Serum Cholesterol(mg/dl)	196.92 (196.07,197.77)	194.70 (193.19,196.21)	0.0042
Serum Uric Acid(mg/dl)	5.40 (5.38,5.43)	5.39 (5.34,5.43)	0.4898
METS-VF	5.90 (5.89,5.92)	6.08 (6.04,6.11)	<0.0001
Gender(%)			<0.0001
Male	50.08 (49.49,50.66)	41.50 (39.84,43.19)	
Female	49.92 (49.34,50.51)	58.50 (56.81,60.16)	
Race(%)			<0.0001
Mexican American	14.00 (12.43,15.74)	10.57 (9.04,12.32)	
White	68.94 (66.66,71.13)	70.78 (68.10,73.33)	
Black	10.31 (9.21,11.54)	12.04 (10.61,13.64)	
Other Race	6.75 (6.11,7.45)	6.61 (5.63,7.73)	
Education Level(%)			0.0126
Less than high school	20.57 (19.51,21.67)	18.46 (16.66,20.40)	
High school	29.00 (28.07,29.95)	28.24 (26.33,30.24)	
More than high school	50.43 (48.97,51.89)	53.30 (51.23,55.36)	
Marital Status(%)			<0.0001
Cohabitation	65.72 (64.68,66.75)	59.23 (57.29,61.14)	
Solitude	34.28 (33.25,35.32)	40.77 (38.86,42.71)	
Alcohol(%)			0.5013
Yes	62.59 (61.09,64.06)	63.64 (61.65,65.58)	
No	20.50 (19.20,21.87)	19.67 (18.03,21.42)	
Unclear	16.91 (16.06,17.80)	16.69 (15.26,18.23)	
High Blood Pressure(%)			<0.0001
Yes	29.34 (28.45,30.25)	34.33 (32.51,36.20)	
No	70.66 (69.75,71.55)	65.67 (63.80,67.49)	
Diabetes(%)			0.0052
Yes	8.12 (7.73,8.54)	9.53 (8.56,10.59)	
No	91.88 (91.46,92.27)	90.47 (89.41,91.44)	
Smoked(%)			0.0001
Yes	45.70 (44.64,46.76)	49.80 (47.67,51.94)	
No	54.30 (53.24,55.36)	50.20 (48.06,52.33)	
Physical Activity(%)			0.6511
Never	29.00 (28.08,29.93)	28.22 (26.60,29.91)	
Moderate	32.03 (31.27,32.80)	32.62 (30.85,34.43)	
Vigorous	38.97 (37.97,39.98)	39.16 (37.08,41.27)	
Blood relatives had asthma(%)			<0.0001
Yes	17.83 (17.22,18.46)	40.44 (38.58,42.33)	

(Continued)

TABLE 1 Continued

Characteristic	Non-asthma formers (n=31957)	Asthma formers (n=4919)	P-value
No	80.35 (79.69,80.98)	56.17 (54.22,58.09)	
Unclear	1.82 (1.65,2.01)	3.39 (2.80,4.11)	
Coronary Artery Disease(%)			0.0194
Yes	3.31 (2.99,3.66)	4.23 (3.49,5.11)	
No	96.69 (96.34,97.01)	95.77 (94.89,96.51)	
Cancers(%)			0.0009
Yes	8.95 (8.50,9.43)	11.15 (9.91,12.52)	
No	91.05 (90.57,91.50)	88.85 (87.48,90.09)	
PIR(%)			<0.0001
<1.3	18.58 (17.61,19.59)	24.10 (22.32,25.98)	
≥1.3<3.5	33.53 (32.46,34.62)	31.49 (29.39,33.67)	
≥3.5	41.42 (39.88,42.98)	38.40 (35.82,41.06)	
Unclear	6.46 (5.93,7.04)	6.00 (5.08,7.08)	
Total Kcal(%)			0.4393
Lower	40.50 (39.69,41.31)	40.10 (38.24,41.99)	
Higher	47.72 (46.78,48.66)	47.20 (45.06,49.34)	
Unclear	11.78 (11.12,12.48)	12.70 (11.31,14.24)	
Total Sugar(%)			0.2664
Lower	38.78 (37.99,39.58)	37.33 (35.67,39.01)	
Higher	40.00 (39.16,40.86)	40.62 (38.88,42.38)	
Unclear	21.22 (20.49,21.96)	22.06 (20.57,23.62)	
Total Water(%)			0.1938
Lower	40.74 (39.88,41.59)	41.55 (39.54,43.59)	
Higher	47.48 (46.52,48.44)	45.75 (43.53,47.98)	
Unclear	11.78 (11.12,12.48)	12.70 (11.31,14.24)	
Total Fat(%)			0.3428
Lower	40.01 (39.20,40.82)	40.17 (38.29,42.07)	
Higher	48.21 (47.30,49.11)	47.13 (45.05,49.22)	
Unclear	11.78 (11.12,12.48)	12.70 (11.31,14.24)	

For continuous variables: survey-weighted mean (95% CI), P-value was by survey-weighted linear regression (svyglm).
METS-VF, Metabolism score for visceral fat; PIR, Poverty income ratio.

3.2 Asthma prevalence was associated with a higher METS-VF index

There was a positive association between METS-VF and asthma prevalence in all models, and this positive association remained stable after adjusting for all covariates (OR = 1.27, 95% CI: 1.22,1.32). Furthermore, when METS-VF was grouped by tertiles, we found that this positive association remained and became more pronounced with increasing METS-VF (P for trend <0.01) (Table 2). An analysis of smooth curve fitting was conducted in order to clarify if there is a nonlinear relationship between METS-VF and asthma prevalence. The results suggested that there is a significant nonlinear relationship (Figure 2). Further, we conducted a threshold effect analysis, and we found that there are two more inflection points before the effective inflection point of 6.9, namely 5.24 and 5.33. After all inflection points, the linear model of METS-VF before and after all inflection points showed a positive correlation with asthma prevalence. Interestingly, there was a negative relationship between METS-VF and asthma prevalence when METS-VF was less than 5.24 (OR = 0.60, 95% CI: 0.33,1.09),

TABLE 2 Logistic regression analysis between METS-VF index with asthma prevalence.

Characteristic	Model 1 OR(95%CI)	Model 2 OR(95%CI)	Model 3 OR(95%CI)
METS-VF Index	1.24 (1.20, 1.28)	1.24 (1.20, 1.28)	1.27 (1.22, 1.32)
Tertiles of METS-VF			
Tertile 1 (4.62-5.25)	1	1	1
Tertile 2 (5.26-6.54)	1.17 (1.08, 1.26)	1.19 (1.10, 1.29)	1.18 (1.09, 1.28)
Tertile 3 (6.55-7.78)	1.55 (1.44, 1.67)	1.56 (1.45, 1.68)	1.61 (1.48, 1.75)
P for trend	< 0.01	< 0.01	< 0.01

Model 1 was adjusted for no covariates;
Model 2 was adjusted for race, marital status and education;
Model 3 was adjusted for covariates in Model 2+diabetes,blood pressure, PIR, total water, total kcal, total sugar, total fat, smoked, physical activity, alcohol use, serum cholesterol, serum uric acid, coronary artery disease, blood relatives had asthma and cancers were adjusted.
METS-VF: Metabolism score for visceral fat.

but the statistical difference was not significant. Once METS-VF exceeded 5.24, all positive correlations between METS-VF and asthma prevalence were stable (Table 3).

3.3 Subgroup analysis

The results of the subgroup analysis suggested that the positive association between METS-VF and asthma prevalence was stable in all populations with different characteristics. And the more significant population characteristics were men (OR = 1.79, 95% CI: 1.20, 2.68), 40-59 years old (OR = 1.35, 95% CI: 1.26, 1.44), Mexican-American (OR = 1.41, 95% CI: 1.28, 1.54), Hypertension (OR = 1.30, 95% CI: 1.23, 1.38), Diabetes mellitus (OR = 1.36, 95% CI: 1.23, 1.50) and the next relative had no history of asthma (OR = 1.29, 95% CI: 1.23, 1.36) (Table 4, Figure 3).

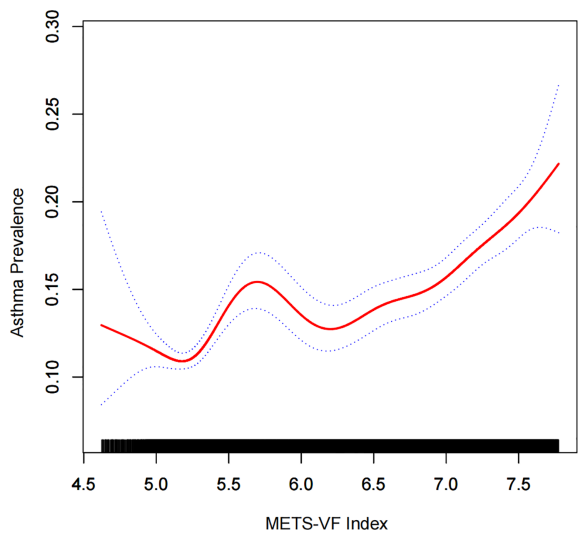

FIGURE 2
Density dose-response relationship between METS-VF index with asthma prevalence. The area between the upper and lower dashed lines is represented as 95% CI. Each point shows the magnitude of the METS-VF index and is connected to form a continuous line. Adjusted for all covariates except effect modifier. METS-VF: Metabolism score for visceral fat.

4 Discussion

This is the first cross-sectional study to assess the association between METS-VF and asthma prevalence, based on a representative sample of US adults. The prevalence of asthma and METS-VF is positively correlated, but the relationship isn't linear. Although there was a trend of negative correlation between METS-VF and asthma prevalence below 5.24, this trend was not statistically different on the premise that this study had sufficient samples.

Asthma is a chronic disease that affects the respiratory system, with the development of medical science, the incidence of asthma has not improved optimistically, and the disease is still closely related to global health burdens (21). Research and clinical staff have always had difficulty doing the primary prevention of asthma compared to timely and effective treatment (22). The bidirectional relationship between obesity and asthma has been recognized (23), and obesity-related asthma has also emerged as a challenge for researchers. Obese patients with asthma were more likely to report continuous symptoms, miss more days of work, and use more medications, among other possibilities. In addition, obese asthmatics were less likely to be in asthma remission and more likely to have severe persistent asthma (24).. However, how to accurately assess the real situation of obesity has also become a puzzle. As mentioned earlier, the researchers recommended BMI as a rough indicator of obesity or overweight (12). The main limitations of BMI include the lack of distinction between fat mass and lean mass, and the lack of interpretation of local fat distribution patterns (16) of local fat distribution. The association between visceral fat abnormalities and worse lung function and inflammation in obesity-related asthma has been further recognized through increased research (25). Increased visceral fat is associated with higher levels of IL-6 (26, 27), and IL-6 disrupts the homeostasis of fatty acid metabolism, causing a state of active inflammation in the body (28, 29). Researchers have recognized a correlation between IL-6 and asthma development (29, 30). Based on the results of this study, the current prevailing view is that visceral fat is associated with a more active inflammatory state.

Additionally, we found that some participants with specific characteristics were more likely to show a positive relationship

TABLE 3 Subgroup analysis between METS-VF index with asthma prevalence.

Characteristic	Model 1 OR (95%CI)	Model 2 OR (95%CI)	Model 3 OR (95%CI)
Stratified by age(years)			
20-39	1.19 (1.12, 1.26)	1.24 (1.17, 1.32)	1.24 (1.15, 1.33)
40-59	1.42 (1.34, 1.51)	1.43 (1.34, 1.51)	1.35 (1.26, 1.44)
60-85	1.27 (1.20, 1.35)	1.28 (1.21, 1.36)	1.32 (1.24, 1.42)
Stratified by gender			
Male	1.69 (1.19, 2.41)	2.30 (1.61, 3.28)	1.79 (1.20, 2.68)
Female	1.24 (1.17, 1.32)	1.27 (1.19, 1.35)	1.26 (1.17, 1.36)
Stratified by race			
Mexican American	1.38 (1.28, 1.49)	1.40 (1.29, 1.51)	1.41 (1.28, 1.54)
White	1.22 (1.16, 1.28)	1.21 (1.15, 1.27)	1.24 (1.17, 1.31)
Black	1.21 (1.13, 1.29)	1.19 (1.11, 1.27)	1.20 (1.11, 1.30)
Other Race	1.28 (1.13, 1.45)	1.27 (1.13, 1.44)	1.36 (1.18, 1.57)
Stratified by hypertension			
Yes	1.27 (1.21, 1.34)	1.26 (1.20, 1.33)	1.30 (1.23, 1.38)
No	1.17 (1.12, 1.22)	1.18 (1.13, 1.24)	1.25 (1.18, 1.31)
Stratified by diabetes			
Yes	1.31 (1.20, 1.42)	1.32 (1.21, 1.43)	1.36 (1.23, 1.50)
No	1.22 (1.17, 1.26)	1.22 (1.17, 1.26)	1.25 (1.20, 1.31)
Stratified by blood relative had asthma			
Yes	1.19 (1.13, 1.26)	1.19 (1.13, 1.26)	1.22 (1.14, 1.30)
No	1.22 (1.17, 1.28)	1.22 (1.17, 1.28)	1.29 (1.23, 1.36)
Unclear	1.05 (0.87, 1.27)	1.08 (0.89, 1.31)	1.25 (1.01, 1.57)

Model 1=no covariates were adjusted.
Model 2=Model 1+race, marital status and education were adjusted.
Mode3=adjusted for all covariates except effect modifier.

between METS-VF and asthma prevalence. Men, for instance, are more troubled by this correlation than women. There are many factors that contribute to the global obesity rate among women, including diet, occupation, activity, and other factors (31). However, the body mass index (BMI) is undoubtedly one measure used. Considering the results we have currently obtained, we believe that differences in sex hormones may have led to differences in fat metabolism and distribution patterns. Previous studies have shown that the interaction between reduced testosterone levels and abdominal obesity in men, while obese

TABLE 4 Two-piecewise linear regression and logarithmic likelihood ratio test explained the threshold effect analysis of METS-VF index with asthma prevalence.

METS-VF Index	ULR Test	PLR Test	LRT test
	OR (95%CI)	OR (95%CI)	P value
<5,24	1.66 (1.15, 2.40)	0.60 (0.33, 1.09)	<0.0001
≥5,24		6.50 (3.12, 13.55)	
<5.53	1.21 (1.13, 1.30)	1.68 (1.27, 2.23)	0.018
≥5.53		1.07 (0.95, 1.21)	
<6.9	1.27 (1.22, 1.32)	1.20 (1.13, 1.27)	0.008
≥6.9		1.66 (1.36, 2.02)	

ULR, univariate linear regression; PLR, piecewise linear regression; LRT, logarithmic likelihood ratio test, statistically significant: p<0.05.

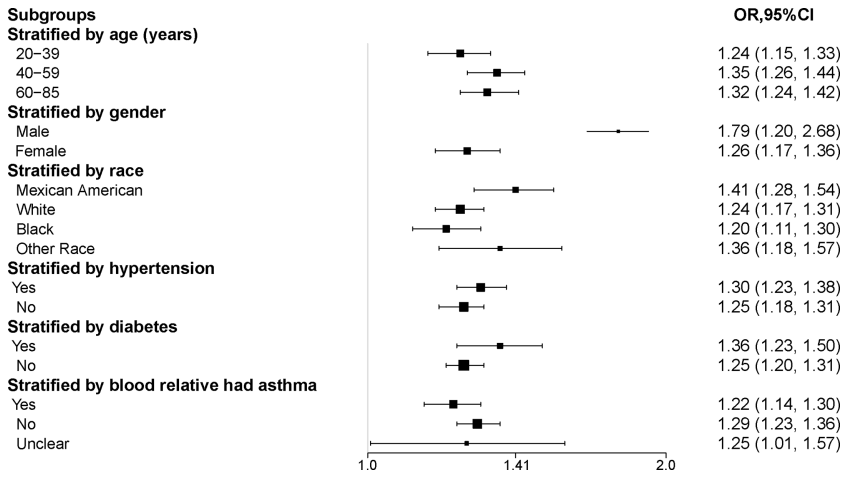

FIGURE 3
Subgroup analysis of the association between METS-VF and asthma. All the covariates in Table 1 were adjusted. In the subgroup analysis stratified by each covariate, the model is not adjusted for the stratification variable itself. METS-VF, Metabolism score for visceral fat.

women show androgen excess (32). A woman's fat distribution is mainly located in her thighs, buttocks, chest, and other peripheral areas because of estrogen (33). In terms of asthma alone, its prevalence also differs between genders. Chowdhury NU mentioned in their review that as children, the prevalence of asthma increased in boys, while in adulthood, the prevalence and severity of asthma increased in females, and that changes in sex hormones were a factor in the variations in the prevalence of asthma by gender (34). In addition, the metabolic syndrome consisting of a series of metabolic abnormalities (such as hypertriglyceridemia and low high-density lipoprotein (HDL) cholesterol) increased in obese patients, especially Mexican American (35), which could also explain the increased susceptibility of the positive association between MEETS-VF and asthma prevalence in the subgroup analysis.

