frontiers | Research Topics

Women in nutritional epidemiology

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Published in
Frontiers in Nutrition

ISSN 1664-8714
ISBN 978-2-83251-606-5
DOI 10.3389/978-2-83251-606-5

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Women in nutritional epidemiology

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Citation

Rodriguez, R. C., Tresserra-Rimbau, A., Castaner, O., Bresciani, L., eds. (2023). *Women in nutritional epidemiology*. Lausanne: Frontiers Media SA.

doi: 10.3389/978-2-83251-606-5

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frontiers | Frontiers in Nutrition

TYPE Editorial
PUBLISHED 03 February 2023
DOI 10.3389/fnut.2023.1147856

OPEN ACCESS

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

RECEIVED 19 January 2023
ACCEPTED 20 January 2023
PUBLISHED 03 February 2023

CITATION
Castañer O, Tresserra-Rimbau A, Bresciani L and Casas R (2023) Editorial: Women in nutritional epidemiology.
Front. Nutr. 10:1147856.
doi: 10.3389/fnut.2023.1147856

Editorial: Women in nutritional epidemiology

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KEYWORDS

women in science, sex differences, biomarkers, cancer, biomedicine, health outcomes, food sustainability

Editorial on the Research Topic
Women in nutritional epidemiology

According to data from the UNESCO Institute for Statistics for 107 countries during 2015–2018, the participation of women represents only a third part of worldwide researchers (1). Gender balance is close to 50% in some regions, such as Central Asia, Latin America and the Caribbean, but this proportion is reduced to 30–35% in other regions, such as Europe and North America (2). Women are still critically underrepresented in scientific professions, and this underrepresentation is greater in some fields such as ICT (information and communications technology), and STEM (Science, Technology, Engineering, and Mathematics) research in particular (3). Throughout history, female researchers have faced a large number of systemic barriers (e.g., gender gap, pay gap, undervalued work or implicit bias) to advance in their global professional careers. Of notice, research women are less likely to be named as authors in the research team and their contributions are less acknowledged than their male counterparts (4). Women have usually received less scientific recognition. An example is Rosalind Franklin, a British scientist, whose contribution to the discovery of the structure of DNA was not recognized until long after her death. In the years since her death, she has won recognition among scientists for her research on the original Crick and Watson paper which had been denied by the scientific community (5). Recently, we can also find other examples such as the case of Jennifer Doudna and Emmanuelle Charpentier, who awarded the Nobel Prize in Chemistry in 2020 (6). They discovered one of gene technology's sharpest tools: the CRISPR/Cas9 genetic scissors. However, their contribution to this discovery had been relegated to a second position until then. It is essential to recognize the achievements of women in science. For that purpose, the aim of this Research Topic, *Women in nutritional epidemiology,* is to acknowledge achievements of women in both health and science fields related to nutritional epidemiology.

The issue collects 9 works presented by 9 excellent women researchers who work in the field of biomedicine and nutrition. The Research Topics include studies that associate food patterns or specific food components to obesity related factors, such as satiety, adiposity, and cardiometabolic factors. It also includes two articles in the context of hormonal-related cancers, such as ovarian and breast cancer, as well as a couple of studies investigating the association

between biomarkers and health outcomes. Finally, two articles evaluated sex differences in nutritional studies and a test on the knowledge of food sustainability among youngsters.

Among the published articles of this Research Topic, two systematic reviews and meta-analysis were published. Liu et al. identified the current knowledge gaps on the impact of different heavy metals on breast cancer. Parilli-Moser et al. found moderate evidence on regular consumption of peanuts and the modulation of lipid metabolism, reducing triglyceride blood levels, without promoting weight gain. A randomized clinical trial led by Hernando-Redondo et al. provided high-quality evidence on both mid-and long-term satiety hormones, as a pertinent approach to weight loss on a weight loss intervention with a hypocaloric Mediterranean diet and physical activity promotion. Gong et al. presented the ovarian cancer follow-up study (OOPS) protocol, which is an on-going hospital-based large prospective longitudinal cohort study better understand the linkage between biospecimens and clinical data collected throughout the patient treatment, and reveal additional information about the prognosis of ovarian cancer. Moreover, three cross-sectional studies were published on this topic. The first one did not show conclusive results, since different levels of association between hemoglobin levels and preterm birth were observed (Elmugabil et al.). The second study, conducted by Laveriano-Santos et al., showed potential therapeutic effects of cocoa flavonoids against obesity, demonstrating an association between high cocoa consumption and lower risk of presenting abdominal obesity and better adiposity parameters. Finally, de Moraes Prata Gaspar et al. pointed out the importance to implement more sustainable practices within the university community. These conclusions were derived from results of 1,220 participants that completed the survey to evaluate the level of knowledge and perceptions of food sustainability in a university community from Spain. Additionally, a case-control study, which included 217 gestational diabetes mellitus (GDM) cases and 217 matched controls conducted by Li et al., suggested that the combinations of circulating fatty acids could be a significant marker of GDM development compared to individual fatty acids or their subgroups. Lastly, Garrabou et al. showed how sex influence is frequently underrated not only in biomedicine, also in nutritional and molecular medicine, leading to bias in scientific analyses.