Its strength lies in the fact that it is the first cross-sectional study to examine the relationship between visceral fat distribution and asthma prevalence, and the sample size is sufficient and representative. It is also important to note that our study has some limitations as well. First of all, cross-sectional studies cannot provide a causal explanation, and whether there is a causal relationship between METS-VF and asthma and whether this causal relation is unidirectional or bidirectional still needs to be determined by further research. A second limitation of this study was that asthma was diagnosed using participants' self-reports, which had unavoidable recall bias, so subsequent prospective studies are needed. Third, the potential influencing factors of asthma and METS-VF are numerous, and although we included as many relevant covariates as possible in the model, there is still no guarantee to exclude the effects of other potential covariates. Finally, based on the results of the smoothed curve fit, there was a negative association between METS-VF and asthma when it was less than 5.24 (no statistically significant difference). The plausibility of this result still needs to be worthy of continued investigation. Since METS-VF as a new index lacks a clear range of normal values, we cannot tell whether individuals with METS-VF less than 5.24 are obese or lean based on previous studies. However, it would be worthwhile to confirm whether the number of participants with METS-VF less than 5.24 is sufficient based on the results of smoothing curve fitting and whether it is due to other potential confounding factors. Despite these limitations, we believe that this study demonstrated a positive association between increased METS-VF and asthma prevalence.

5 Summary

An increase in the METS-VF index is associated with an increase in the incidence of asthma. The hypothesis is that treatment and management of obesity at a young age may delay, ameliorate or reduce the onset of asthma, but a causal relationship cannot be clearly established, but this is of clinical concern nonetheless.

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 author.

Ethics statement

The NCHS Research Ethics Review Committee approved the NHANES survey protocol, and all participants of the study provided informed written consent. The NHANES database is open to the public and therefore the ethical review of this study was exempt.

Author contributions

Data analysis and manuscript writing: QL, LH. Study design and statistical advice: QL, XH. Manuscript editing: XH, YC, LH.

Validation and review: YC, YG, WY. Quality control: LH. All authors agreed on the journal to which the article was to be submitted and agreed to take responsibility for all aspects of the work. All authors contributed to the article and approved the submitted version.

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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

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Hepatokines, bile acids and ketone bodies are novel Hormones regulating energy homeostasis

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Current views show that an impaired balance partly explains the fat accumulation leading to obesity. Fetal malnutrition and early exposure to endocrine-disrupting compounds also contribute to obesity and impaired insulin secretion and/or sensitivity. The liver plays a major role in systemic glucose homeostasis through hepatokines secreted by hepatocytes. Hepatokines influence metabolism through autocrine, paracrine, and endocrine signaling and mediate the crosstalk between the liver, non-hepatic target tissues, and the brain. The liver also synthetizes bile acids (BAs) from cholesterol and secretes them into the bile. After food consumption, BAs mediate the digestion and absorption of fat-soluble vitamins and lipids in the duodenum. In recent studies, BAs act not simply as fat emulsifiers but represent endocrine molecules regulating key metabolic pathways. The liver is also the main site of the production of ketone bodies (KBs). In prolonged fasting, the brain utilizes KBs as an alternative to CHO. In the last few years, the ketogenic diet (KD) became a promising dietary intervention. Studies on subjects undergoing KD show that KBs are important mediators of inflammation and oxidative stress. The present review will focus on the role played by hepatokines, BAs, and KBs in obesity, and diabetes prevention and management and analyze the positive effects of BAs, KD, and hepatokine receptor analogs, which might justify their use as new therapeutic approaches for metabolic and aging-related diseases.

KEYWORDS

bile acids, fasting, GPBAR1, hepatokines, ketogenic diet

1 Introduction

In humans and other large mammals, energy homeostasis depends on several complex pathways that control the balance between energy intake and energy expenditure. For a long time, obesity was considered the effect of an imbalance between energy expenditure and intake, but actual data support a much more complex picture. The "dogma" of impaired balance explains only part of the mechanisms responsible for body fat excess.

Growing evidence indicates that fetal malnutrition (1, 2), early exposure to endocrine-disrupting chemicals (EDC) (3–6), and early exposure to EDC contribute to obesity, visceral fat accumulation (3, 7–11), and insulin resistance (10, 12, 13).

Involved mechanisms include gene-environment crosstalk (2, 14, 15) and epigenetic changes of DNA methylation (16), histone acetylation/deacetylation, and non-coding mRNA (16). The early and/or prolonged exposure to an "obesogenic" environment, together with environmental factors (2, 17) activates pathways that lead to inflammation and immune system dysfunction involved in chronic low-grade inflammation and insulin resistance (18, 19). Insulin resistance occurs when insulin binding to its receptor is not followed by (1) adequate glucose uptake into target tissues such as the skeletal muscle and adipose tissues (2), suppression of glucose production by the hepatocytes, and/or (3) decreasing of adipose tissue lipolysis (20).

In the present review, we will focus on possible roles played by the liver as a source of molecules playing endocrine function and regulating energy homeostasis.

2 The role of the liver in hepatokines, ketone bodies, bile acids synthesis, and metabolism

The liver plays a major role in systemic glucose homeostasis. It senses nutrient availability and increases or decreases glucose production and glycogen storage in the transition from fasting to the post-prandial phase. After an overnight fast, glucose supply to the brain and muscles is ensured by increased hepatic glycogenolysis and gluconeogenesis (21, 22). By contrast, in the post-prandial phase, hepatic glucose production is reduced and hepatic glucose uptake is increased (enhanced glycogen-synthesis and the novo lipogenesis) because circulating levels of glucose are sufficient for the energy needs of non-hepatic organs (23, 24).

In humans and other mammalian species, the liver is also the main site of the production of bile acids (BAs), ketone bodies (KB), and lipids (Figures 1, 2). The liver supplies KB derived by lipids oxidation and very low-density lipoproteins (VLDL) to target tissues.

During short-term fasting, mammalian species produce and use KB as a surrogate fuel to compensate for the decrease in carbohydrate (CHO) availability and the increase in fatty acid availability (28, 29). In prolonged fasting, the brain utilizes KB as an alternative to CHO. In prolonged energy deprivation, either eukaryotes or archea and bacteria uses KB (28).

Hepatokines are proteins secreted by the hepatocytes. They influence metabolism through autocrine, paracrine, and endocrine signaling and are the main mediators in the crosstalk between the liver, non-hepatic target tissues, and the brain. Hepatocytes secrete more than 500 hepatokines (30).

Many hepatokines have been linked to the induction of metabolic dysfunction. Altogether, hepatokines represent the hepatocyte secretome, which is consistently modified in

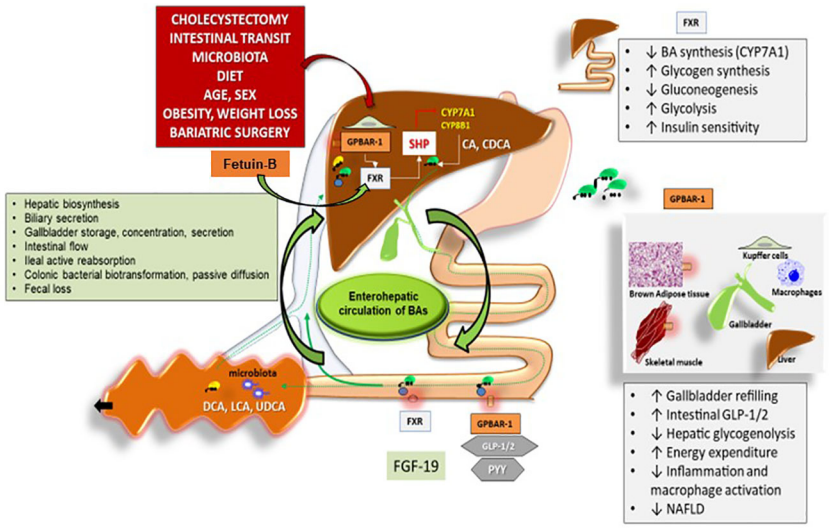

FIGURE 1
Interaction between hepatokines and BAs in nutrient and energy homeostasis. Legend for Figure 1 Overall events involved in the function of bile acids (BAs) acting as signaling molecules and receptor ligands of farnesoid X receptor (FXR) and the G protein–coupled receptor 1 (GPBAR–1). In the left box, the steps involved in BA biosynthesis, secretion, storage, intestinal flow, absorption, colonic biotransformation and fecal loss are listed, as part of the enterohepatic circulation of BAs. The location of FXR and GPBAR–1 (TGR5) are shown in the liver, intestine, and several other tissues with respect to the control of BAs on: 1. hepatic biosynthesis of primary BAs *via* the rate–limiting enzyme 7a–hydroxylase (CYP7A1) and CYP8B1 controlled negatively by the small heterodimer partner (SHP): cholic acid (CA) and chenodeoxycholic acid (CDCA), 2. intestinal release of FXR–mediated secretion of the fibroblast growth factor 19 (FGF19, known as FGF15 in mice), which circulates to the liver and reduces the expression of CYP7A1 to inhibit hepatic BA synthesis through FXR, 3. Intestinal GPBAR–1–mediated release of peptide YY (PYY), glucagon–like peptide 1 (GLP–1) and glucagon–like peptide 2(GLP–2). Recent studies show that Fetuin–B is playing additional roles through its interaction with Farnesoid X Receptor (FXR). In studies utilizing microarray technology Fetuin–B acts as a FXR agonist–regulated gene. Several metabolic functions (boxes on the right). Adapted from 25 modified by Garruti et al. for Front Endocrinol.

pathological conditions. In liver steatosis, increased intrahepatic depots of triglycerides are associated with changes in transcription and endoplasmic reticulum (ER) folding and the transport of some hepatokines and are ultimately causing the increase in secretion of some of them and the decrease in secretion of some others. This imbalance in hepatokines levels is associated with insulin resistance, glucose intolerance, ectopic lipid accumulation, inflammation, and impaired insulin secretion. Robust data indicate that Fetuin A, Fetuin B, Retinol-binding protein 4 (RBP4), and Selenoprotein P are key players in metabolic dysfunction (31, 32). The present review will focus on some of the hepatokines playing a role in obesity, diabetes, and non-alcoholic fatty liver disease (NAFLD) not only in animal models but also in humans.

3 Hepatokines

In this section, we will consider some of the hepatokines already studied in humans that are related to obesity, insulin resistance, and/or diabetes, and NAFLD. Angiopoietin-like proteins (ANGPTL) are eight subtypes of glycoproteins secreted by the liver. They share with the angiopoietin proteins the same C-terminal fibrinogen-like domain and the N-terminal coiled-coil domain (33). ANGPTL3 is one of the most studied ANGPTLs in humans. The liver is the only site of ANGPTL3 synthesis. Cohorts of subjects with loss of function alleles for ANGPTL3 show reduced circulating levels of both triglycerides (TGL) and cholesterol (LDL and HDL) (26, 34). Similar findings exist in knockout mice for ANGPTL3 that exhibit reduced blood levels of TGL and free fatty acids because of the increased activity of LPL (35, 36). The interaction between ANGPTL3 and LPL induces the dissociations of LPL dimers to monomers. The transformation of the active to the inactive LPL accounts for the enhanced deposition of FFA derived by lipoproteins into white adipocytes (37). Interestingly, the liver X receptor (LXR) upregulates ANGPTL3. By contrast insulin, peroxisome proliferator-activated receptor (PPAR) beta and leptin downregulate it. Some recent studies indicate that thyroid hormones and statins are also negatively regulating ANGPTL3 and might explain its effects on lipid and CHO metabolism (38, 39).

Evinacumab is a synthetic ANGPTL3 agonist. In recent studies, combining Evinacumab with either statins or PCSK inhibitors, rodents showed a more significant improvement in hyperlipidemia compared with statins alone (40).

ANGPTL3 is not only regulating lipid patterns but is also involved in glucose homeostasis. In insulin-resistant subjects, ANGPTL3 levels directly correlated with HOMA-IR as well as with glucose and insulin circulating levels (41).

ANGPTL6 is expressed in different tissues at very low levels but hepatocytes are the major source of its secretion. In animal models, overexpression of ANGPTL6 is associated with increased energy expenditure, protection of hepatic steatosis, resistance to high-fat diet-induced overweight, and increased insulin sensitivity compared with controls. By contrast, knockout mice for ANGPTL6 show an increased incidence of obesity, insulin resistance, and increased fat content in peripheral organs (especially the liver and skeletal muscle) (42). Another effect of ANGPTL6 on glucose metabolism is represented by the reduced expression in glucose-6 phosphatase and the following decrease in gluconeogenesis (43, 44). Most recent data in humans indicate that subjects with obesity and/or type 2 diabetes have higher blood levels of ANGPTL6 compared with controls (45).

In both humans and mice, fasting–induced adipose factor (FIAF) is another very important hepatokine. It is mainly expressed in adipocytes and hepatocytes, but it might also be detected in cardiomyocytes and skeletal muscle myocytes (46, 47). It is also known as ANGPTL4. Fasting was the first condition known to induce a robust increase in hepatic levels of mRNA for FIAF while refeeding was able to suppress FIAF expression (48). It stimulates adipose tissue lipolysis, inhibits LPL activity, and blocks the clearance of triglyceride–rich lipoproteins. These mechanisms account for the increase in plasma FFA and triglycerides (49, 50). During food deprivation, there is a decrease in LPL concentrations in adipose tissue capillaries and a prevailing triglyceride uptake in oxidative target tissues (myocytes of skeletal muscle) (51). Very recently, research has demonstrated that FIAF expression increases during exercise, and triglyceride uptake is shifted from the adipose tissue to the muscle with the same mechanism activated during fasting (52). Contrasting results exist on FIAF circulating levels in obesity and type 2 diabetes (53). In knockout mice for FIAF, a high–fat diet is associated with a higher increase in visceral fat compared with controls (54). The phenotype of mice with the overexpression of FIAF is interesting and characterized by liver steatosis but there is also a better insulin sensitivity in the liver and other major target tissues (55). FIAF is not only playing a role in energy homeostasis but is involved in angiogenesis and cancer cell infiltration.

Fetuins are abundant fetal serum α–globulins that belong to the cystatin family of cysteine protease inhibitors (56). The α2–Heremans and Schmid glycoprotein (AHSG) are also known as Fetuin–A. Human Fetuin–B (382 amino acids) shares 22% sequence similarity with Fetuin–A. Fetuin–A is coded by the AHSG gene and Fetuin–B by the Fetub gene. Fetuin–A was first described because of its role in osteogenesis and the inhibition of vascular calcification and bone reabsorption (57, 58). Unexpectedly, Fetuin–A might also negatively regulate the activity of the insulin–receptor tyrosine kinase in the liver, adipose tissue, and skeletal muscles (59–61). Fetuin–B is also able to inhibit calcium phosphate precipitation, but since in humans its circulating levels are low, its role in osteogenesis and bone reabsorption seems limited. In wild–type mice, Fetuin–B inhibits the activity of ovastacin, a metalloproteinase, which is responsible for the hardening of the zona pellucida. By contrast, female Fetuin–B deficient mice are infertile because of the enhanced activity of ovastacin (62).

Recent studies show that Fetuin–B plays an additional role through its interaction with Farnesoid X Receptor (FXR or NR1H4). In studies utilizing microarray technology, Fetuin–B acts as an FXR agonist–regulated gene (Figure 1). FXR belongs to a nuclear receptor family, is mainly activated by bile acids (BAs), and is abundantly expressed in the adrenal gland, intestine, liver, and kidney (63, 64). Recent data support the role of Fetuin–B in insulin resistance. Adult subjects with liver steatosis and/or type 2 diabetes have increased levels of Fetuin–B. In cell cultures of hepatocytes and myocytes, Fetuin–B impairs insulin activity. In

support of the insulin–resistant role of Fetuin–B, there are also animal studies showing that mice treated with Fetuin–B develop impaired glucose tolerance (31).

Recent studies demonstrate that Fetuin–A and adiponectin have opposite effects on insulin sensitivity. Adiponectin is a cytokine playing a role in chronic low–grade inflammation and positively correlates with insulin sensitivity (65). By contrast, Fetuin–A positively correlates with insulin resistance. Fetuin–A inhibits adiponectin and vice versa, adiponectin is a negative regulator of Fetuin–A through the AMPK signaling cascade. In subjects diagnosed with metabolic syndrome, the increased circulating levels of Fetuin–A might be due to the decreased expression of adiponectin (66). Studies on type 2 diabetic patients show that Fetuin–A concentrations are decreased by pioglitazone, as well as three months of reduced caloric intake causing a reduction in intra–abdominal body fat, arterial blood pressure levels, and fasting glycemia, together with an improvement in lipid patterns (67, 68).