Relevant scientific topics related to nutritional epidemiology leaded by women are highlighted in this Research Topic, which aims to encourage other women to continue contributing in this field, as well as in other scientific fields.

Author contributions

OC and RC wrote the draft. All authors revised, discussed, and modified the text, agreed on the content, contributed to the article, and approved the submitted version.

Funding

This work was supported by MICIN/AEI/FEDER, UE (grant CEX2021-001234-M).

Acknowledgments

We want to thank all research women that have encouraged to continue breaking gender barriers and inspire the youngest researcher women to share their scientific contributions in this field.

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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SYSTEMATIC REVIEW
published: 01 April 2022
doi: 10.3389/fnut.2022.853378

Effect of Peanut Consumption on Cardiovascular Risk Factors: A Randomized Clinical Trial and Meta-Analysis

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OPEN ACCESS

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

Received: *12 January 2022*
Accepted: *14 March 2022*
Published: *01 April 2022*

Citation:
Parilli-Moser I, Hurtado-Barroso S, Guasch-Ferré M and Lamuela-Raventós RM (2022) Effect of Peanut Consumption on Cardiovascular Risk Factors: A Randomized Clinical Trial and Meta-Analysis. Front. Nutr. 9:853378. doi: 10.3389/fnut.2022.853378

Although numerous studies have reported the protective effect of nut consumption on cardiovascular risk, evidence for the role of peanuts in maintaining cardiometabolic health is inconclusive. Presented here are the results from the ARISTOTLE study, a parallel randomized controlled trial evaluating the impact of regular peanut intake on anthropometric, biochemical, and clinical measurements. The 63 healthy subjects that completed the study consumed their habitual diet plus either: a) 25 g/day of skin roasted peanuts (SRP, $n = 21$), b) two tablespoons (32 g)/day of peanut butter (PB, $n = 23$) or c) two tablespoons (32 g)/day of a control butter based on peanut oil (CB, $n = 19$) for 6 months. In addition, a meta-analysis of clinical trials, including data from the ARISTOTLE study, was carried out to update the evidence for the effects of consuming peanuts, including high-oleic peanuts, and peanut butter on healthy subjects and those at high cardiometabolic risk. After a systematic search on PubMed, Web of Science, Cochrane Library and Scopus databases up to July 2021, 11 studies were found to meet the eligibility criteria. In the ARISTOTLE study, lower total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios were found in the SRP group compared to the CB group ($p = 0.019$ and $p = 0.008$). The meta-analysis of clinical trials revealed that peanut consumption is associated with a decrease in triglycerides (MD: −0.13; 95% CI, −0.20 to −0.07; $p < 0.0001$) and that healthy consumers had lower total cholesterol and LDL-cholesterol/HDL-cholesterol ratios compared to the control groups (MD: −0.40; 95% CI, −0.71 to −0.09; $p = 0.01$ and MD: −0.19; 95% CI, −0.36 to −0.01; $p = 0.03$, respectively). However, individuals at high cardiometabolic risk experienced an increase in body weight after the peanut interventions (MD: 0.97; 95% CI, 0.54 to 1.41; $p < 0.0001$), although not in body fat or body mass index. According to the dose-response analyses, body weight increased slightly with higher doses of peanuts. In conclusion, a regular consumption of peanuts seems to modulate lipid metabolism, reducing triglyceride blood levels.

Systematic Review Registration: https://osf.io/jx34y/, identifier: 10.17605/OSF.IO/MK35Y.

Keywords: ARISTOTLE, lipid profile, nuts, cardiometabolic risk, health, triglycerides

INTRODUCTION

Peanuts are the most consumed nuts worldwide. In 2018, the global consumption of peanuts increased to ∼42.6 million metric tons, which is 10-fold higher than that of tree nuts (1). The sustainability and low cost of peanut production makes them more affordable than other nuts (2). Numerous studies indicate that peanut consumption may have a positive effect on cardiometabolic biomarkers, and reduce the risk of total cardiovascular and coronary heart disease (3–8). Peanuts are a rich source of nutritious and bioactive components, including protein, fiber, folate, niacin, magnesium, selenium, arginine, α-tocopherol, manganese, monounsaturated fatty acids, and phytochemicals such as polyphenols and phytosterols, which have a protective affect against cardiovascular disease (9–11).

Studies evaluating the effects of peanut consumption on cardiovascular risk factors have reported conflicting results, possibly due to differences in sample size, intervention products or study duration. Therefore, our aim was i) to evaluate the health impact of peanut products in a 6-month parallel randomized clinical trial, which was carried out between November 2019 and June 2020, and ii) to update the existing evidence for the effects of consuming peanuts, including high-oleic peanuts, and peanut butter on cardiometabolic risk by conducting a meta-analysis of controlled trials.

METHODS

Study Design

The ARISTOTLE study is a three-arm parallel-group randomized controlled trial (NCT04324749), approved by the Ethics Committee of Clinical Investigation of the University of Barcelona (Barcelona, Spain) and conducted according to the principles of the Declaration of Helsinki. The 63 healthy volunteers, aged between 18 and 33 years, who completed the ARISTOTLE study were recruited from the Food and Nutrition Torribera Campus of the University of Barcelona and surrounding area and signed an informed consent prior to the start of the trial. The exclusion criteria were as follows: a history of chronic diseases (cardiovascular diseases, cancer, diabetes, and others), peanut allergy or intolerance, body mass index (BMI) over 25 kg/m2, active smoking, high alcohol consumption and other toxic habits.

At baseline, participants were randomized to three intervention groups, consuming either a) 25 g/day of skin roasted peanuts (SRP) or b) two tablespoons (32 g)/day of peanut butter (PB) or c) two tablespoons (32 g)/day of a control butter based on peanut oil, free of fiber and polyphenols (CB). The intervention period was 6 months, but due to the COVID-19 pandemic, in some cases it was extended to 7 months. The volunteers were supplied with the three intervention products and requested to follow a peanut-free diet for 2 weeks before the start of the study. During the intervention, they followed their habitual diet excluding wine, grapes, dark chocolate (<70%) and berries (due to their high content of resveratrol, also present in peanuts), as well as nuts (due to a similar lipid content).

Outcome Measurements

At baseline and the end of the intervention, participants attended the research center under fasting conditions (between 8:00 and 10:30 a.m.) to have anthropometric measurements taken by trained staff. BMI was calculated as weight divided by height squared (kg/m^2). Height was measured in the standing position using a portable stadiometer. Weight and body fat were measured using a tetrapolar OMRON BF511 bioelectrical device, with the participants wearing light clothes and no shoes. Waist circumference was measured using an inelastic flexible tape positioned equidistantly between the lowest rib and the iliac crest. Blood pressure was measured in triplicate in the sitting position using a digital monitor OMRON M6. Biochemical markers in serum and plasma (glucose and lipid profile, respectively) were measured in an external laboratory (Cerba internacional, Barcelona, Spain) using enzymatic methods. For that, blood was extracted via venipuncture into tubes containing ethylenediaminetetraacetic acid (EDTA). Serum and plasma were separated after centrifugation at 3,000 g for 10 min at 4°C and at 1,500 g for 15 min at 4°C, respectively.

In addition, diet and physical activity were recorded by trained staff through a 151-item semi-quantitative food frequency questionnaire (FFQ) and a Spanish validated version of the Minnesota Leisure-Time Physical Activity Questionnaire, respectively (12, 13). Both questionnaires were conducted at baseline and at the end of the intervention.

Statistical Analysis

The sample size was calculated to ensure a significance level of 0.05 and statistical power of 80%, as well as 5% of loss for follow-up were included. The normality of distribution was analyzed by the Shapiro–Wilk test, and due to the Non-normality of most variables, Non-parametric tests were used. The Kruskal Wallis test followed by Dunn's *post hoc* test were applied to detect any differences between interventions at baseline. Chi-square was used for categorical variables to detect differences in participant characteristics between the three groups at baseline. A generalized estimating equation based on a Poisson regression model for repeated measures and adjusted for age and sex was used to estimate the effect of the interventions. The Wilcoxon signed-rank test was applied to evaluate any differences at the end of the study with respect to the baseline in each arm group. Continuous variables were expressed as mean ± standard deviation and categorical variables as number (n) and proportion (%). Differences were considered significant when the p value was lower than 0.05. All statistical analyses were conducted by intention-to-treat using STATA software version 16.0 (StataCorp, College Station, TX, USA).

Meta-Analysis

Protocol Register

The protocol of this systematic review and meta-analysis was registered in the platform OSF (https://osf.io/jx34y/). In addition, this study was carried out according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analysis, **Supplementary Table 1**), following the Cochrane Group recommendations (14).

Systematic Search and Selection of Studies

PubMed, Web of Science, Cochrane Library and Scopus databases were used for the systematic search (all years up to July 2021). Both Medical Subject Heading (MeSH) and free-text search terms were used according to the Cochrane Group recommendations. The search strategy included: (peanut OR Arachis OR Groundnut OR "Ground Nut" OR "peanut butter") AND ("Insulin Resistance" OR "Insulin Sensitivity" OR Insulin OR Glucose OR "Glucose Intolerance" OR "Glucose Tolerance" OR "blood glucose" OR "glycemic index" OR "Waist Circumference" OR "Sagittal Abdominal Diameter" OR "Quetelet index" OR "Body Mass Index" OR adiposity OR obesity OR overweight OR "body weight" OR "weight gain" OR "weight loss" OR "body fat" OR "body composition" OR "body constitution" OR cholesterol OR Triacylglycerol OR Triglycerides OR "plasma lipid" OR "Blood Pressure" OR "Arterial Pressure" OR "Diastolic Pressure" OR "Systolic Pressure"). In addition, there were no language restrictions in the search.

The titles and abstracts identified in the systematic search were independently reviewed by I.P-M and S.H-B. Potentially relevant full texts were selected by the same two authors (I.P-M and S.H-B).