Hepatocytes are the major site of synthesis and secretion of another hepatokine and fibroblast growth factor 21 (FGF21). FGF21 is defined as a fasting hormone, and it is a key mediator of lipid metabolism during fasting ketosis (69) (Figure 2). It is regulated by the nuclear receptor peroxisome proliferator–activated receptor alpha (PPARα). In high–fat–fed Rhesus macaque monkeys, FGF21 administration reduced body weight without changing energy intake (70). In mouse models of insulin–resistance treatment, FGF21 increased energy expenditure, reduced plasma circulating levels of glucose, improved liver steatosis, and improved both leptin and insulin sensitivity (71, 72). FGF21 binds the co–receptor b–klotho (KLB) and facilitates the formation of the FGF receptor 1/KLB complex, which is involved in the phosphorylation of ER1/2 (73). Recently, research demonstrated that FGF21 controls the expression of some genes involved in cellular aging and energy homeostasis like acetyl–Coa carboxylase (ACC1) and adipose triglyceride lipase. AMPK is the downstream protein of FGF21 and promotes lipolysis. However, FGF21 also regulates the expression of ACC1 and SREBP1c, thus inhibiting lipid synthesis. The anti–aging effects of FGF21 might also account for AMPK activation which plays a key role in the regulation of mTORC1 gene and NF–kB genes involved in autophagy and inflammation, respectively (74). FGF21 is considered a mediator of the transition from the fasted to the refed state because it stimulates insulin–mediated glucose uptake (32). In mice and humans, the maximal increase in plasma levels of FGF21 occurs after a high intake of simple CHO combined with low–protein intake (75). However, Schumann and co–workers demonstrated that FGF21 synthesis also increases after alcohol consumption (76). In subjects with obesity and/or type 2 diabetes and NAFLD, FGF21 circulating levels are higher than those measured in healthy age–matched subjects. The possibility exists that these metabolic conditions are characterized by FGF21–resistance and increased levels of FGF21 might be a

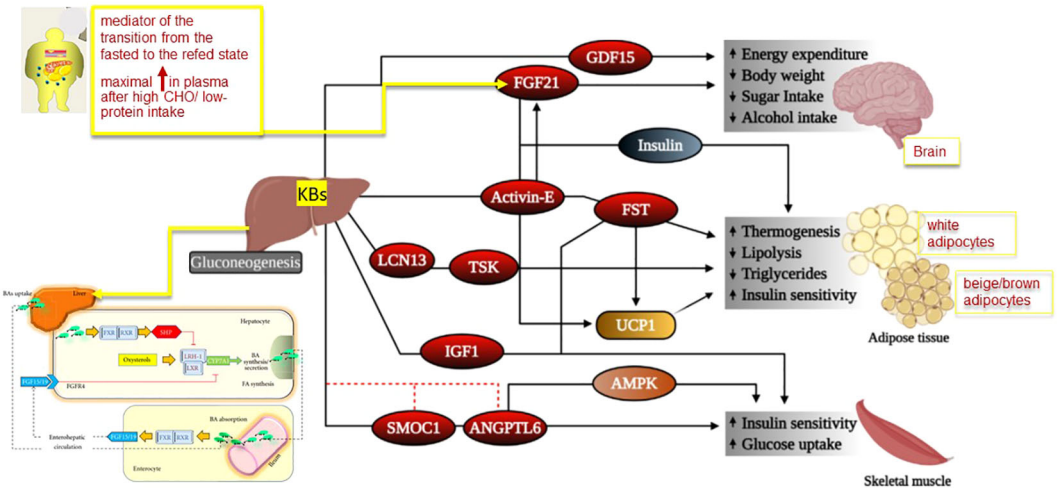

FIGURE 2
Mechanism for physiological effects of hepatokines in target tissues. Legend for Figure 2 Some hepatokines signal the brain to modulate body weight homeostasis and food intake, while others act on adipose tissues and muscle to regulate lipid and glucose homeostasis. However, liver is also the main site of production of bile acids (BAs). In the figure we depicted the potential molecular mechanisms of crosstalk between nuclear receptors LXR and FXR–SHP–LRH–1 regulatory cascade in the liver and intestine. Bile acids are the natural ligands for FXR, which regulates transcription by binding as a heterodimer with RXRs. This step results in increased SHP expression. SHP in turn inhibits LRH–1, preventing the activation of target genes that participate in bile acid and fatty acid synthesis. In the absence of bile acids, LRH–1 acts together with LXR to stimulate bile acid synthesis. The important pathways in the intestine that contribute to modulation of bile acid synthesis are also depicted. There is a bile–acid–mediated activation of intestinal FXR and, as a result, the release of FGF15 in the small intestine. The secreted FGF15 by the intestine circulates to the liver, likely through the portal circulation or lymph flow and induces the activation of FGFR4 in the liver. The FGF15/FGFR4 pathway synergizes with SHP in vivo to repress CYP7A1 expression. Abbreviations: AMPK, adenosine monophosphate–activated protein kinase, ANGPTL6, angiopoietin–like 6,BAs: bile acids, FGF: fibroblast growth factor, FGFR4: FGF receptor 4, FGF21, fibroblast growth factor 21, FST, follistatin, FXR: farnesoid X receptor, GDF15, growth differentiation factor 15, IGF1, insulin–like growth factor 1, LCN13, lipocalin 13, LRH–1: liver receptor homologue–1, LXR: liver X receptor, RXR: retinoid X receptors, SHP: short heterodimer partner, SMOC1, SPARC–related modular calcium–binding protein–1, TSK, Tsukushi, UCP1, uncoupling protein 1 Adapted from Reference 26 and 27 modified by Garruti et al. for Front Endocrinol.

compensatory mechanism (74). Some analogs of FGF21 already exist. Their administration to obese subjects with type 2 diabetes is associated with weight loss, a decrease in fasting insulin levels, and improvement in triglyceride circulating levels (74, 77). FGF21 analogs or FGF21 receptor agonists might represent an alternative treatment for NAFLD, obesity–associated type 2 diabetes, and other aging–associated metabolic diseases. FGF21 also plays an important role in the brain, involving potential effects in metabolic regulation, neuroprotection, and cognition (78).

Lipasin is an angiopoietin–like protein (ANGPTL8 or betatrophin). In humans and mice, it is expressed in the liver and adipose tissue. Food intake–mediated insulin secretion, FFA, and thyroid hormones regulate the levels of the expression of lipasin (79). Lipasin plays an anorectic effect by regulating the activity of NPY in the dorso–medial hypothalamus (80). It seems to negatively regulate glucose and lipid metabolism. Lipasin forms a complex with ANGPTL3 which robustly inhibits LPL. When ANGPTL4 is bounded to lipasin, ANGPTL4 loses its ability to inactivate LPL.

Selenoproteins are glycoproteins that are also secreted by the hepatocyte. Selenium is essential in balanced nutrition in humans and animal models. It was originally studied because of its role in thyroid diseases. Very recently, it was also found to play a role in fat and CHO metabolism. In the blood, selenium is bound to cysteine (selenocysteine) or proteins (selenoproteins). Selenoprotein P carries and donates selenium to peripheral target organs (81). Circulating levels of selenoprotein are positively regulated by selenium and glucose intake. Adiponectin and insulin reduce the expression of selenoprotein P (82, 83). Animal models helped to understand the role of selenoprotein in insulin resistance. The treatment of wild–type mice with selenoprotein P was associated with the appearance of impaired insulin sensitivity and impaired glucose tolerance. This treatment improved both insulin sensitivity and glucose tolerance in knockout mice for selenoprotein P (82). In patients diagnosed with type 2 diabetes, obesity or NAFLD serum levels of selenoproteins are higher compared with those measured in healthy subjects (83, 84). Prestigious studies show increased circulating levels of selenoprotein P associated with hypertriglyceridemia and insulin resistance (84).

4 Ketone bodies and ketogenic regimens

In mammals, several physiological conditions are characterized by the oxidation of ketone bodies to produce energy. In humans and other mammalian species, the liver is the major site of production of ketone bodies (KB). The liver diverts KB derived by lipids oxidation and very low–density lipoproteins (VLDL) to target tissues.

During short–term fasting, mammalian species might utilize KB as a surrogate supply to compensate for decreased CHO availability and increased fatty acid availability (28, 29). In prolonged fasting, the brain also utilizes KB as an alternative to CHO.

In healthy humans, the daily hepatic production of KB is around 300 grams. Prolonged starvation induces robust ketone body production and utilization, but KB production is also followed by intense exercise. In adults, normal circulating levels of KB range from 100 to 250 μM. After prolonged exercise or 24h food deprivation, KB increases 10 times (28), and in pathological conditions such as ketoacidosis, they might increase 200 times (28).

Studies in subjects undergoing ketogenic diets show that KBs are important mediators of inflammation and oxidative stress and might influence the post–translation re–arrangement of proteins (85, 86).

In the last 40 years, the ketogenic diet (KD) became a promising dietary intervention in some pathological conditions. The main outcome of this dietary strategy is to reach nutritional ketosis, but KD is usually assimilated to very low–carbohydrate diets where carbohydrates (CHO) are ranging from 5 to 10% of a 24h caloric intake independent of the total caloric intake (87, 88). It is not possible to establish a general cut–off level for CHO and kilocalories (Kcal) under which all individuals enter ketosis because this cut–off is subjective (89). In a high–fat ketogenic diet (HFKD), the daily CHO intake represents less than 50 g, but there are no limits for fat and caloric intake. HFKD was originally studied as a non–pharmacologic therapy in children and adolescents diagnosed with refractory epilepsy. Unfortunately, fatal cardiac arrhythmias (prolonged QT interval) and cardiomyopathy occurred in a few epileptic children treated with HFKD, supplying less than 800 kcal/day (VLCD) (90). More than 40 years ago, some retrospective studies concerning adult obese subjects assuming liquid–protein–modified fast diets poor in selenium reported some cases of sudden death associated with prolonged QT interval (91). Because of these sudden deaths, the European Food Safety Authority published precise guidelines stating that VLCKD should supply adequate selenium intake not less than 30 to 50 g/day of CHO but not more than 15 to 30% of fats for total kcal/day (92). In 2019, in a prospective cohort study, KD was associated with an increased risk of arrhythmia (atrial fibrillation). However, this KD was a high–fat/low–CHO diet (93).

A ketogenic diet with low CHO and high–fat content mimics the metabolic state of starvation. Recent trials considered a ketogenic diet as a therapeutic strategy in children with intractable epilepsy (94). Even if this therapeutic approach was successful in reducing epileptic crisis, it was also associated with thyroid dysfunction (95).

Most recent formulas of KD are represented by very low–calorie KD (VLCKD) supplying 400 to 800 kcal/day, but they are always supplying adequate selenium supplementation (96, 97). They are actually promising weight loss strategies for overweight/obese people. The majority of VLCKD is also known as protein–sparing modified fasting because they contain increased protein intake, mainly vegetable protein intake, which counterbalances the reduced CHO content (98).

The most intriguing observational studies analyzed the effects of ketone bodies on the myocardium. In mice, preliminary studies demonstrated that failing hearts consumed higher amounts of ketone bodies than healthy hearts (99). The same result was confirmed in humans (100–102). In experimental models of ischemia following reperfusion, KBs seem to be protective (103). Circulating levels of KBs were increased in subjects with heart failure, but the mechanisms involved are still unknown (104, 105).

Recently, the current treatment of type 2 diabetes with inhibitors of tubular sodium/glucose co–transporter–2 (GLT2i)

highlighted other important roles played by KBs. In both mice and humans, the inhibition of sodium/glucose co–transporter–2 at the level of the proximal tubules of the kidney was associated with an increase in liver ketogenesis, which accounted for the increased blood levels of KB (106–108). The treatment of type 2 diabetes with the new GLT2i is associated with a reduction in cardiovascular mortality and risk of hospitalization for heart failure. Therefore, GLT2i–induced ketosis might represent one of the beneficial effects of these drugs, together with reduced plasma volume due to osmotic diuresis, decreased body weight, reduced arterial blood pressure levels, and improvement in sympathetic nervous system activity, glycemia, and hyperuricemia (109–111).

5 Bile acids are nutrient signaling and thermogenic hormones

Bile acids (BAs) are the major lipid components of bile. The liver synthetizes BAs from cholesterol and secretes them into bile (Figure 1). The gallbladder (GB) represents the physiologic storage compartment of BAs. After food consumption, the entero–hormone cholecystokinin (CCK) stimulates GB to release bile into the duodenum where the BAs–mediated digestion and absorption of fat–soluble vitamins and lipids occurs (112, 113). Cyclic changes in serum BAs concentrations accompany the fasting to fed transition. In healthy subjects, fasting serum BAs concentrations are 0.2 to 0.7 μM, and after meals, they reach 4 to 5 μM (114–116). One part of the BA is re–absorbed in the ileum and returned to the liver through the portal vein. The recycling of BAs is a negative feedback regulatory mechanism that inhibits further hepatic BAs synthesis. Another part of BA escapes from intestinal reabsorption, enters the colon, and gives rise to secondary BA by the resident gut microbiota (25).

Recent studies unequivocally pointed out that bile is not simply a fat emulsifier. BAs indeed represent signaling molecules regulating different metabolic pathways involving the nuclear receptors farnesoid X receptor (FXR), pregnane X receptor (PXR), vitamin D receptor (VDR), G–protein coupled receptors (TGR5 or GPBAR1), c–Jun N–terminal kinase (JNK), and extracellular signal–regulated kinase (ERK). Through these interactions, BAs are able to affect energy, glucose, lipid, and lipoprotein metabolism (117).

FXR is mainly activated by BAs and is abundantly expressed in the adrenal gland, intestine, liver, and kidney (63, 64).

FXR is mainly involved in cholesterol and triglyceride metabolism. However, some reports emphasized FXR activity in glucose homeostasis (118). Knockout mice for the FXR gene show impaired insulin sensitivity (118, 119). FXR negatively regulates lipogenesis and glucose production in the liver (120, 121) (Figure 1). Bile acids (BAs) are not only the natural ligand of FXR but also the major ligand for GPBAR–1 (TGR5). FXR is usually interacting with DNA by forming a heterodimer with inverted repeat–1 (IR1) elements like Retinoid X receptor (RXR). FXR is the principal BA sensor in the liver and ileum (122, 123). FXR activation increases the expression of fibroblast growth factor 19 (FGF19) in the human intestine and that of fibroblast growth factor 15 (FGF15) in mice intestines. When the circulating FGF19 enters the portal vein and reaches the liver, it induces a reduction in the expression of hepatic cholesterol 7D–hydroxylase in BAs' additional synthesis. (124).

BAs are also the natural ligands of GPBAR–1 largely expressed in brown adipocytes, Kupffer cells, entero–endocrine cells of the intestine, macrophages, monocytes, and skeletal muscle. The effects of the binding of BAs with GPBAR1 are site–specific (Figure 1). In ileal L–cells, the binding of BAs to GPBAR–1 increases the secretion of PYY, Glucagon–like peptide (GLP)–1, and GLP2 with anorexigenic effect (111). In macrophages and Kupffer cells, GPBAR–1 activation blocks LPS–induced cytokine production. In skeletal muscle and brown adipocytes, GPBAR–1 signaling results in paracrine local activation of type II iodothyronine deiodinase (DIO2) which catalyzes the transformation of thyroxine (T4) to the active triiodothyronine (T3) responsible for increased energy expenditure (125). Recent research has demonstrated additional roles of BAs beyond their digestive function (114). By considering these additional effects, BAs are unequivocal mediators of glucose/insulin homeostasis and might explain some positive effects of bariatric surgery which appear independently of body fat loss (65, 113, 125, 126). Bariatric surgery represents an interesting model to re–evaluate the function of BAs in the pathophysiology of weight loss following surgical procedures in obesity (127).

Several papers demonstrated that plasma BA levels increase after bariatric surgery and might induce GLP–1 secretion in the intestine (128). Nonetheless, BAs were demonstrated to activate thyroid hormones (126, 129–133). This view is mainly supported by animal studies. GPBAR–1 knockout mice fed with a high–fat diet show a higher amount of body fat mass compared with wild–type mice. Interestingly, by increasing GPBAR–1 expression with the GPBAR–1 agonist INT–777, HFD–induced obesity is blunted (128, 134, 135).

Unexpectedly, at the level of brown adipose tissue and skeletal muscle, BAs induce energy expenditure by locally activating the type II iodothyronine deiodinase (DIO2), which transforms the inactive thyroxine (T4) to active thyroid hormone (T3), a key regulator of metabolism and energy homeostasis (125, 126). BAs seem to be the mediator of diet–induced thermogenesis by activating the BA–GPBAR–1– cAMP–DIO2 (type II iodothyronine deiodinase) signaling pathway. Diet–induced thermogenesis is probably impaired in obesity. BAs stimulation of brown adipocytes and myocytes increases their oxygen consumption, but this thermogenic effect is lost in DIO2 knockout mice (125).

An interesting model of bariatric surgery is represented by ileal interposition with or without Vertical sleeve gastrectomy (VSG). After this surgical procedure, obese Zucker rats display increased levels of circulating BAs. The intestinal adaptation after bariatric surgery is associated with increased recycling of BAs, which plays a protective role against obesity–related comorbidities (129, 136).