Selection Criteria

The inclusion criteria were the following: 1) healthy or suffering metabolic syndrome (MetS) or at high risk of MetS subjects; 2) intervention based on intake of peanuts (including high-oleic peanuts) or peanut butter (studies evaluating the effects of peanut oil consumption were excluded); 3) health outcomes that referred to anthropometric measurements, biochemical analyses (related to glucose and lipid metabolism) and clinical parameters (blood pressure); 4) randomized controlled trial (RCT) design. Details about PICOS strategy are described in **Supplementary Table 2.**

Data Extraction

After the study selection, I.P-M and S.H-B extracted the data. For each study, the following data were collected: i) author and year, ii) number and characteristics of participants, iii) study design (including intervention length), iv) control group, v) intervention group(s), vi) health outcomes [body weight, BMI, waist circumference, body fat, glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol, systolic blood pressure (SBP) and diastolic blood pressure (DBP)].

Quality Assessment

I.P-M and S.H-B independently checked the quality of the included studies. The revised Cochrane risk-of-bias tool for randomized trials (RoB-2) was used to evaluate the risk of bias in each study (15). According to the design of the RCT, the specific template of the Rob-2 was assayed: i) individually randomized parallel-group trial, ii) cluster-randomized parallel-group trial or iii) individually randomized cross-over or other matched design. The tool assesses five domains of bias: the randomization process, deviation from the intended interventions, missing outcome data, measurement of the outcome and selection of the reported result. The overall risk of bias assessment for each study was summarized within each domain. A low, unclear, or high risk of bias was established for each study considering all the domains.

In addition, I.P-M and S.H-B independently checked the quality of evidence for each outcome. Thus, the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) framework (16) was assayed using the software GRADEpro. The following domains were evaluated: risk of bias, inconsistency, indirectness, imprecision, and publication bias. The overall certainty of evidence was calculated considering all the domains. Very low, low, moderate, or high certainty was established for each outcome.

Statistical Analysis

The mean difference was calculated for each outcome considering the mean and standard deviation of control and intervention groups in the studies. For that, the data for each variable were converted to specify units. A mixture of change-from-baseline and final values were included (17). Each intervention phase of a crossover study was treated as an independent arm of a parallel study. In studies on the consumption of peanut products (peanuts, peanut butter, or high-oleic peanuts) with two or more experimental arms, a combined arm was calculated for a comparison with the control group. The meta-analysis was performed by pooling mean differences if $\geq$2 studies reported data for specific outcomes. Moreover, subgroup analyses were carried out according to the health status of the participants and the interventions. First, healthy subjects and patients at risk of metabolic syndrome were analyzed separately. Second, high-oleic peanuts were analyzed independently of peanut and peanut butter interventions. The random-effect model was used in all cases due to the high variability of the studies and the low number of studies meta-analyzed. The I^2 test, Tau^2, and 95% prediction intervals were used to evaluate the heterogeneity across studies. Finally, we estimated the dose-response effect of peanut consumption using the doresmeta package in R version 4.1.1. Meta-analyses were performed with the software Review Manager 5.4.

RESULTS

Enrollment and Baseline Characteristics of Participants

Of the 90 healthy subjects that were randomized and enrolled, 63 completed the study (**Supplementary Figure 1**). The average age of the 63 subjects was 22.71 $\pm$ 3.13 years; around 70% were female and 36% had graduated from a 4-year degree course. At baseline, no significant differences were reported in the participant characteristics, except in HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ($p = 0.006$ and $p = 0.031$, respectively) (**Table 1**).

Health Outcomes

Lower total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios were observed in the SRP than in the CB group ($p = 0.019$ and 0.008, respectively).

TABLE 1 | Participant characteristics at baseline.

	CB ($n = 19$)	SRP ($n = 21$)	PB ($n = 23$)	*p-value*
Female, *n* (%)	12 (63)	14 (66)	18 (78)	0.528
Age (years)	22.42 ± 3.29	22.28 ± 3.20	23.43 ± 2.90	0.247
Education level, *n* (%)				0.512
University students	12 (63%)	11 (52%)	11 (48%)	–
Graduated	7 (37%)	10 (48%)	12 (52%)	–
Physical activity (mets/week)	4,607 ± 1,728	4,850 ± 2,124	4,703 ± 2,381	0.954
Anthropometric measurements				
Body weight (kg)	63.78 ± 10.04	63.26 ± 10.12	60.10 ± 7.72	0.412
BMI (kg/m^2)	22.59 ± 2.67	22.12 ± 3.52	22.19 ± 2.60	0.679
Waist circumference (cm)	74.68 ± 5.99	72.73 ± 8.31	71.28 ± 5.53	0.228
Body fat (%)	26.22 ± 7.99	26.66 ± 8.07	28.45 ± 7.88	0.628
Glucose metabolism				
Glucose (mmol/L)	4.47 ± 0.24	4.54 ± 0.44	4.59 ± 0.35	0.581
Lipid profile				
TG (mmol/L)	0.80 ± 0.25	0.71 ± 0.20	0.85 ± 0.35	0.341
TC (mmol/L)	4.09 ± 0.64	4.33 ± 0.52	4.60 ± 0.88	0.137
LDL-c (mmol/L)	2.30 ± 0.50	2.22 ± 0.39	2.60 ± 0.69	0.142
HDL-c (mmol/L)	1.50 ± 0.30^a	1.75 ± 0.30^b	1.69 ± 0.40^b	**0.006**
TC/HDL-c	2.76 ± 0.38	2.52 ± 0.32	2.79 ± 0.57	0.056
LDL-c/HDL-c	1.56 ± 0.35^a	1.29 ± 0.29^b	1.59 ± 0.53^{ab}	**0.031**
Blood pressure				
SBP (mmHg)	110 ± 11.83	111 ± 7.34	109 ± 8.87	0.451
DBP (mmHg)	70 ± 8.73	72 ± 7.63	72 ± 6.20	0.415
Dietary intake				
Energy (kcal/day)	2,596 ± 477.97	2,770 ± 594.50	2,705 ± 602.17	0.588
Carbohydrates (g/day)	246.74 ± 59.49	257.43 ± 80.73	241.26 ± 73.92	0.867
Sugar (g/day)	113.89 ± 41.02	115.86 ± 34.83	111.65 ± 35.04	0.906
Fiber (g/day)	38.93 ± 15.07	45.17 ± 21.95	42.12 ± 14.65	0.768
Protein (g/day)	107.75 ± 27.51	103.72 ± 29.47	110.17 ± 31.86	0.598
Total fat (g/day)	129.53 ± 28.96	144.55 ± 29.17	141.83± 35.35	0.249
SFAs (g/day)	36.81 ± 13.02	37.61 ± 10.00	38.18 ± 11.04	0.871
MUFAs (g/day)	59.46 ± 15.87	70.37 ± 16.12	69.06 ± 17.17	0.093
PUFAs (g/day)	23.59 ± 6.59	25.91 ± 6.76	23.99 ± 7.25	0.541

Data are expressed as mean ± SD.

CB, control butter; SRP, skin roasted peanuts; PB, peanut butter; BMI, Body mass index; DBP, Diastolic blood pressure; MUFAs, Monounsaturated fatty acids; PUFAs, Polyunsaturated fatty acids; SFAs, Saturated fatty acids; SBP, Systolic blood pressure; TC, Total cholesterol; TG, Triglyceride; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.

p column refers to differences between groups at baseline. P values <0.05 are statistically significant (a and b superscripts) and were calculated by the Kruskal–Wallis test. Values shown in bold are statistically significant.

A significant decrease in physical activity was reported after the CB and SRP interventions compared to baseline ($p = 0.034$ and 0.012) due to the pandemic situation, but no changes between groups were observed. No differences were observed in other lipid parameters, body composition, glucose or blood pressure. The nutritional intake had not changed after the intervention or between groups (**Table 2**).

Meta-Analysis

Selected Studies and Their Participants

A total of 4,100 articles were identified from the databases and 3,130 articles were screened after the removal of duplicates. Finally, 10 of the 29 potentially eligible full-text articles were included in the systematic review and meta-analysis. The reasons for study exclusion are set out in **Supplementary Table 3**. In addition, data from the ARISTOTLE study were included in this updated meta-analysis (**Supplementary Figure 2**). The number of selected articles dealing with each outcome was the following: 8 for body weight, 7 for BMI, 5 for body fat, 7 for waist circumference, 8 for glucose, 4 for insulin, 9 for total cholesterol, 9 for HDL-cholesterol, 9 for LDL-cholesterol, 9 for triglycerides, 5 for total cholesterol/HDL-cholesterol, 7 for LDL-cholesterol/HDL-cholesterol, 3 for SBP and 3 for DBP (**Supplementary Table 4**).

TABLE 2 | Health outcomes, physical activity, and nutritional intake of healthy adults from the ARISTOTLE study.