Similar effects are observed in rats with diet–induced obesity (130). Data obtained from the Ussing chamber (an electro–physiologic experimental model) show that BAs bind GPBAR–1 located in the basolateral membrane of the GLP–1–secreting L–cells. However, the GLP–1 release might occur only after the initial BA absorption across the intestinal epithelium (133) and effective stimulation of GPBAR–1. A translational study performed on rats

and patients demonstrates that endothelial dysfunction which is typically found in obesity is rapidly reversed by gastric bypass according to Roux (RYGBP), which is able to induce a GLP–1–mediated restoration of the endothelium–protective properties of HDL (137).

VSG is a surgical procedure that is associated with an increase in serum BAs and a decrease in gene expression of the GPBAR–1 receptor in the white adipose tissue, independent from dietetic variations (138). The effects of RYGBP on BAs were extensively studied in morbidly obese subjects. Total fasting BAs display a bimodal rise after RYGB: ursodeoxycholic acid (UDCA) and its glycine and taurine conjugate increase after 1 month (with insulin–sensitizing effects), while primary unconjugated BAs, as well as deoxycholic acid and its glycine conjugate increases after 24 months (139–141). According to Watanabe et al., the increase in the circulating BA levels after bariatric surgery might activate the thyroid hormone–stimulated pathway involving TGR5 (or GPBAR–1) and enhance thermogenesis and weight loss (126).

When BAs bind ileal GPBAR–1, peptide YY (PYY) circulating levels as well as GLP–1 and GLP–2 increase playing an anorexigenic effect (i.e., appetite reduction), as well as GLP–1 and GLP–2 (142). An additional mechanism is the activation of the BA–GPBAR–1–GLP–1 axis which is followed by the GLP–1–induced insulin release, the decrease in glucagon secretion from the liver, and the inhibition of gastrointestinal motility and food assumption (143). In the same line of evidence, Yu, et al. demonstrated that the levels of chenodeoxycholic acid (CDCA) in obese patients with type 2 diabetes undergoing RYGBP might be considered a prognostic marker of diabetes remission after this bariatric procedure (144). To explain these results, the additional BAs signaling involving FXR might be taken into account. Shen, et al. reported that CDCA is able to increase intracellular glucose transport in adipocyte cell lines by activating GLUT4 transcription *via* the FXR–FXR response element (FXRE) signaling (145). The same mechanisms might control GLUT4 transcription in hepatocytes and ileal cells where BAs also act as signaling agents of the nuclear receptor FXR. After bariatric surgery, BAs/FXR axis might also clarify several effects on glucose and lipid metabolism (146). Düfer and co–workers showed that BAs/FXR interaction might acutely increase β–cell function and insulin secretion (147).

The gut microbiota might also be involved in this crosstalk between BAs and glucose and lipid metabolism (140). In obese FXR knockout mice, the beneficial effects of VSG are not completely explained by the mechanical restriction of the stomach per se but are also due to both increased circulating levels of BAs and associated changes in gut microbiota. Furthermore, the surgery–induced reduction of body weight and the improvement in glucose tolerance is blunted after the silencing of FXR (146).

Gut microbiota is responsible for the deconjugation, oxidation, sulfation, and dihydroxylation of the primary BAs to the secondary BAs (Figure 1). The possibility exists that the modified gut microbiota following bariatric surgery may modify the BAs' composition and kinetic and metabolic function. Several data demonstrate that it is possible to transfer the gut microbiota from mice with RYGBP to germ–free mice, inducing body weight loss in germ–free mice. This result and others support the view that gut microbiota composition plays an important role in the beneficial effects observed after RYGBP. The overall mechanisms responsible for the increase in plasma BA concentrations after bariatric surgery is not completely clarified. Moreover, not all studies on bariatric surgery are in favor of the beneficial effect of BAs on glucose homeostasis and energy metabolism.

Kohli, et al. (148) found that RYGBP is indeed associated with increased circulating BAs and GPBAR–1 signaling (i.e., the increased peak of GLP–1 after a meal and decreased serum TSH). BAs do not appear to be simultaneous to the early increment in GLP–1 and gut peptide secretion in obese subjects undergoing bariatric surgery (RYGBP or VSG). GLP–1 and PYY circulating levels increase significantly 1 week and 3 months after the surgery. By contrast, both basal and postprandial levels of BAs increase more slowly and progressively, reaching significant rises only one year after the surgery (149). Unexpectedly, changes in circulating levels of BAs do not correlate with meal–mediated insulin response, insulin sensitivity, or basal thermogenesis.

Robust data show that the beneficial metabolic effects on glucose tolerance might occur by ileal interposition without intestinal resection (150–155). In this procedure, a part of the terminal ileum is interposed into the proximal jejunum. This puts nutrients prematurely in contact with the ileal mucosa and stimulates the L cells to produce GLP–1 and PYY. The increased delivery of BAs to the distal L cells and the altered GPBAR–1 receptor activation, however, do not seem to play a master role in the early increases in the intestinal secretion of GLP–1 and PYY, as seen after other procedures of bariatric surgery (156–163).

A list of the most studied hepatokines is reported in Table 1. Recent studies show that Fetuin–B plays additional roles through its interaction with Farnesoid X Receptor (FXR or NR1H4) (Figure 1). In studies utilizing microarray technology, Fetuin–B acts as an FXR agonist–regulated gene. The interaction between BAs and Fetuin–B needs further studies in humans, especially in models of ketogenic diets, because they activate the same nuclear receptor pathway. However, from the present data, it seems that the connecting point between hepatokines, BAs, and KBs is represented by the liver itself but in the contest of the microbiota–gut–brain system.

Conclusions

The liver has recently acquired the dignity of an endocrine organ, especially for its ability to express hepatokines. Some hepatokines are promising markers of metabolic abnormalities. The liver is also involved in KBs and BAs' production. Both KBs and BAs are involved in endocrine, paracrine, and autocrine effects already confirmed in humans. Analogs for some hepatokine receptors are gradually being valued and could become new therapeutic approaches for metabolic diseases. BAs might probably be considered with major attention in the context of obesity prevention, treatment, and management because they play

TABLE 1 List of some of the hepatokines expressed in humans.

Name	Target organs	Role	Circulating levels	Reference
ANGPTL3	Liver, Ads, SkM, Brain, Heart	increases InsR inhibits LPL inactives LPL	⇧	(164–169)
ANGPTL6	Several	regulates InsS regulates glucose and lipid metabolism	⇧	(42–44, 170, 171)
FIAF (ANGPTL4)	Liver, Ads, SkM, Brain	increases glucose production	⇳	(49–51, 54, 172–174)
Fetuin A	Liver, Ads, SkM	inhibits insulin receptor phosphorylation	⇧	(28, 33, 63–65)
Fetuin B	Liver, SkM,	increases InsR increases AdT inflammation inhibits insulin receptor phosphorylation interacts with FXR	⇧	
FGF21	Ads, Brain	increases EE increases InsS decreases sugar intake decreases circulating TGL	⇧	(72, 175–177)
Lipasin (ANGPTL8)	Liver, Ads, SkM, Brain, Heart	increases InsR inhibits LPL binds ANGPTL3 downregulates FIAF	⇳	(3, 178, 179)
Selenoprotein P	Several	increases glucose utilization impairs Insulin signaling	⇧	(180, 181)

Ads, adipocytes; ANGPTL, Angiopoietin–like proteins; BW, Body weight; EE, Energy expenditure; InsR, Insulin resistance; InsS, Insulin sensitivity; SkM, Skeletal muscles. ⇧ Increased ⇩ decreased ⇳ contrasting results.

an important role in modulating the microbiota–brain interaction as suggested by studies performed in animal models of bariatric surgery and humans undergoing bariatric metabolic surgery. The current treatment of type 2 diabetes with inhibitors of tubular sodium/glucose co–transporter–2 (GLT2i) highlighted additional important therapeutic roles played by KBs.

Author contributions

GG wrote the review and prepared the figure, JB revised the manuscript, AC added references, SP revised the manuscript, FG critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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.

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Dipterocarpus tuberculatus as a promising anti-obesity treatment in *Lep* knockout mice

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Introduction: The therapeutic effects and mechanisms of *Dipterocarpus tuberculatus* (*D. tuberculatus*) extracts have been examined concerning inflammation, photoaging, and gastritis; however, their effect on obesity is still being investigated.

Methods: We administered a methanol extract of *D. tuberculatus* (MED) orally to *Lep* knockout (KO) mice for 4 weeks to investigate the therapeutic effects on obesity, weight gain, fat accumulation, lipid metabolism, inflammatory response, and β-oxidation.

Results: In *Lep* KO mice, MED significantly reduced weight gains, food intake, and total cholesterol and glyceride levels. Similar reductions in fat weights and adipocyte sizes were also observed. Furthermore, MED treatment reduced liver weight, lipid droplet numbers, the expressions of adipogenesis and lipogenesis-related genes, and the expressions of lipolysis regulators in liver tissues. Moreover, the iNOS-mediated COX-2 induction pathway, the inflammasome pathway, and inflammatory cytokine levels were reduced, but β-oxidation was increased, in the livers of MED-treated *Lep* KO mice.

Conclusion: The results of this study suggest that MED ameliorates obesity and has considerable potential as an anti-obesity treatment.

KEYWORDS

anti-obesity, *D. tuberculatus*, lipogenesis, lipolysis, inflammasome, β-oxidation

1 Introduction

Southeast Asia has a large distribution of the medicinal herb *Dipterocarpus tuberculatus* (*D. tuberculatus*), particularly in Bangladesh, Thailand, Cambodia, Laos, Myanmar, and Vietnam (1). The parts of *D. tuberculatus* continue to be used as traditional medicines; for example, its leaf gum is used as an anti-venom, and its roots have anti-inflammatory and anti-dysenteric effects (1). Consequently, the therapeutic effects and underlying mechanisms of *D. tuberculatus* have been investigated for their effects on anti-inflammatory response, anti-photoaging, and promotion of osseointegration. An ethanolic extract of *D. tuberculatus* strongly suppressed macrophage-mediated inflammatory responses *in vitro* and *in vivo* downregulated PDK1/NK-κB signaling pathway and suppressed EtOH/HCl-induced acute gastric lesions (2). Moreover, it inhibited lipopolysaccharide (LPS) induced activator protein-1 (AP-1)-mediated inflammatory reaction in macrophages and protected against acute liver injury (3). Furthermore, a methanolic extract of *D. tuberculatus* (MED) exhibited significant anti-photoaging effects by inhibiting apoptosis, cell cycle arrest, age-related extracellular matrix structural changes, and inflammation in UV-irradiated cells and nude mice (4), protective effects in blue light-induced retinal degeneration (5), and was also found to stimulate focal cell adhesion via the MCL2/FAK/Akt signaling pathway (6) and induce the proliferation and adhesion of osteoblasts, which led to bone formation and regeneration in tibia implantation models when coated on the surfaces of titanium plates (7). However, the effects of *D. tuberculatus* on obesity have not been elucidated.

The greatest healthcare challenge in our generation is regulating excessive body fat (8). Over the past 50 years, the global obesity rate has more than tripled, according to World Health Organization (9, 10). Obesity goes far beyond just being overweight; it promotes alarming rates of diabetes, which is associated with an increased risk of type I diabetes, hypertension, hyperlipidemia, cardiovascular disease, and certain cancers, which are major causes of premature death (11, 12). Numerous factors contribute to the onset and progression of obesity and its complications. Among these, hormones such as leptin, adiponectin, and visfatin play a significant role in the regulation of food consumption and energy expenditure by hormones such as leptin, adiponectin, and visfatin are important factors (13–18). Moreover, high serum fatty acid (FA) and triglyceride (TG) levels trigger fat accumulation in adipocytes and lead to oxidative stress, hypertriglyceridemia, lipotoxicity, diabetes, and various metabolic syndromes (19). Thus, reductions in circulating and stored fat levels, lipase inhibition, appetite suppression, energy expenditure stimulation, adipocyte differentiation inhibition, and lipolysis activation are considered key anti-obesity strategies (8, 20) and have been widely utilized to investigate the therapeutic effects of natural products *in vitro* and *in vivo*.

In this study, we investigated the anti-obesity effect and mechanism of action of a MED and its mechanism of action in an obese mouse model. Our findings provide the first evidence that the anti-obesity effects of MED are attributable to the regulation of multiple targets associated with lipid metabolism, including lipid accumulation, lipolysis, the inflammasome, cytokine expression, and the expressions of related signaling molecules in *Lep* knockout mice.

2 Materials and methods

2.1 Preparation and methanol extraction

The International Biological Material Research Center of the Korea Research Institutes of Bioscience and Biotechnology (Daejeon, Republic of Korea) provided a lyophilized sample of MED (FBM 213-075). Briefly, dried stem powder of *D. tuberculatus* was mixed with methanol in a ratio of 1:10 (wt/vol). The mixture was sonicated for 15 min and incubated for 2 h at room temperature. The process was repeated 10 times daily for 3 days and filtered using a 0.4 μm filter. The extracted solution was concentrated using a rotary evaporator (N = 1000 SWD, EYELA, Bohemia, NY, USA) and lyophilized using a speed vacuum concentrator (Modulspin 40, Biotron Co., Marysville, WA, USA). The MED obtained was dissolved in 1×PBS buffer to the required concentrations for administration to mice.

2.2 Liquid chromatography-electrospray ionization mass spectrometry analysis

The bioactive compounds in MED were identified as previously described by Lee et al. (4). Liquid chromatography-mass spectrometry (LC-MS) analysis was performed with a BEH C18 Column (2.1 × 100 mm, 1.7 μm) (Waters, Milford, MA, USA) using an Agilent 1290 Infinity HPLC system (Agilent Technologies, Waldbronn, Germany). Mass spectra were obtained in the negative mode electrospray ionization (ESI) using MassHunter software (Agilent Technologies).

2.3 Experiments on *Lep* KO mice

Lep KO mice are a useful model for metabolic studies as they display hyperphagia, early-onset obesity, and symptoms of metabolic disease, including increased fatty acid synthesis in fat and liver, impaired glucose tolerance, insulin sensitivity, and hepatic steatosis (18, 21).

The animal experimental protocol for *Lep* KO mice (C57BL/6-*Lep*^{em1Shwl}/Korl) was approved by the Institutional Animal Care and Use Committee of Pusan National University (PNU-Institutional Animal Care and Use Committee (IACUC); Approval Number PNU-2021-0072). *Lep* KO and wild-type (WT, C57BL/6/Korl) mice were maintained at the Pusan National University-Laboratory Animal Resources Center, which is accredited by the Korea Food and Drug Administration

(Accredited Unit Number: 000231) and the Association for the Assessment and Accreditation of Laboratory Animal Care International (Accredited Unit Number: 001525). Four-week-old *Lep* KO (n = 21) and WT (n = 7) mice were kindly provided by the Department of Laboratory Animal Resources at the National Institute of Food and Drug Safety Evaluation (NIFDS, Chungju, Korea). Animals were allowed *ad libitum* access to filtered water and a standard irradiated chow diet (Samtako BioKorea Co., Osan, Korea) (crude protein 20%, crude fat 4.5%, crude fiber 6%, crude ash 7%, calcium 0.5%, Phosphorus 1%) throughout the experimental period. Mice were maintained under specific pathogen-free (SPF) conditions at 23 ± 2°C and 50 ± 10% relative humidity (RH) under a daily light cycle (lights on at 08:00 h and off at 20:00 h). The genotype of *Lep* KO mice was identified using DNA-PCR, as previously reported in the literature (22, 23).

Mice were classified into two groups: WT mice (the WT group, n = 7) and obese mice (the *Lep* KO group, n = 21). Mice in the *Lep* KO group were divided into the following three groups; (1) the 1x PBS administrated group (the Vehicle-treated Lep KO group, n = 7), (2) the 100 mg/kg of MED administrated group (the LMED-treated *Lep* KO group, n = 7), and (3) the 200 mg/kg of MED administrated group (the HMED-treated *Lep* KO group, n = 7). The dosages for MED treatment were decided based on results from previous research using identical extract (4, 5). The same volume of Vehicle (PBS) or MED solution was administered orally daily for 4 weeks. Mice were euthanized with CO_2 at 24 h following final treatments after a 12 h fasting. Tissue samples and sera were acquired and stored in Eppendorf tubes at −70°C until required for the assay.

2.4 Measurement of body and organ weights

Mouse weights were measured daily at 10:00 a.m. using an electronic balance (Mettler Toledo, Greifensee, Switzerland), according to KFDA guidelines. In addition, weights of livers and abdominal fat were obtained after sacrifice using the same method.

2.5 Serum biochemical analysis

Blood samples from an abdominal vein were incubated for 30 min at room temperature (RT) in serum-separating tubes (BD Containers, Franklin Lakes, NJ, USA). Serum samples were obtained by centrifugation at 1,500 ×g for 15 min, and serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels were determined using an BS-120 Automatic Chemical Analyzer (Mindray, Shenzhen, China). All assays were conducted in duplicate using a fresh serum.

2.6 Histopathological analysis

Liver and fat tissues were fixed overnight in 10% neutral buffered formalin (pH 6.8), embedded in paraffin wax, and sectioned (4 μm) using a Leica microtome (Leica Microsystems, Bannockburn, IL, USA). Sections were collected on glass slides, deparaffinized with xylene (DaeJung, Gyeonggi-do, Korea), and rehydrated with graded ethanol (100 to 70%) and distilled water. Sections were then stained with hematoxylin and eosin (H&E; Sigma Aldrich Co., St. Louis, MO, USA), and numbers of lipid droplets in liver tissues were counted using the Leica Application Suite (Leica Microsystems, Heerbrugg, Switzerland). Also, areas of adipocytes in fat sections were measured using Image J 1.52a (NIH, Bethesda, ML, USA).

2.7 Quantitative reverse transcription polymerase chain reaction analysis

The mRNA levels of PPARγ, C/EBPα, aP2, FAS, adenylyl cyclase (AC), PDE4, CPT1, PPARα, NF-κB, TNF-α, IL-6, and IL-1β in liver tissues were measured by RT-qPCR, as previously described (24). Briefly, total RNA in liver tissues was purified using RNAzol (TelTest Inc., Friendswood, TX, USA) and quantified using a NanoDrop system (Biospecnano, Shimadzu Biotech, Kyoto, Japan), complementary DNA (cDNA) was synthesized using a mixture of total RNA (5 μg), oligo-dT primer (Invitrogen, Carlsbad, CA, USA), dNTP, and reverse transcriptase (Superscript II, Invitrogen). qPCR was conducted with a cDNA template, 2× Power SYBR Green (Toyobo Co., Osaka, Japan), and specific primers (Supplementary Table S1) using the following cycle: 15 sec at 95°C, 30 sec at 55°C, and 60 sec at 70°C. Fluorescence intensities were determined at the end of the extension phase of each cycle. Values measured during the exponential phase of PCR amplification were used to define threshold cycles (Ct). The expressions of target genes were normalized versus β-actin (housekeeping gene) based on Ct values at constant fluorescence intensity, as described by (25).

2.8 Western blot analysis

Liver total protein was extracted using Pro-Prep Protein Extraction Solution (iNtRON Biotechnology, Seongnam, Korea), and quantified using a SMARTTM BCA Protein Assay Kit (Thermo Scientific). Total proteins (20-30 μg) were loaded and separated by 4–20% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for 2 h, after which resolved proteins were transferred to nitrocellulose membranes for 2 h at 40 V. Membranes were then incubated separately overnight at 4°C with specific primary antibodies (Supplementary Table S2). Probed membranes were then washed with washing buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 0.05% Tween 20) and incubated with 1:2,000 diluted horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Invitrogen) at RT for 1 h. Finally, the membrane blots were developed using Amersham ECL Select Western Blotting detection reagent (GE Healthcare, Little Chalfont, UK). Chemiluminescence signals originating from specific bands were detected using FluorChemi®FC2 (Alpha Innotech Co., San Leandro, CA, USA).

2.9 Statistical analysis

The statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA). The significance of intergroup differences was determined by one-way analysis of variance (ANOVA) and Tukey's *post hoc* t-test for multiple comparisons. Unless otherwise specified, error bars represent SEMs. P values of <0.05 were considered statistically significant. Individual P values are provided in the figure legends. All experiments were performed twice independently.

3 Results

3.1 Chemical profile of MED

Firstly, we analyzed the distribution of the bioactive components in MED to predict its potential for anti-obesity activity. LC-ESI-MS analysis in the negative ion mode was performed to investigate the chemical profile of MED. A representative total ion chromatogram (TIC) of LC-ESI-MS is shown in Figure 1A. Among the various peaks of TIC, seven bioactive compounds were identified. In addition, it was confirmed through their extracted ion chromatogram (XIC) that each peak was identified as gallic acid, bergenin, ellagic acid, ϵ-viniferin, asiatic acid, oleanolic acid, and 2α-hydroxyursolic acid (Figures 1B–H and Table S3). These results show that MED is potentially used as an anti-obesity treatment.

3.2 Inhibitory effect of MED on obesity phenotypes in *Lep* KO mice

The alterations in body weight, food intake, and serum lipid profiles were measured over the 4-week administration period of MED administration to determine whether MED could ameliorate obesity phenotypes in *Lep* KO mice (Figure 2A). A significant difference in body weight was observed between the WT and Vehicle-treated *Lep* KO groups. However, body weights were dose-dependently lower in the MED-administered groups than in the Vehicle-treated *Lep* KO group from experimental days 14 to 28. Body weight gains in the LMED and HMED groups were significantly lower (by 8.2% and 24.7%, respectively) than in the Vehicle-treated *Lep* KO group (Figures 2B, C). Moreover, food intake was higher in the Vehicle-treated *Lep* KO group than in the WT group during the experiment, and dramatically reduced dose-dependently after MED administration (Figure 2D).

Furthermore, TG and TC serum concentrations in the LMED and HMED groups were significantly lower than in the Vehicle-treated *Lep* KO group, although the concentrations of HDL-C were significantly higher (Figure 2E). These results demonstrate that MED treatment for 4 weeks can suppress body weight gain and improve serum TC, TG, and HDL-C levels.

3.3 Inhibitory effect of MED on fat accumulation in abdominal fat tissue

Next, the effect of MED on abdominal fat tissue was investigated. Abdominal fat mass, including epididymal and retroperitoneal fat,

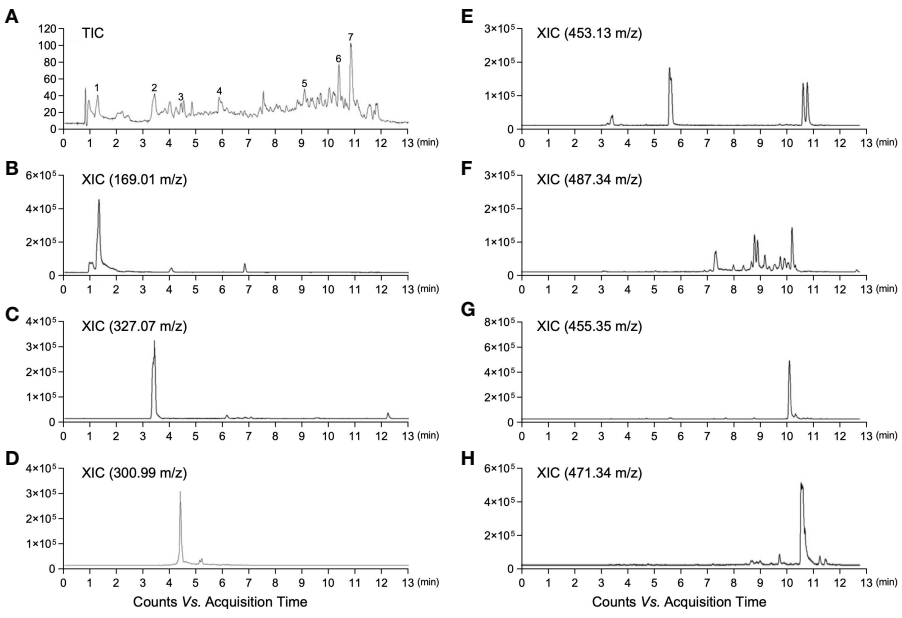

FIGURE 1
LC-ESI-QTOF-MS chromatograms of MED. **(A)** Total ion chromatogram (TIC) of MED obtained by LC-MS; **(B)** Gallic acid: extracted ion chromatogram (XIC) of m/z 169.01 in negative mode; **(C)** Bergenin: XIC of m/z 327.07 in negative mode; **(D)** Ellagic acid: XIC of m/z 300.99 in negative mode; **(E)** Viniferin: XIC of m/z 453.13 in negative mode; **(F)** Asiatic acid: XIC of m/z 487.34 in negative mode; **(G)** Oleanolic acid: XIC of m/z 455.35 in negative mode; and **(H)** Hydroxyursolic acid: XIC of m/z 471.34 in negative mode.

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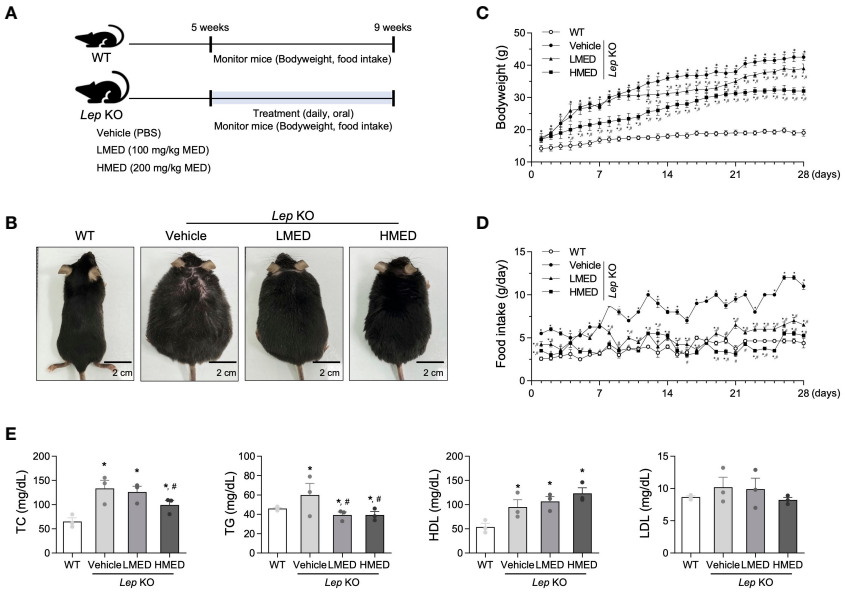

FIGURE 2
Body weights, food intakes, and serum lipid profiles of *Lep* KO mice treated with MED. **(A)** A schematic representation of the animal experiment. **(B)** Representative images of wild-type (WT) mice and *Lep* KO mice treated with 1x PBS (Vehicle), 100 mg/kg of MED (LMED), or 200 mg/kg of MED (HMED), n=7, size bar = 2 cm. The body weights **(C)** and daily food intakes **(D)** of WT mice and *Lep* KO mice treated with Vehicle, LMED, or HMED during the 4-week experiment. **(E)** TC, TG, HDL, LDL level analyses in the serum of WT mice and Lep KO mice treated with Vehicle, LMED, or HMED after 4-week experiment. Results are presented as means ± SEMs (n = 10). *$p < 0.05$ vs. the WT group; $^{\#}p < 0.05$ vs. the Vehicle-treated *Lep* KO group (*t*-test). TC, total cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein.

was significantly (11.7-fold) more prevalent in the Vehicle-treated *Lep* KO group compared to WT controls (Figures 3A, B). The mean weights of abdominal fat in the LMED and HMED groups were 2.56 ± 0.25 g and 1.38 ± 0.44 g, respectively. This was significantly lower than the mean weight of the Vehicle-treated *Lep* KO group (3.02 ± 0.29 g). Also, the average area of adipocytes in H&E-stained fat tissues was significantly and dose-dependently lower in the LMED and HMED groups compared to the Vehicle-treated *Lep* KO group (Figures 3C, D). These results indicate that the suppressive effects of MED on obesity phenotypes are closely associated with the inhibition of fat accumulation in *Lep* KO mice.

3.4 Inhibitory effect of MED on hepatic steatosis in liver tissues of *Lep* KO mice

The changes in liver weight and pathological characteristics were measured to investigate whether MED was associated with a

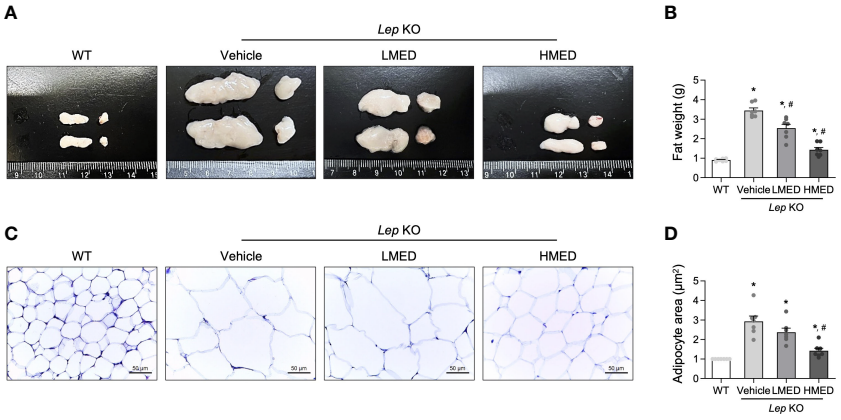

FIGURE 3
Weights and average areas of adipocytes in the fat tissues of *Lep* KO mice treated with MED. **(A)** Representative fat image showing epididymal (left) and retroperitoneal (right) fat harvested from the abdominal region of mice in the WT, Vehicle-treated *Lep* KO, LMED, and HMED groups. **(B)** Fat weights were calculated by adding the weights of epididymal and retroperitoneal fat. Fat tissues were collected from 7 mice per group, and fat tissue weights were measured in duplicate for each tissue type. **(C)** The representative image of H&E-stained epididymal fat tissues in all groups at 200×, size bar = 50 μm. **(D)** Plots of average adipocyte areas in epididymal fat tissues as determined using Image J. H&E-stained fat tissues of seven mice per group were examined, and adipocyte areas were measured in duplicate on each slide. Results are presented as means ± SEMs. *$p < 0.05$ vs. the WT group; $^{\#}p < 0.05$ vs. the Vehicle-treated *Lep* KO group (*t*-test).

reduction in hepatic steatosis. The liver weights of the Vehicle-treated *Lep* KO group were significantly greater (by 3.7-fold) than those of the WT group (Figures 4A, B). However, compared to the Vehicle-treated *Lep* KO group, MED supplementation dose-dependently reduced liver weights by 23.8% and 56.1% in the LMED and HMED groups, respectively. Furthermore, average numbers of lipid droplets in liver tissue sections (a surrogate of fat accumulation in the liver) showed a similar pattern of suppression. Figures 4C, D show that lipid drop numbers in liver tissue were significantly and dose-dependently lower in the LMED and HMED groups than in the Vehicle-treated KO group, although they were considerably higher in the Vehicle-treated *Lep* KO group than in the WT group. These findings suggest that the ameliorating effects of MED on obesity phenotypes are associated with the inhibition of hepatic steatosis in *Lep* KO mice.

3.5 Effect of MED on adipogenesis, lipogenesis, and lipolysis in liver tissue

Subsequently, we examined whether the inhibitory properties of MED on hepatic steatosis could be accompanied by an alteration in lipid metabolism. Four adipogenesis and lipogenesis-related genes (PPARα, C/EBPα, aP2, and FAS) had higher mRNA levels in the liver of the Vehicle-treated *Lep* KO group than in the WT group. However, MED-treated groups showed significant and dose-dependent decreases in the mRNA levels of PPARα, C/EBPα, aP2, and FAS in the liver tissue (Figure 5A). Moreover, MED had similar effects on lipolysis. The mRNA levels of two genes, AC and PDE4 (both positively associated with lipolysis), were analyzed using specific primers in the liver tissue. The mRNA levels of AC in liver tissues were greater in the MED-treated groups than in the Vehicle-treated KO group, although levels remained higher than in the WT group. Interestingly, PDE4 mRNA levels exhibited the opposite pattern (Figure 5B). In addition, the increases in AC mRNA levels observed in the liver tissue of MED-treated mice matched those of three lipogenic proteins in these mice. MED-treated mice had dose-dependently higher levels of ATGL mRNA expression than in the WT group. Additionally, MED increased the phosphorylation of HSL and perilipin in the liver, but at lower levels than in the WT group (Figure 5C). Consequently, the findings suggest that the inhibitory effects of MED on hepatic steatosis are associated with the inhibition of adipogenesis and lipogenesis and the stimulation of lipolysis in the livers of *Lep* KO mice.