	CB			SRP			PB		p^1	p^2 *SRP vs. CB*	p^2 *PB vs. CB*
	Pre-intervention	Post-intervention	P^1	Pre-intervention	Post-intervention	p^1	Pre-intervention	Post-intervention			
Anthropometric measurements											
Body weight (kg)	63.78 ± 10.04	63.67 ± 10.97	0.930	63.26 ± 10.12	63.13 ± 10.91	0.850	60.10 ± 7.72	59.37 ± 7.90	0.742	0.896	0.600
BMI (kg/m^2)	22.59 ± 2.67	22.5 ± 2.93	0.895	22.12 ± 3.52	21.99 ± 3.46	0.940	22.19 ± 2.56	21.94 ± 2.71	0.835	0.982	0.672
Waist circumference (cm)	74.68 ± 5.98	73.84 ± 6.84	0.599	72.73 ± 8.31	71.81 ± 7.79	0.706	71.28 ± 5.53	70.24 ± 5.70	0.560	0.962	0.893
Body fat (%)	26.22 ± 7.99	25.66 ± 8.26	0.855	26.66 ± 8.07	26.16 ± 8.22	0.910	28.45 ± 7.88	27.77 ± 8.57	0.838	0.844	0.512
Glucose metabolism											
Glucose (mmol/L)	4.47 ± 0.24	4.58 ± 0.26	0.238	4.54 ± 0.43	4.76 ± 0.30	0.087	4.59 ± 0.35	4.65 ± 0.29	0.875	0.266	0.266
Lipid profile											
TG (mmol/L)	0.80 ± 0.25	0.79 ± 0.24	0.594	0.71 ± 0.20	0.76 ± 0.22	0.876	0.85 ± 0.35	0.81 ± 0.30	0.505	0.557	0.847
TC (mmol/L)	4.09 ± 0.64	4.23 ± 0.64	0.807	4.33 ± 0.52	4.49 ± 0.70	0.498	4.60 ± 0.88	4.66 ± 0.86	0.975	0.968	0.709
LDL-c (mmol/L)	2.30 ± 0.50	2.49 ± 0.50	0.404	2.22 ± 0.39	2.45 ± 0.44	0.150	2.60 ± 0.69	2.80 ± 0.76	0.672	0.837	0.917
HDL-c (mmol/L)	1.50 ± 0.30	1.42 ± 0.20	0.629	1.75 ± 0.30	1.68 ± 0.31	0.519	1.69 ± 0.40	1.59 ± 0.31	0.740	0.919	0.886
TC/HDL-c	2.76 ± 0.38	2.99 ± 0.40	0.121	2.52 ± 0.32	2.69 ± 0.30	0.099	2.79 ± 0.57	2.97 ± 0.62	0.207	**0.019**	0.819
LDL-c/HDL-c	1.56 ± 0.35	1.76 ± 0.37	0.125	1.29 ± 0.29	1.48 ± 0.29	0.072	1.59 ± 0.53	1.80 ± 0.61	0.191	**0.008**	0.727
Blood pressure											
SBP (mmHg)	110 ± 11.83	110 ± 15.65	0.715	111 ± 7.34	111 ± 18.45	0.624	109 ± 8.87	106 ± 15.00	0.317	0.982	0.982
DBP (mmHg)	70 ± 8.73	70 ± 12.83	0.693	72 ± 7.63	73 ± 12.71	0.734	72 ± 6.20	73 ± 9.38	0.886	0.487	0.487
Physical activity											
Physical activity (mets/week)	4,607 ± 1,728	3,330 ± 1,983	**0.034**	4,850 ± 2,124	3,269 ± 1,613	**0.012**	4,703 ± 2,381	3,736 ± 1,837	0.144	0.416	0.290
Nutritional intake											
Energy (kcal/day)	2,596 ± 477	2,640 ± 324	0.474	2,770 ± 594	2,663 ± 499	0.753	2,705 ± 602	2,668 ± 478	0.750	0.120	0.450
Carbohydrates (g/day)	246 ± 59.49	227 ± 46.34	0.373	257 ± 80.73	238 ± 65.18	0.443	241 ± 73.92	226 ± 53.41	0.462	0.864	0.678
Sugar	113 ± 41.02	93.25 ± 28.47	0.118	115 ± 34.83	101 ± 33.12	0.163	111 ± 35.04	95.69 ± 28.03	0.127	0.370	0.426
Fiber	38.93 ± 15.07	34.97 ± 10.55	0.457	45.17 ± 21.95	43.80 ± 18.22	0.734	42.12 ± 14.65	40.56 ± 10.07	0.818	0.202	0.302
Protein (g/day)	107 ± 27.51	115 ± 25.65	0.194	103 ± 29.47	105 ± 26.77	0.753	110 ± 31.86	111 ± 24.13	0.974	0.159	0.158
Total fat (g/day)	129 ± 28.96	148 ± 22.71	0.084	144 ± 29.17	146 ± 28.43	0.642	141 ± 35.35	151 ± 31.07	0.386	0.080	0.168
SFAs (g/day)	36.81 ± 13.02	38.04 ± 10.03	0.965	37.61 ± 10.00	36.76 ± 10.62	0.950	38.18 ± 11.04	37.37 ± 10.71	0.575	0.285	0.301
MUFAs (g/day)	59.46 ± 15.87	67.29 ± 14.62	0.088	70.37 ± 16.12	67.76 ± 15.90	0.811	69.06 ± 17.17	69.73 ± 15.96	0.957	0.200	0.141
PUFAs (g/day)	23.59 ± 6.59	20.69 ± 4.59	0.140	25.91 ± 6.76	22.45 ± 4.80	0.076	23.99 ± 7.25	21.90 ± 4.87	0.318	0.716	0.678

Data are expressed as mean ± SD.

CB, control butter; SRP, skin roasted peanuts; PB, peanut butter; BMI, Body mass index; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; MUFAs, Monounsaturated fatty acids; PUFAs, Polyunsaturated fatty acids; SFAs, Saturated fatty acids; TG, Triglyceride; TC, Total cholesterol; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol.

p1 represents the p value at the end of the intervention compared to baseline, calculated by Wilcoxon's test. p2 represents the p value between SRP and PB vs. CB at 6 months adjusted by sex and age, calculated by the generalized estimating equation (GEE). p values <0.05 are statistically significant. Values shown in bold are statistically significant.