3.6 Inhibitory effect of MED on hepatic inflammation

Hepatic steatosis can be accompanied by an alteration in inflammation (26). We examined the expression level of key proteins in the iNOS-induced COX-2 mediated pathway and the NLRP3 inflammasome pathway, in addition to the transcription level of inflammatory cytokines in liver tissue after the administration of MED. The protein levels of iNOS and COX-2 were higher in the liver tissues of Vehicle-treated *Lep* KO mice than in WT controls. Figure 6A shows that MED administration reduced both and dose-dependently reduced iNOS expression. Furthermore, the inhibitory effects of MED were detected in the NLRP3 inflammasome pathway, which includes NLRP3 (NLR family pyrin domain containing 3), ASC (apoptosis-associated speck-like protein containing a CARD), and cleaved Cas-1. The protein levels of NLRP3, ASC, and cleaved Cas-1 were remarkably higher in Vehicle-treated *Lep* KO mice than in WT controls, but they were significantly lower in *Lep* KO mice treated with MED than in Vehicle-treated *Lep* KO mice (Figure 6B). Furthermore, MED had a similar inhibitory effect on the mRNA levels of inflammatory-

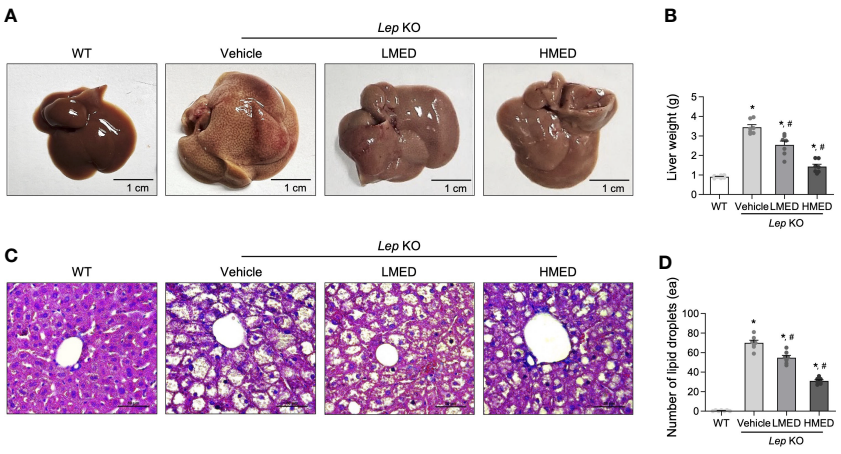

FIGURE 4
Hepatic lipid accumulation in *Lep* KO mice treated with MED. **(A)** Representative liver images of a WT and *Lep* KO mouse treated with Vehicle, LMED, or HMED, size bar = 1 cm. **(B)** Measurement of liver weights. Livers were collected from 7 mice per group and weights were measured twice. **(C)** Representative images of H&E stained liver sections, size bar = 50 μm. **(D)** Circular lipid droplets in H&E-stained sections reflected lipid accumulations. Total numbers of lipid droplets were measured in H&E-stained fat sections at 200× using the Leica Application Suite. Numbers of lipid droplets in H&E-stained tissues of 7 mice per group were counted in duplicate. Results are presented as means ± SEMs. *$p < 0.05$ vs. the WT group; $^{\#}p < 0.05$ vs. the Vehicle-treated *Lep* KO group (*t*-test).

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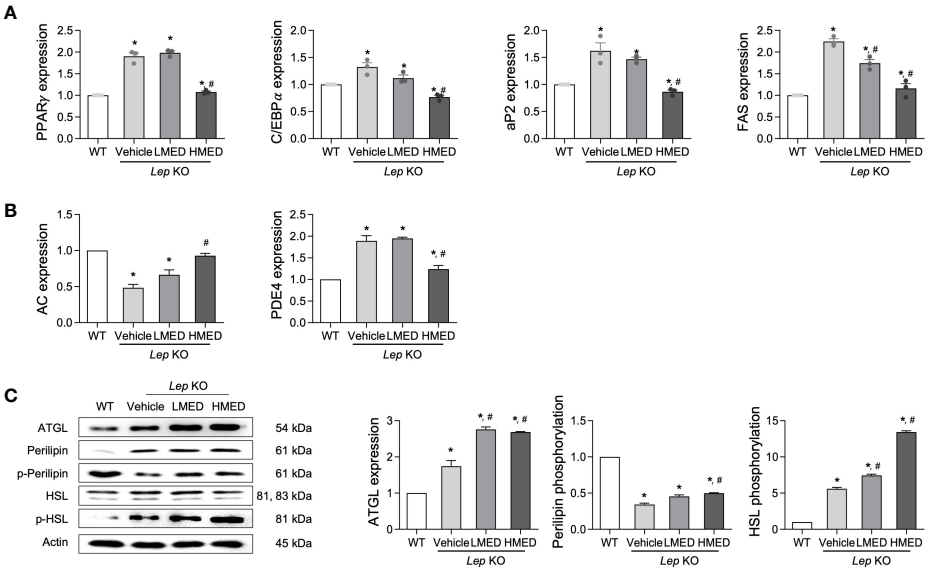

FIGURE 5
Expressions of proteins related to the lipid metabolism pathway in the liver tissues of *Lep* KO mice treated with MED. Expressions of lipogenesis-related genes. **(A)** Transcript levels of PPARγ, C/EBPα, aP2, and FAS were measured by RT-qPCR in WT mice and *Lep* KO mice treated with Vehicle, LMED, or HMED; and **(B)** Expression levels of lipogenesis-related genes. Transcript levels of AC and PDE4 were analyzed by using RT-qPCR in WT mice and Vehicle-treated *Lep* KO, LMED, and HMED mice. **(C)** Expression levels of proteins related to lipid metabolism. ATGL, perilipin p-perilipin, HSL, and p-HSL were detected using specific antibodies, and actin was used as the internal control. Results are presented as means ± SEM (n=5). $^{*}p < 0.05$ vs. the WT group; $^{\#}p < 0.05$ vs. the Vehicle-treated *Lep* KO group (*t*-test).

related cytokines, and IL-1β, IL-6, TNF-α, and NF-κB mRNA levels were higher in the Vehicle-treated *Lep* KO group than in the WT group. Moreover, the administration of MED significantly decreased the expressions of these cytokine levels compared to the Vehicle-treated KO group (Figure 6C). These results suggest that the inhibitory effects of MED on hepatic steatosis are associated with the suppression of the inflammatory response through the downregulations of the iNOS-COX-2 pathway, the NLRP3 inflammasome pathway, and the expression of inflammatory cytokines.

3.7 Stimulatory effects of MED on β-oxidation in liver tissue

Finally, we explored whether the inhibition effects of MED on hepatic steatosis can be accompanied with stimulation of β-oxidation. The level of β-oxidation-related factors was analyzed in the liver of *Lep* KO mice after treatment with MED. The mRNA levels of PPARα and CPT in the liver tissue were significantly lower in Vehicle-treated KO mice than in WT controls. MED treatment dose-dependently increased these mRNA levels (Figure 7A). In addition, significant increases were detected in protein levels of two β-oxidation-related proteins (ACADs and ACO) in the liver tissue after MED treatment. However, MED administration dose-dependently reduced ATPCL phosphorylation in the liver tissue (Figure 7B). These results demonstrate that the inhibitory effects of MED on hepatic steatosis are associated with enhanced β-oxidation via the regulations of CPT and PPARα.

4 Discussion

Over the past half-century, tremendous progress has been made in managing metabolic diseases closely related to obesity. However, anti-obesity medications have questionable safety and often prove ineffective (8). Recently, several studies have focused on discovering natural compounds and extracts with disease-preventing or health-promoting effects (20). Some bioactive components with few side effects have been investigated for their anti-obesity effects and the mechanisms responsible (20, 27–31). *D. tuberculatus* has attracted considerable interest as a potential treatment for a number of diseases, particularly those associated with inflammation and anti-photoaging. We were interested in providing scientific evidence on the anti-obesity effects of MED, as considerations of lipid metabolism and related-inflammatory responses are crucial for treating obesity. In this study, we found that the primary therapeutic effects of MED are inhibiting body weight gain, food intake, liver weight, fat accumulation, lipid metabolism, inflammation, and the stimulation of β-oxidation in *Lep* KO mice.

Body weight gain, fat tissue, and serum lipid profile can be crucial indicators for assessing the anti-obesity effect (32). Oral administration of MED (100 and 200 mg/kg, daily) for four weeks reduced body weight gain, fat weight, and adipocyte area in a dose-

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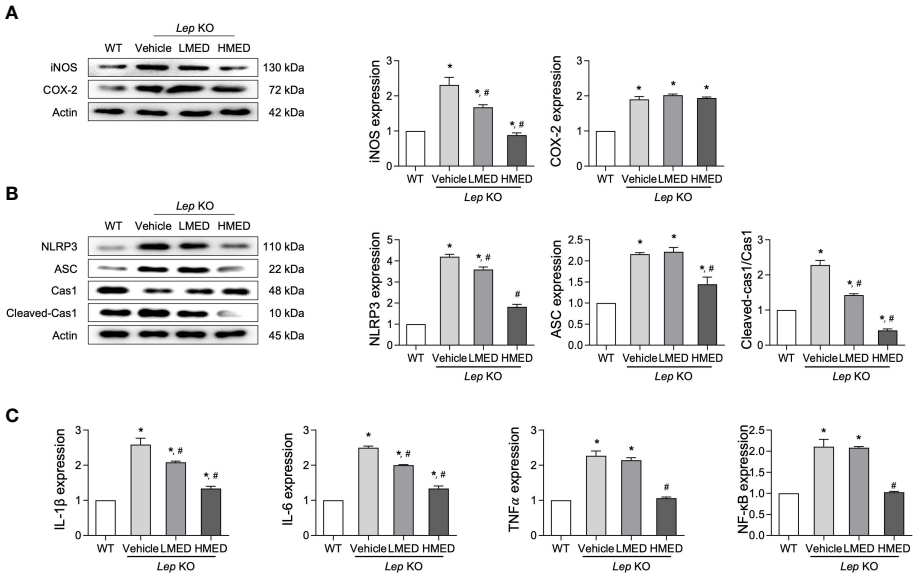

FIGURE 6
Expressions of inflammatory regulators in the liver tissues of MED-treated *Lep* KO mice. **(A)** Expression levels of proteins in the iNOS-mediated COX-2 induction pathway. Levels of iNOS and COX-2 proteins were detected in the liver tissues of WT and MED-treated *Lep* KO mice using specific antibodies. **(B)** Expression levels of proteins in the NLRP3 inflammasome pathway. Levels of the NLRP3, ASC, Cas-1, and cleaved Cas-1 were detected in the liver tissues of WT and *Lep* KO mice treated with MED using specific antibodies. Actin was used as the internal control. **(C)** Transcriptional levels of inflammatory cytokines. Levels of IL-1β, IL-6, TNF-α, and NF-κB mRNA were detected in the liver tissues of WT and MED-treated *Lep* KO mice using specific primers. Results are presented as means ± SEMs (n=5). **p* < 0.05 vs. the WT group; $^{\#}p$ < 0.05 vs. the Vehicle-treated *Lep* KO group (*t*-test).

dependent manner. The increased levels of serum TC and TG in *Lep* KO mice were effectively inhibited after MED administration. Although no significant change was detected in LDL-C levels, our results are consistent with previously reported results of anti-obesity studies. Furthermore, the MED-treated group exhibited a reduction in food intake comparable to that of the Vehicle group in *Lep* KO mice, showing similar to the WT group. However, we did not observe any indications that the reduced food intake was due to the unpalatable nature of MED. There were no noticeable differences in the appearance or behavior of the MED-treated mice compared to the WT control group, and the MED-treated mice showed no signs of aversion to the MED.

The liver is the most important organ in lipid metabolism, which involves lipid absorption, synthesis, transportation, and degradation. Fat accumulates in hepatocytes during obesity-induced lipid metabolic disorders, and the number of lipid

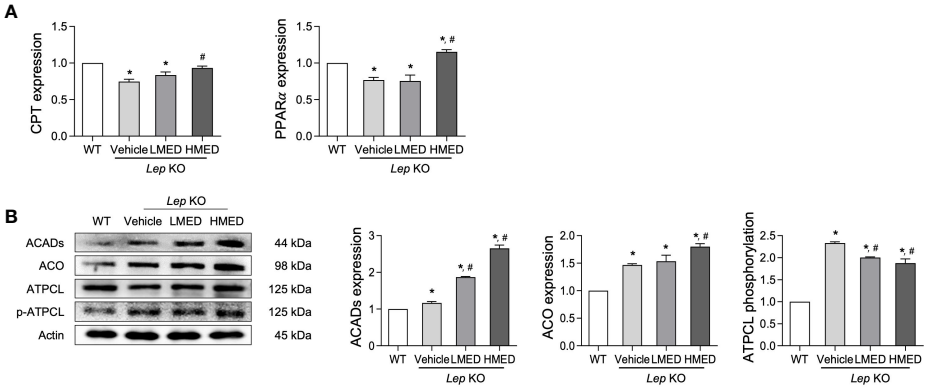

FIGURE 7
Levels of the β-oxidation regulators in the liver tissues of *Lep* KO mice treated with MED. **(A)** Levels of transcription factors. Levels of CPT and PPARγ mRNAs were assessed in the liver tissues of WT and *Lep* KO mice treated with MED using specific primers. **(B)** Expression levels of β-oxidation enzymes. Levels of ACADs, ACO, ATPCL, and p-ATPCL proteins were detected in the liver tissues of WT and *Lep* KO mice treated with MED using specific antibodies. Actin was used as the internal control. Results are presented as means ± SEMs (n = 5). **p* < 0.05 vs. the WT group; $^{\#}p$ < 0.05 vs. the Vehicle-treated *Lep* KO group (*t*-test).

droplets in the liver increases, leading to hepatocellular steatosis, a severe pathologic feature in the obese (33). These pathological features were observed in the present study, and in particular, many fat droplets accumulated in the liver acini of *Lep* KO mice. In our study, oral administration of MED reduced liver steatosis, as determined by liver weights and histological findings, and the number of lipid droplets was significantly reduced in the livers of *Lep* KO mice.

Obesity results from an imbalance between lipogenesis and lipolysis (34). Adipose tissues store energy as TGs within lipid droplets formed by lipogenesis, and fatty acids (FAs) are released from these stores via lipolysis. However, both processes are significantly elevated in obese patients (35). The regulatory effects of several herbs on lipogenesis and lipolysis have been investigated. *Garcinia* (36) and *Citrus depressa Hayata* (37) inhibited lipogenesis by downregulating lipogenesis-related genes. *Salix matsudana leaves* (38), *Actinidia arguta* roots (39), and *Zicao* roots (40) have been reported to induce lipolysis in adipocytes. The rhizomes of Curcuma longa were found to affect both mechanisms, specifically, they suppressed lipogenesis and increased lipolysis by upregulating lipases like adipose TGs, lipase, hormone-sensitive lipase, adiponectin, and AMP-activated protein kinase (41). In addition, the roots and stems of *Salacia reticulate* upregulated lipogenesis genes and downregulated lipolysis genes through AMPkα activation in adipocytes (42). Adipogenesis is a process for the differentiation of preadipocytes into mature adipocytes (lipid-accumulating and insulin-responsive adipocytes) and is regulated by adipogenic transcription factors (PPARγ and C/EBPα) (43, 44). When these factors are activated, lipogenesis is induced by lipogenic genes (aP2 and FAS) and the maintenance of adipocyte phenotypes during the late adipocyte differentiation (45, 46). In our study, MED administration regulated adipogenesis and lipogenesis by suppressing related transcriptional factors and enhanced the expression of lipolytic proteins in *Lep* KO mice. The results obtained for the effects of MED on lipogenesis and lipolysis are similar to those of previous studies that investigated the lipogenesis-inhibiting and lipolysis-stimulating effects of natural products.

Inflammatory responses can damage hepatocytes because proinflammatory cytokines can cause adipocyte hypertrophy and adipose tissue disorders (26), and thus regulators of inflammatory responses are considered targets of anti-obesity therapy (47). In particular, NLRP3 inflammasome is activated by cellular stress response and obesity-induced inflammatory response in adipocytes and promotes the autoactivation of Cas-1, which induces the activations of mediators of immune response (48, 49). Also, IL-1β stimulates the secretions of IL-6 and TNF-α to regulate the migration and infiltration of immune cells, while IL-18 promotes the recruitment and activation of immune cells (48, 50). Interestingly, adipose tissue-derived inflammation in a high-fat diet-induced model of obesity was effectively suppressed by several natural products (29–31). Representative, Mulberry leaf and fruit extract significantly decreased hepatic levels of TNF-α and IL-1β, though iNOS levels were not significantly suppressed (29). Also, another natural product, *Malus hupehensis* administration, promotes reducing serum levels of TNF-α, IFN-γ, IL-1β, and IL-6 (31). MED suppressed the transcript levels of the proinflammatory cytokines IL-1β, IL-6, TNF-α, and NF-κB and also reduced the mRNA expressions of three inflammasome regulators in the livers of *Lep* KO mice. Our results provide the first evidence that MED acts by targeting obesity-induced inflammatory response in *Lep* KO mice.

The changes in β-oxidation-related protein levels provide a method for evaluating the anti-obesity effects of natural products. The β-oxidation of FAs produces energy by breaking them down into CO_2 and ketone bodies. A number of several enzymes, including acyl-CoA oxidase-1 (ACO-1), carnitine palmitoyltransferase-1 (CPT-1), and acyl-CoA dehydrogenases (ACADs), which are expressional regulated by PPARα, are involved in this process (51). According to reports, several natural products stimulate the β-oxidation of FAs in high fat diet-induced obese animal models (30, 31). The water-soluble extract of *Cucurbita moschata* was found to increase ACO-1 and CPT-1 by upregulating PPARα (30), while the treatment of Mulberry leaf and fruit extracts was reported to significantly upregulate PPARα and CPT1 mRNA expression in liver (29). In the present study, MED increased the protein expressions of ACADs and ACO in *Lep* KO mice by upregulating the mRNA levels of PPARα. The effects of MED on β-oxidation stimulation are similar to those previously reported for other natural products. Additionally, our findings indicate that MED acts as a β-oxidation stimulator.

Even though the anti-obesity effects of MED are proved in various targets in this study, since MED is a mixture of complex compounds, it is hard to explain the mechanism of its action on adipose tissue clearly. According to the chemical profile of MED, gallic acid, bergenin, ellagic acid, ϵ-viniferin, asiatic acid, oleanolic acid, and 2α-hydroxyursolic acid are regarded as the main components, and these individual components have been reported to show anti-obesity effects. Gallic acid decreased body weights and adipose tissue weights of peritoneal and epididymal tissues in addition to the serum TAG, phospholipid, total cholesterol, and LDL-cholesterol in high-fat diet-fed rats (52). Bergenin, olanolic acid, and ϵ-viniferin also lowered body weight gain and the weight of adipose tissue in obese mice (53–55). Moreover, asiatic acid attenuated body weight gain, tissue lipids, mRNA levels of PPAR γ, FAS, aP2, and inflammatory factor TNF-α in HFD-fed rats (56). Considering that all of these components are included in MED, MED has a high potential to be a novel treatment for obesity.

5 Conclusions

This study was performed to determine the therapeutic effects of MED on obesity using *Lep* KO mice. MED-induced alterations in body weights, food intakes, serum lipid profiles, and lipid accumulation were analyzed in *Lep* KO mice after treatment for 4 weeks. In addition, we investigated changes in the expression of genes involved in lipid accumulation, inflammation, and β-oxidation. Our results provide scientific evidence that MED has considerable potential to prevent obesity and ameliorate its effects.

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

The animal study was reviewed and approved by Institutional Animal Care and Use Committee of Pusan National University (PNU-IACUC).

Author contributions

Conceptualization, DH. Methodology, DH and SC. Software, SL. Validation, SL. Formal analysis, SL. Investigation, SL, YJ, JK, YR, AS, HS, JP and SP. Resources, BD and OS. Data curation, SL and YJ. Writing and original draft preparation, DH. Writing, reviewing, and editing, SC. Visualization, DH and SC. Supervision, DH. Project administration, DH. Funding acquisition, DH. All authors contributed to the article and approved the submitted version.

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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/fendo.2023.1167285/full#supplementary-material

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Higher visceral adiposity index was associated with an elevated prevalence of gallstones and an earlier age at first gallstone surgery in US adults: the results are based on a cross-sectional study

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Objective: We sought to evaluate the association between visceral adiposity index (VAI) and the incidence of gallstones and the age at first gallstone surgery in adults in the United States.

Methods: We selected individuals from the National Health and Nutrition Examination Survey (NHANES) database from 2017 to 2020 and evaluated the association between VAI and gallstone incidence and age at first gallstone surgery using logistic regression analysis, subgroup analysis, and dose–response curves.

Results: A total of 7,409 participants aged >20 years were included in our study; 767 had a self-reported history of gallstones. After adjustment for all confounding factors, for each unit of VAI after Ln conversion, gallstone prevalence increased by 31% (OR = 1.31, 95% CI: 1.17, 1.48), while the first gallstone surgery was 1.97 years earlier (β = −1.97, 95% CI: −3.35, −0.42). The dose–response curves showed a positive correlation between VAI and gallstone prevalence. There was a negative correlation between increased VAI and age at first gallstone surgery.

Conclusion: A higher VAI is positively associated with the prevalence of gallstones and may lead to an earlier age at first gallstone surgery. This is worthy of attention, although causality cannot be established.

KEYWORDS

gallstone prevalence, VAI, cross-sectional study, age at first gallstone surgery, metabolic syndrome

Introduction

Gallstones are one of the most common diseases of the digestive system worldwide and are a clear risk factor for gallbladder cancer (1, 2). Gallstones are a significant health care burden in America, affecting up to 15% of the population (3, 4). Epidemiologic evidence suggests that the prevalence of gallstones is 10% to 15% in adult Caucasians and as high as 70% in American Indians (5, 6). However, the prevalence in Asian populations is low (7, 8). Gallstones are mainly divided into cholesterol stones, melanin stones, and mixed stones, among which cholesterol stones and cholesterol components, mainly mixed stones, account for more than 80% of all stones (9, 10). In general, gallstones do not cause any symptoms, but 10% to 25% of patients may have specific symptoms such as biliary pain and acute cholecystitis, of which 1% to 2% may have major complications (1, 11, 12), causing endless pain and even being life-threatening to patients. Although previous studies have reported risk factors for gallstone formation, there is still a lack of reliable clinical indicators to prevent the occurrence of gallstones.

Current evidence suggests that non-modifiable risk factors for the development of gallstones include race, female gender, pregnancy, and age over 40 years, with a 4- to 10-fold increased risk of gallbladder disease (3, 4). Women of childbearing age are about twice as likely as men to develop gallstone disease (4). The most important modifiable risk factor for the development of gallstones is metabolic syndrome (13). This includes obesity, dyslipidemia, type 2 diabetes mellitus, insulin resistance, etc. Obesity, especially abdominal obesity (about 25% of the population has abdominal obesity), is closely related to the occurrence of gallstones (3). Some studies have shown that obesity is a risk factor for gallstones (14–16), and the incidence of gallstones has increased 1.63 times for every five-unit increase in body mass index (15). Although obesity is strongly associated with the occurrence of gallstones, reliable obesity indicators to predict and assess the risk of gallstones are severely lacking.

As the main form of energy storage in the human body, adipose tissue is the regulator of lipid metabolism and glucose balance (17). Studies have shown that visceral adipose tissue is more closely associated with metabolic diseases such as hypertension, diabetes, and cardiovascular disease than is subcutaneous fat (18–20). The Visceral Fat Index (VFI), an indicator of abdominal fat distribution and adipose tissue function that indirectly expresses visceral fat function based on waist circumference, BMI, triglycerides, and HDL cholesterol, is a novel specificity index (21). It has a lower practice cost and better applicability than the traditional methods used to assess body fat content and distribution (magnetic resonance imaging, computed tomography, dual-energy X-ray absorptiometry, etc.). Some scientists believe that VAI has higher sensitivity and specificity than traditional body parameters such as body mass index and waist circumference. Studies have shown that the clinical application of VAI can significantly improve the risk assessment of obesity-related cardiovascular diseases (21–23). In conclusion, we speculate that there is a relationship between VAI and the occurrence of gallstones, and therefore, in this study, we aimed to evaluate the value of VAI in the occurrence of gallstones in the US adult population.

Materials and methods

Study design and participants

The baseline clinical data evaluated in this study were from the 2017–2020 NHANES. We retained information on participants who explicitly answered whether they had gallstones and their age at first gallstone surgery. A total of 15,560 individuals completed the questionnaire. Exclusion criteria were as follows (Figure 1). Finally, a total of 7,409 cases were included in this study, including 767 self-reported histories of gallstones.

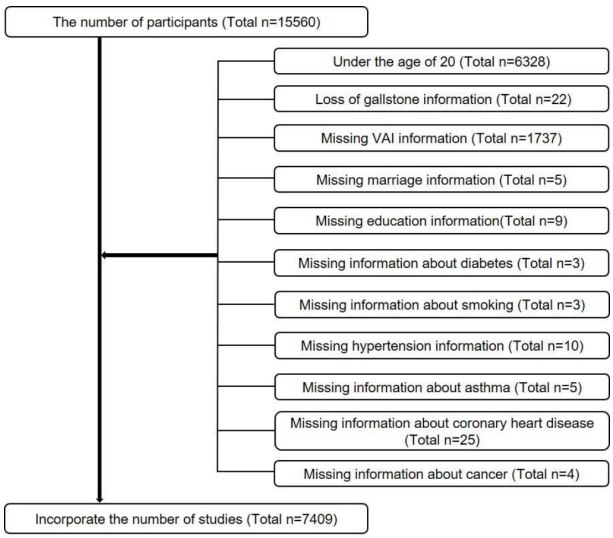

FIGURE 1
The participants selection flow chart.

Collection and definition of data

The VAI was designed as an exposure variable and was calculated using the following sex-specific equations, where the units for WC, BMI, TG, and HDL are cm kg/m^2 and mmol/L: males: VAI = [WC/[39.68 + (1.88 × BMI)]) × (TG/1.03) × (1.31/HDL); women: VAI = [WC/[36.58 + (1.89 × BMI)]) × (TG/0.81) × (1.52/HDL)]. The concentrations of triglycerides and fasting blood glucose were determined enzymatically using an automated biochemical analyzer. Serum triglyceride concentrations were measured using a Roche Modular P and a Roche Cobas 6000 chemistry analyzer. A questionnaire was used to assess the presence of gallstones and age at first gallstone surgery. The development of gallstones and age at first gallstone surgery were used as outcome variables.

Potential covariates that could confound the association between VAI and gallstones were summarized in the multivariable-adjusted model. Covariates in our study included sex (male/female), age (years), ethnicity, education level, poverty income ratio (PIR), marital status (married or living with a partner/single), alcohol consumption (drinking alcohol/not drinking alcohol), physical activity (vigorous/moderate/less than moderate), cholesterol level (mg/dl), smoking status, hypertension, diabetes, asthma, hypertension, cancer, and dietary intake factors, including energy intake, fat intake, sugar intake, and water intake. In 2017–2020, all participants had 24-hour dietary recalls; our analysis will use the average consumption rate for the two recalls. All detailed measurement procedures using the study variables are publicly available at www.cdc.gov/nchs/nhanes/. All NHANES protocols were conducted in accordance with the US Department of Health and Human Services (HHS) Human Research Subjects Protection Policy and were reviewed and standardized annually by the NCHS Research Ethics Review Board. All subjects participating in the study signed an informed consent form. All data in this study were freely released by NHANES without additional permission or ethical review.

Statistical methods

The NHANES sampling weights, stratification, and clustering provided in the study were applied to all statistical analyses to illustrate the complex, multistage sampling design used to select a representative non-institutionalized U.S. population. Continuous variables were expressed as weighted survey means and 95% CIs, and categorical variables were expressed as weighted survey means and 95% CIs. As VAI have skewed distributions, LN transformations convert them into normal distributions. We first screened all covariates for VIF collinearity and removed them if VIF values greater than 5 were considered collinear. As VAI have skewed distributions, LN transformations convert them into normal distributions. According to the guidelines (24, 25), the multiple logistic regression model was used to examine the relationship between VAI, different VAI tiles, gallstone prevalence, and age of first gallstone surgery in the three different models. Model 1 was unadjusted for covariates. Model 2 was adjusted for sex, age, ethnicity, marital status, and education level. Model 3 was adjusted for all variables. Smoothing curve fitting (penalty spline method) and generalized additive model (GAM) regression were performed to further evaluate the relationship between VAI and gallstone prevalence and age at first gallstone surgery. The inflection point values were obtained by the natural ratio test when the presence of nonlinear relationships was determined. Multiple regression analysis was then performed, stratified by sex, age, race, hypertension, and diabetes. A $p < 0.05$ was considered statistically significant. All analyses were performed using Empower software (www.empowerstats.com; X&Y Solutions, Inc., Boston, MA, USA) and R version 4.0.2 (http://www.R-project.org, The R Foundation).

Results

Participant characteristics

The baseline demographic characteristics of the included participants are shown in Table 1. Ln (VAI) was 2.69 (2.37, 3.01) in the gallstone group, higher than the normal group of 2.17 (2.06, 2.27), P <0.01.

Logistic regression results between VAI and gallbladder stones

VIF collinearity screened all covariates with VIF values <5, and all variables were included in the final regression model. For gallstones, a positive correlation was observed between VAI and gallstones. This positive correlation remained stable in the fully adjusted model (model 3) (OR = 1.31, 95% CI: 1.17, 1.48), indicating that each unit increase in Ln-converted VAI was associated with a 31% increase in gallstone prevalence. We also converted VAI from continuous to categorical variables (tertiles) for sensitivity analysis. A significant 0.8-fold increase in gallstone incidence was observed in tertile 3 compared with the lowest VAI tertile (tertile 1) (OR = 1.80, 1.45, 2.25) (Table 2).

VAI's dose–response and threshold effect on gallbladder stone prevalence

A generalized additive model and smooth curve fitting were used to further explore the relationship between Ln (VAI) and gallstone incidence. Our results indicated a non-linear relationship between Ln (VAI) and gallstone incidence (Figure 2; Table 3). Considering the effect of the saturation threshold between them, the likelihood natural ratio test found the best Ln-transformed VAI threshold at 0.6.

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TABLE 1 Baseline characteristics of participants, weighted.

Characteristic	Non-stone formers (n = 6,642)	Stone formers (n = 767)	*P*-value
Age (years)	47.17 (46.01, 48.33)	56.67 (55.38, 57.97)	<0.0001
Serum Creatinine (mg/dl)	0.88 (0.87, 0.89)	0.86 (0.83, 0.88)	0.0688
Ln (VAI)	2.17 (2.06, 2.27)	2.69 (2.37, 3.01)	0.0062
Race (%)			0.0004
Mexican American	8.53 (6.34, 11.37)	7.86 (5.54, 11.02)	
White	70.50 (65.96, 74.66)	77.36 (71.74, 82.13)	
Black	11.13 (8.45, 14.51)	6.59 (4.83, 8.95)	
Other Race	9.85 (7.94, 12.15)	8.19 (5.49, 12.06)	
Physical Activity (%)			<0.0001
Vigorous	50.01 (47.99, 52.04)	33.53 (28.80, 38.62)	
Moderate	27.89 (25.63, 30.27)	36.49 (31.01, 42.34)	
Never	22.09 (20.66, 23.59)	29.98 (25.58, 34.77)	
Marital Status (%)			0.0001
Cohabitation	62.39 (59.70, 65.01)	64.97 (59.22, 70.31)	
Solitude	17.63 (16.24, 19.12)	22.89 (18.75, 27.63)	
Never married	19.98 (17.93, 22.20)	12.14 (9.01, 16.18)	
Alcohol (%)			<0.0001
Yes	13.93 (12.62, 15.36)	26.76 (21.67, 32.55)	
No	76.61 (74.85, 78.28)	62.62 (58.15, 66.87)	
Unclear	9.46 (8.24, 10.84)	10.63 (7.89, 14.17)	
High Blood Pressure (%)			<0.0001
Yes	30.31 (28.09, 32.64)	48.46 (42.31, 54.66)	
No	69.69 (67.36, 71.91)	51.54 (45.34, 57.69)	
Diabetes (%)			<0.0001
Yes	10.05 (9.14, 11.04)	20.20 (17.43, 23.29)	
No	89.95 (88.96, 90.86)	79.80 (76.71, 82.57)	
Asthma (%)			0.0749
Yes	14.84 (13.52, 16.26)	18.29 (14.43, 22.90)	
No	85.16 (83.74, 86.48)	81.71 (77.10, 85.57)	
Coronary Heart Disease (%)			0.0001
Yes	3.72 (2.53, 5.42)	7.31 (5.21, 10.17)	
No	96.28 (94.58, 97.47)	92.69 (89.83, 94.79)	
Cancers (%)			<0.0001
Yes	10.17 (9.26, 11.17)	17.95 (13.91, 22.84)	
No	89.83 (88.83, 90.74)	82.05 (77.16, 86.09)	
Smoked (%)			0.0402
Yes	42.24 (40.35, 44.15)	47.76 (41.31, 54.29)	
No	57.76 (55.85, 59.65)	52.24 (45.71, 58.69)	
PIR			0.0021
<1.3	16.26 (14.79, 17.85)	15.67 (11.87, 20.41)	
≥1.3–<3.5	30.06 (27.38, 32.88)	39.67 (32.55, 47.25)	
≥3.5	42.81 (39.69, 45.99)	35.55 (31.01, 40.36)	
Unclear	10.86 (9.32, 12.63)	9.11 (6.65, 12.35)	
Total Sugar (%)			0.0185
Lower	41.96 (39.96, 43.99)	39.96 (35.22, 44.91)	
Higher	40.18 (38.56, 41.83)	46.16 (41.43, 50.96)	
Unclear	17.86 (16.24, 19.60)	13.88 (11.48, 16.68)	
Total Kcal (%)			0.0003
Lower	38.50 (36.61, 40.43)	47.62 (42.52, 52.77)	
Higher	43.64 (42.30, 44.99)	38.50 (33.10, 44.21)	
Unclear	17.86 (16.24, 19.60)	13.88 (11.48, 16.68)	
Total Fat (%)			0.0004
Lower	38.05 (36.65, 39.46)	47.02 (41.76, 52.35)	
Higher	44.09 (42.64, 45.55)	39.10 (33.68, 44.80)	

(Continued)

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TABLE 1 Continued

Characteristic	Non-stone formers (n = 6,642)	Stone formers (n = 767)	*P*-value
Unclear	17.86 (16.24, 19.60)	13.88 (11.48, 16.68)	
Total Water (%)			0.0208
Lower	35.20 (33.30, 37.15)	41.25 (36.63, 46.04)	
Higher	46.94 (44.73, 49.16)	44.87 (39.30, 50.57)	
Unclear	17.86 (16.24, 19.60)	13.88 (11.48, 16.68)	

Data of continuous variables are shown as survey-weighted mean (95% CI), P-value was calculated by survey-weighted linear regression. Data of categorical variables are shown as survey-weighted percentage (95% CI), P-value was calculated by survey-weighted Chi-square test.