The data about the health outcomes of peanut interventions reported by the studies included in the systematic review and meta-analysis are presented in **Supplementary Table 5**. A total of 643 participants (316 males and 327 females) aged between 18 and 84 years from Asia, North America, Europa, South America, and Australia took part in these studies. Their health status was variable: healthy ($n = 110$) or suffering MetS or at high risk of MetS, with overweight or obesity, diabetes mellitus type II and hypercholesterolemia ($n = 533$). Interventions included peanuts, peanut butter and high oleic peanuts in variable concentrations and duration. The administered doses ranged between 25 and 200 g/d, with follow-up periods of 2–24 weeks. Different control diets were used: a hypocaloric diet, the habitual diet excluding peanuts (of equal or lower energy than the peanut intervention) or the American Diabetes Association meal plan without peanuts or a substitute snack (grain bar, white rice bar, candy, or almonds). In addition, an isocaloric control containing peanut oil was used in the ARISTOTLE study (free of fiber and polyphenols). The analyzed outcomes were body weight, BMI, waist circumference, body fat, glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol, systolic blood pressure and diastolic blood pressure. Regarding the study design, 8 parallel RCTs and 3 crossover RCTs were included **Table 3**).

Anthropometric Measurements

A total of nine studies analyzed body composition parameters (body weight, BMI, body fat and/or waist circumference). In general, no significant changes were detected in the anthropometric measurements (**Figure 1**), but a significant increase in body weight was observed in the subjects with or at risk of MetS included in the six studies analyzed separately (MD: 0.97; 95% CI: 0.54 to 1.41; $P < 0.0001$) (**Supplementary Table 6**). The dose response meta-analyses showed a significant but slight effect of peanut intake (evaluated as g/day) on body weight [curve (estimate): 0.033 kg; 95% CI: 0.000 to 0.066 kg; $P = 0.049$]. No significant trends were observed for the other anthropometric parameters (**Supplementary Figure 3**).

Glucose Metabolism

No changes were observed in fasting blood glucose or insulin in subjects that consumed peanut products compared to control interventions (**Figure 2**). Nor were differences found when analyzing subgroups according to health status or peanut type intake (**Supplementary Tables 6**, **7**). Regarding the dose-response analyses, no significant effects of peanut intake on glucose metabolism were observed (**Supplementary Figure 4**).

Lipid Profile

As shown in **Figure 3,** the level of triglycerides in blood decreased significantly after interventions with peanut products compared to the control interventions (MD: −0.13; 95% CI: −0.20 to −0.07; $p < 0.0001$). This reduction was most acute in healthy subjects (MD: −0.13; 95% CI: −0.25 to −0.00; $p = 0.04$) and in those who consumed peanuts or peanut butter (MD: −0.14; 95% CI: −0.20 to −0.07; $p < 0.0001$) (**Supplementary Tables 6**, **7**). Although no significant changes were observed in the other lipid analytes, healthy subjects that consumed peanut products had lower total cholesterol and LDL-cholesterol/HDL-cholesterol ratio (MD: −0.40; 95% CI: −0.71 to −0.09; $p = 0.010$ and MD: −0.19; 95% CI: −0.36 to −0.01; $p = 0.030$, respectively) in comparison with control groups (**Supplementary Table 6**). Nevertheless, no significant trend was observed in the dose-response analyses of the effect of peanut intake on blood lipids (**Supplementary Figure 5**).

Blood Pressure

No significant changes were observed in SBP or DBP in peanut product consumers compared to the control groups (**Figure 4**). Similar results were obtained when analyzing subgroups according to the health status of participants and type of peanut intake (**Supplementary Tables 6, 7**). Regarding the dose-response analyses, no significant effects of peanut intake on blood pressure were observed (**Supplementary Figure 6**).

Quality of Studies and Overall Strength of Evidence

The overall risk of bias was high in two studies (5, 22), unclear in seven studies (8, 18, 20, 21, 24–26), as well as in the ARISTOTLE study, and low in one study (23). The main concerns regarding bias were the randomization process and outcome measurement. In addition, an unclear risk was identified in some studies regarding the deviation from the intended intervention domain (**Supplementary Figure 7**). The strength of evidence varied from very low to moderate, depending on the outcomes. Evidence quality for the effects of ingesting peanut products was very low regarding body fat, insulin, total cholesterol/HDL-cholesterol, DBP and SBP, and low for body weight, BMI, total cholesterol, HDL-cholesterol, LDL-cholesterol and LDL-cholesterol/HDL-cholesterol. In the case of waist circumference, glucose, and triglycerides, the quality of evidence was rated as moderate. Evidence quality was reduced by: i) heterogeneity among the participants, ii) differences in participants, comparator groups and follow-up duration, iii) small sample size (<400 participants), iv) heterogeneity in the intervention and v) bias arising from the effect estimate (**Supplementary Table 8**).

DISCUSSION

The results of the ARISTOTLE study, a randomized controlled trial, provide evidence that peanut consumption may improve lipid profiles, as the total cholesterol/HDL-cholesterol and LDL-cholesterol/HDL-cholesterol ratios were slightly lower in the SRP group compared to the CB group after the 6-month intervention. An improvement in blood lipids was also found in the meta-analysis of nine studies evaluating this cardiovascular risk factor in peanut consumers. The main finding was a reduction in triglyceride levels after peanut product consumption, this effect being greater in healthy subjects than in patients with or at high risk of MetS. The LDL-cholesterol/HDL-cholesterol ratio was also lower in healthy peanut product consumers. In addition, subgroup analyses showed that triglyceride levels were significantly lower after the interventions with peanuts and peanut butter but not high-oleic peanuts.

TABLE 3 | Summary of studies included in the systematic review and meta-analysis evaluating the effect of peanut product intake on health outcomes.