Subgroup analysis

Subgroup analyses were performed to assess the robustness of the association between Ln (VAI) and gallstone incidence. The results were as follows (Table 4):

- Female group (OR = 1.39, 95% CI: 1.20, 1.62),
- Age group 20–39 years (OR = 1.62, 95% CI: 1.21, 2.16),
- Age group 40–59 years (OR = 1. 26, 95% CI: 1.04, 1.54),
- White group (OR = 1.32, 95% CI: 1.12, 1.56),
- Other population groups (OR = 1.61, 95% CI: 1.19, 2.18),
- Hypertension population group (OR = 1. 27, 95% CI: 1.07, 1.50),
- Non-hypertensive population group (OR = 1.33, 95% CI: 1.12, 1.57),
- Diabetic population group (OR = 1.31, 95% CI: 1.02, 1.67),
- Non-diabetic population group (OR = 1.31, 95% CI: 1.15, 1.50).

Elevated VAI may be associated with earlier age at first gallbladder stone surgery

The unit of Ln (VAI) was 1.97 years earlier (β = −1.97, 95% CI: −3.35, −0.42) in fully adjusted model 3 (Table 5).

Analysis of the dose–response and threshold effects of VAI on age at first gallbladder stone surgery

To further investigate the relationship between VAI and age at first gallstone surgery, a generalized additive model and smooth curve fitting were used. Our results indicated a negative nonlinear correlation between VAI and age at first gallstone surgery (Figure 3). The threshold for the effect of VAI on age at first gallstone surgery after Ln conversion was 0.8, according to the similar natural ratio test (Table 6).

Discussion

To our knowledge, this study is the first comprehensive analysis of the association between VAI and gallstones. Two-cycle population study (2017–2020) based on the NHANES database. The results showed a positive association between VAI and gallstone incidence, with each unit increase in VAI increasing the incidence by 31% (OR = 1.31, 95% CI: 1.17, 1.48). Furthermore, we found that increased VAI was associated with an earlier age of first gallstone surgery, with each unit increase in VAI leading to an earlier age of 1.97 years (beta = −1.97, 95% CI: −3.35, −0.42). At the same time, we converted VAI from a continuous variable to a categorical variable (tertiles) and found that the probability of gallstones in individuals with the highest VAI was 0.8 times higher than the lowest quantile, and the prevalence of individuals with the highest VAI was 0.61 times higher than the lowest quantile. Furthermore, in the stratified analysis, we found a higher risk of gallstone disease in the female group (OR = 1.39, 95% CI: 1.20, 1.62), 20–39 (OR = 1.39, 95% CI: 1.04, 1.54:1.32, 1.62), and other population groups (OR = 1.56) (OR = 1.61, 95% CI: 1.19, 2.18). Moreover, the incidence of gallstones was positively associated with increased VAI in hypertensive, non-hypertensive, diabetic, and non-diabetic groups. These results strongly support the value of VAI as a predictor of gallstone development.

The formation of gallstones results from a combination of genetic factors and environmental stimuli. In particular, dietary factors may directly or indirectly lead to the occurrence of gallstones, such as overweight, obesity (26), insulin resistance (13), and metabolic syndrome (27). While VAI is a more specific index than BMI and triglycerides, our results suggest that the female group and white group

TABLE 2 Logistic regression analysis between VAI with gallbladder stone prevalence.

Characteristic	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Ln (VAI)	1.52 (1.37, 1.68)	1.41 (1.26, 1.57)	1.31 (1.17, 1.48)
Categories			
Tertile 1	1	1	1
Tertile 2	2.02 (1.64, 2.49)	1.73 (1.40, 2.14)	1.61 (1.30, 2.00)
Tertile 3	2.52 (2.06, 3.09)	2.08 (1.68, 2.56)	1.80 (1.45, 2.25)

Model 1 = no covariates were adjusted.
Model 2 = Model 1 + age, gender, race education, and marital status were adjusted.
Model 3 = Model 2 + diabetes, blood pressure, education, PIR, asthma, total water, total kcal, total fat, total sugar, smoked, physical activity, alcohol use, serum creatinine, serum cholesterol, cancers, and CVD were adjusted.

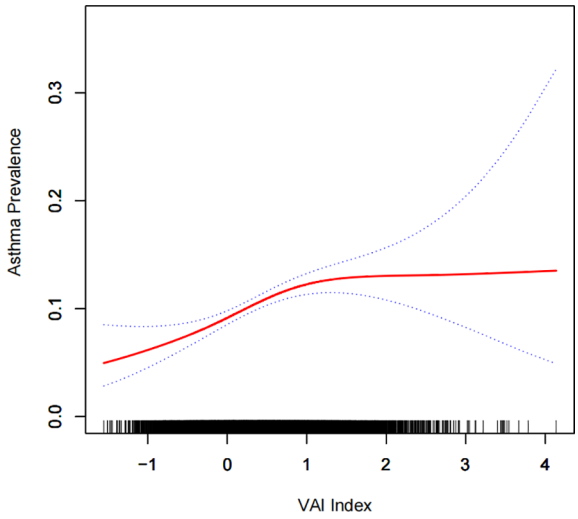

FIGURE 2
Density dose-response relationship between the Ln (VAI) index and gallstone prevalence. The area between the upper and lower dashed lines is represented as the 95% CI. Each point shows the magnitude of the Ln (VAI) index and is connected to form a continuous line. Adjusted for all covariates except the effect modifier.

increased VAI levels in the age group, 60-year-old group, hypertensive/non-hypertensive group, and diabetic/non-diabetic group after adjusting for all confounding factors. Numerous previous studies have shown that women have a higher prevalence of gallstones than men (28, 29). Estrogen increases gallstone formation by increasing hepatic cholesterol synthesis and secretion and decreases bile salt synthesis by upregulating estrogen receptor 1 and G protein-coupled receptor 30 (30). Progestational sex hormones are thought to put women at greater risk for disease, and estrogen can increase the release of cholesterol into the bile, leading to cholesterol saturation and gallbladder stones (31). This may partly explain why the prevalence of gallstones was higher in women than in men. In addition, the results of a Korean study showed that high VAI levels were associated with a high prevalence of asymptomatic cerebral infarction in a healthy population, especially in the female population (32). Although this study was not related to gallstones, it also shows the reliability of our study results.

In the United States, the prevalence of gallstones was 16.6% in white women and 8.6% in men, compared with 13.9% in black women and 5.3% in men (7, 33). In developed countries, 10% to 15% of white adults have gallstones, compared with a lower incidence of gallstones in the black population (5). These results suggest that white Americans have a higher prevalence of gallstones, which may explain our findings. In fact, age as a risk factor for gallstones is controversial. Gallstones were once thought to be associated with pigment stones that occur only in cases of hemolysis, but they are increasingly common in children (34). The incidence of gallstones increases with age and rises significantly after the age of 40, with a 4- to 10-fold increase in the elderly (5). Although gallstones are usually clinically asymptomatic, symptoms and serious complications increase with age, leading to cholecystectomy in over 40% of people over 40 years of age (35). The high prevalence of gallstones may occur in older women (70 to 79 years): 57% have a history of cholecystectomy or current gallstones (8). Therefore, the influence of age on gallstone formation should be further investigated.

In hepatocytes, insulin resistance induces abnormal expression of the transcription factor forkhead box protein O1 (FOXO1) through the ABCG5 and ABCG8 genes to promote cholesterol secretion (36). This mechanism may explain the high prevalence of gallstones in diabetic patients, which is consistent with our findings. Interestingly, our results showed that elevated VAI levels were also positively associated with increased gallstone incidence in the non-diabetic group, with its OR value equal to the statistical results in the diabetic group. In a cross-sectional study by Ali, 204 patients with gallstones were included, of whom 74 were diabetic, 79 were non-diabetic, 51 were pre-diabetic, one had well-controlled diabetes, and one had poorly controlled diabetes (37). The results indicate that diabetes is a risk factor for gallstones, and non-diabetes also seem to be associated with the occurrence of gallstones. Furthermore, in a

TABLE 3 Two-piecewise linear regression and logarithmic likelihood ratio test explained the threshold effect analysis of VAI with gallbladder stone prevalence.

Ln (VAI)	ULR Test	PLR Test	LRT test
	OR (95% CI)	OR (95% CI)	P-value
<0.6 umol/L	1.28 (1.14, 1.44)	1.72 (1.34, 2.21)	0.006
≥0.6 umol/L		1.02 (0.84, 1.25)	

ULR, univariate linear regression; PLR, piecewise linear regression; LRT, logarithmic likelihood ratio test, statistically significant: p <0.05.

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TABLE 4 Subgroup analysis between VAI with gallbladder stone prevalence.

Characteristic	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Stratified by age (years)	Non-adjusted	Adjust I	Adjust II
20–39	1.71 (1.37, 2.14)	1.95 (1.50, 2.52)	1.62 (1.21, 2.16)
40–59	1.37 (1.15, 1.62)	1.37 (1.14, 1.65)	1.26 (1.04, 1.54)
60–80	1.44 (1.24, 1.68)	1.30 (1.10, 1.52)	1.19 (0.99, 1.42)
Stratified by gender	Non-adjusted	Adjust I	Adjust II
Male	1.27 (1.07, 1.50)	1.27 (1.06, 1.54)	1.22 (0.99, 1.50)
Female	1.70 (1.49, 1.93)	1.53 (1.33, 1.75)	1.39 (1.20, 1.62)
Stratified by race	Non-adjusted	Adjust I	Adjust II
Mexican American	1.16 (0.89, 1.52)	1.04 (0.75, 1.43)	1.07 (0.75, 1.51)
White	1.49 (1.30, 1.72)	1.45 (1.25, 1.68)	1.32 (1.12, 1.56)
Black	1.67 (1.32, 2.12)	1.48 (1.15, 1.91)	1.31 (0.99, 1.72)
Other Race	1.53 (1.17, 2.00)	1.52 (1.15, 2.01)	1.61 (1.19, 2.18)
Stratified by hypertension	Non-adjusted	Adjust I	Adjust II
YES	1.40 (1.21, 1.62)	1.31 (1.12, 1.54)	1.27 (1.07, 1.50)
NO	1.48 (1.28, 1.70)	1.38 (1.18, 1.62)	1.33 (1.12, 1.57)
Stratified by diabetes	Non-adjusted	Adjust I	Adjust II
YES	1.40 (1.14, 1.72)	1.29 (1.03, 1.63)	1.31 (1.02, 1.67)
NO	1.44 (1.28, 1.61)	1.34 (1.18, 1.53)	1.31 (1.15, 1.50)

Model 1 = no covariates were adjusted.
Model 2 = Model 1 + age, gender, race education, and marital status were adjusted.
Model 3 = adjusted for all covariates except effect modifier.

TABLE 5 Analysis between VAI with age at the first gallbladder stone operation.

Characteristic	Model 1 β (95% CI)	Model 2 β (95% CI)	Model 3 β (95% CI)
Ln (VAI)	−0.65 (−2.30, 1.01)	−0.94 (−2.52, 0.64)	−1.97 (−3.53, −0.42)

Model 1 = no covariates were adjusted.
Model 2 = Model 1 + gender, race education, and marital status were adjusted.
Model 3 = Model 2 + diabetes, blood pressure, education, PIR, asthma, total water, total kcal, total fat, total sugar, smoked, physical activity, alcohol use, serum creatinine, serum cholesterol, cancers, and CVD were adjusted.

Korean study, metabolic syndrome was associated with gallstone development in non-hypertensive and non-diabetic patients (38). The study by Chen (39) showed that the elevated metabolic syndrome specific index was associated with increased asthma prevalence in non-hypertensive and non-diabetic populations, while Shen's study found a correlation between the METS-IR index and the prevalence of kidney stones in non-hypertensive and non-diabetic populations (40). Our results showed a strong correlation between the prevalence of gallstones in the non-hypertensive group (OR = 1.33, 95% CI: 1.12, 1.57) and in the hypertensive group (OR = 1.27, 95% CI: 1.07, 1.50). The above two research topics are not specific to gallstones. However, they also demonstrate the reliability of our experimental results. In addition, we found an interesting result: for every 1 unit of VAI, the age of first gallstone surgery was 1.97 years earlier, and the smooth curve fitting showed a non-linear negative correlation between VAI and the age of first gallstone surgery. This result has not been reported before, and if it is confirmed by more studies, it reminds us that more attention and management of VAI at a young age will help to reduce the occurrence of gallstones.

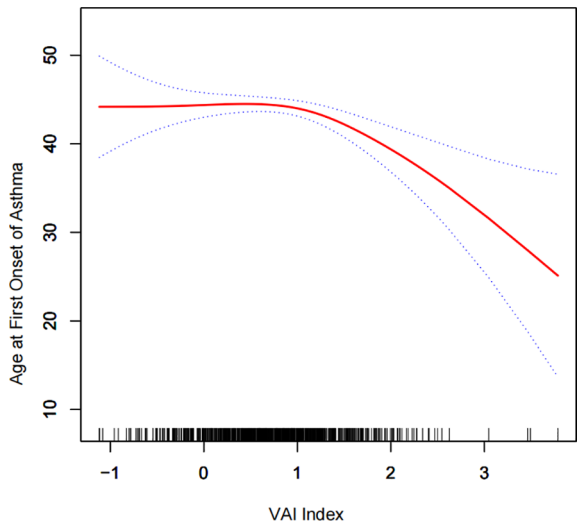

FIGURE 3
Density dose–response relationship between Ln (VAI) index and age at first gallstone surgery. The area between the upper and lower dashed lines is represented as the 95% CI. Each point shows the magnitude of the VAI and is connected to form a continuous line. Adjusted for all covariates except the effect modifier.

TABLE 6 Two-piecewise linear regression and logarithmic likelihood ratio test explained the threshold effect analysis of VAI with age at the first gallbladder stone operation.

Ln (VAI)	ULR Test	PLR Test	LRT test
	OR (95% CI)	OR (95% CI)	P-value
<0.8 umol/L	−1.97 (−3.53, −0.42)	0.55 (−1.55, 2.66)	<0.001
≥0.8 umol/L		−8.59 (−12.64, −4.54)	

ULR, univariate linear regression; PLR, piecewise linear regression; LRT, logarithmic likelihood ratio test, statistically significant: p <0.05.

Our study has several strengths. First, the study participants in NHANES are a representative sample of the U.S. who strictly followed the carefully designed study protocol with strict quality control and assurance to ensure that our conclusions are reliable. Second, we adjusted for confounding variables and performed subgroup analysis to ensure that our results apply to a broader range of individuals. However, our study has several limitations. First, our study was a cross-sectional study, which did not allow us to clarify the causal relationship between VAI and gallstones. Second, all the survey data were based on questionnaires, and there may be recall bias. Despite these limitations, this paper is the first to reveal the relationship between VAI and the prevalence of gallstones.

Conclusion

This study showed an association between the modifiable risk factor VAI, the prevalence of gallbladder stones, and age at first gallbladder stone surgery. A higher VAI was associated with an increased prevalence of gallbladder stones and an earlier age for first gallbladder stone surgery. Our findings suggest that weight control and a healthy lifestyle may improve or reduce the occurrence of gallbladder stones, and although the causal relationship between the two cannot be clearly established, it is still of interest.

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 in the article/supplementary material.

Ethics statement

The NCHS Research Ethics Review Committee approved the NHANES survey protocol. The patients/participants provided their written informed consent to participate in this study.

Author contributions

GZ and ZD: Conceptualization, methodology, and software. JY, TW, and LT: Data curation and writing original draft. JC and CZ: Writing—review and editing. All authors contributed to the article and approved the submitted version.

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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

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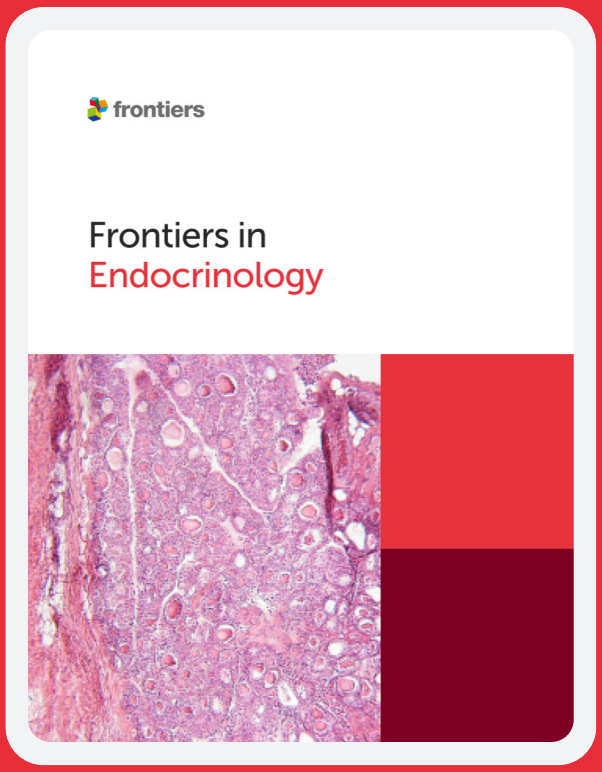

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