Author (year)	Number and characteristics of participants	Study design (length of the intervention)	Control group	Intervention group(s)	Health outcomes
Alves et al. (18)	65 overweight or obese men (18–50 years)	Parallel RCT (4 weeks)	Hypocaloric diet	Hypocaloric diet including 56 g/day of unpeeled roasted peanuts (CVP or HOP)	Body weight, BMI, waist circumference, body fat (%)
Alves et al. (19)	65 overweight or obese men (18–50 years)	Parallel RCT (4 weeks)	Hypocaloric diet	Hypocaloric diet including 56 g/day of unpeeled roasted peanuts (CVP or HOP)	Glucose, insulin, TC, LDL-c, HDL-c, triglycerides, TC/HDL-c, LDL-c/HDL-c
Barbour et al. (8)	61 overweight or obese men or postmenopausal women (50–75 years)	Crossover RCT (12 weeks)	Habitual diet without peanuts or nuts	Habitual diet adding roasted unsalted HOP: 84 g/day in men and 56 g/day in women 6 days per week	Body weight, BMI, waist circumference, body fat (%), glucose, insulin, TC, LDL-c, HDL-c, triglycerides, LDL-c/HDL-c
Claesson et al. (5)	25 healthy adults (19–30 years)	Parallel RCT (2 weeks)	20 kcal/kg/day of candy	20 kcal/kg/day of roasted and salted peanuts (~200 g/day)	Body weight, BMI, waist circumference, body fat (%), glucose, insulin, TC, LDL-c, HDL-c, triglycerides, LDL-c/HDL-c
Ghadimi Nouran et al. (20)	54 hypercholesterolaemic men (25–65 years)	Crossover RCT (4 weeks)	Habitual diet	Habitual diet adding roasted and salted peanuts (20% of total energy = 60 g/day−93 g/day)	Body weight, TC, LDL-c, HDL-c, triglycerides, TC/HDL-c, LDL-c/HDL-c, SBP, DBP
Hou et al. (21)	25 adults with type 2 diabetes Mellitus (40–80 years)	Parallel RCT (12 weeks)	Low-carbohydrate diet supplemented with unsalted almonds with skin (55 g/day in men and 45 g/day in women)	Low-carbohydrate diet supplemented with unsalted peanuts with skin (60 g/day in men and 50 g/day in women)	BMI, glucose, TC, LDL-c, HDL-c, triglycerides
Johnston et al. (18)	44 overweight or obese adults (20–65 years)	Parallel RCT (16 weeks)	40 g/day of grain bar	28 g/day of peanuts	Body weight, waist circumference, body fat (%), glucose, insulin
Kris-Etherton et al. (22)	22 healthy adults (21–54 years)	Crossover RCT (24 days)	Average American diet	MUFA-rich diet based on peanuts and peanut butter	TC, LDL-c, HDL-c, triglycerides, TC/HDL-c, LDL-c/HDL-c
Wang et al. (23)	224 adults with metabolic syndrome (MetS) or at risk of MetS (20–65 years)	Parallel RCT (12 weeks)	White rice snack bar	56 g/day of roasted salted peanuts	Body weight, BMI, waist circumference, glucose, TC, LDL-c, HDL-c, triglycerides, SBP, DBP
Wien et al. (24)	60 adults with type 2 diabetes Mellitus (34–84 years)	Parallel RCT (24 weeks)	ADA meal plan without peanuts and tree nuts	ADA meal plan + 46 g/day of salted peanuts and/or peanut butter with salt and oil (without other tree nuts)	Body weight, BMI, waist circumference, glucose, TC, LDL-c, HDL-c, triglycerides, TC/HDL-c, LDL-c/HDL-c

ADA, American Diabetes Association; BMI, body mass index; CVP, conventional peanuts; DBP, diastolic blood pressure; HDL-c, high-density lipoprotein cholesterol; HOP, high oleic peanuts; LDL-c, low-density lipoprotein cholesterol; MUFA, monounsaturated fatty acids; RCT, randomized controlled trial; SBP, systolic blood pressure; TC, total cholesterol.

In agreement with our findings, Alper et al. reported a 24% lower triglyceride level in 15 normolipidemic adults after regular peanut consumption in a 30-week trial (27). Elsewhere, acute peanut intake (85 g) within a high-fat meal improved the postprandial triglyceride response and preserved endothelial function in 15 healthy overweight or obese men (28). Healthy consumers of peanuts had 7.2 and 20% less total cholesterol and triglycerides, respectively, after an 8-week intervention (3). Moreover, a recent systematic review and meta-analysis found an enhancement of HDL-cholesterol in healthy subjects consuming peanut products, particularly high-oleic peanuts, for periods longer than 12 weeks (9). In a parallel study with 118 adults who randomly consumed 56 g of peanuts in different forms, an increase in HDL-cholesterol and a reduction of the triglycerides/HDL-cholesterol ratio (considered a predictive marker for higher small dense (sd)-LDL-cholesterol and an independent predictor of cardiovascular risk) were reported Post-intervention compared to baseline (7, 29, 30). Notably, the participants with high total cholesterol, mainly those who had a high LDL-cholesterol level, experienced a significantly greater

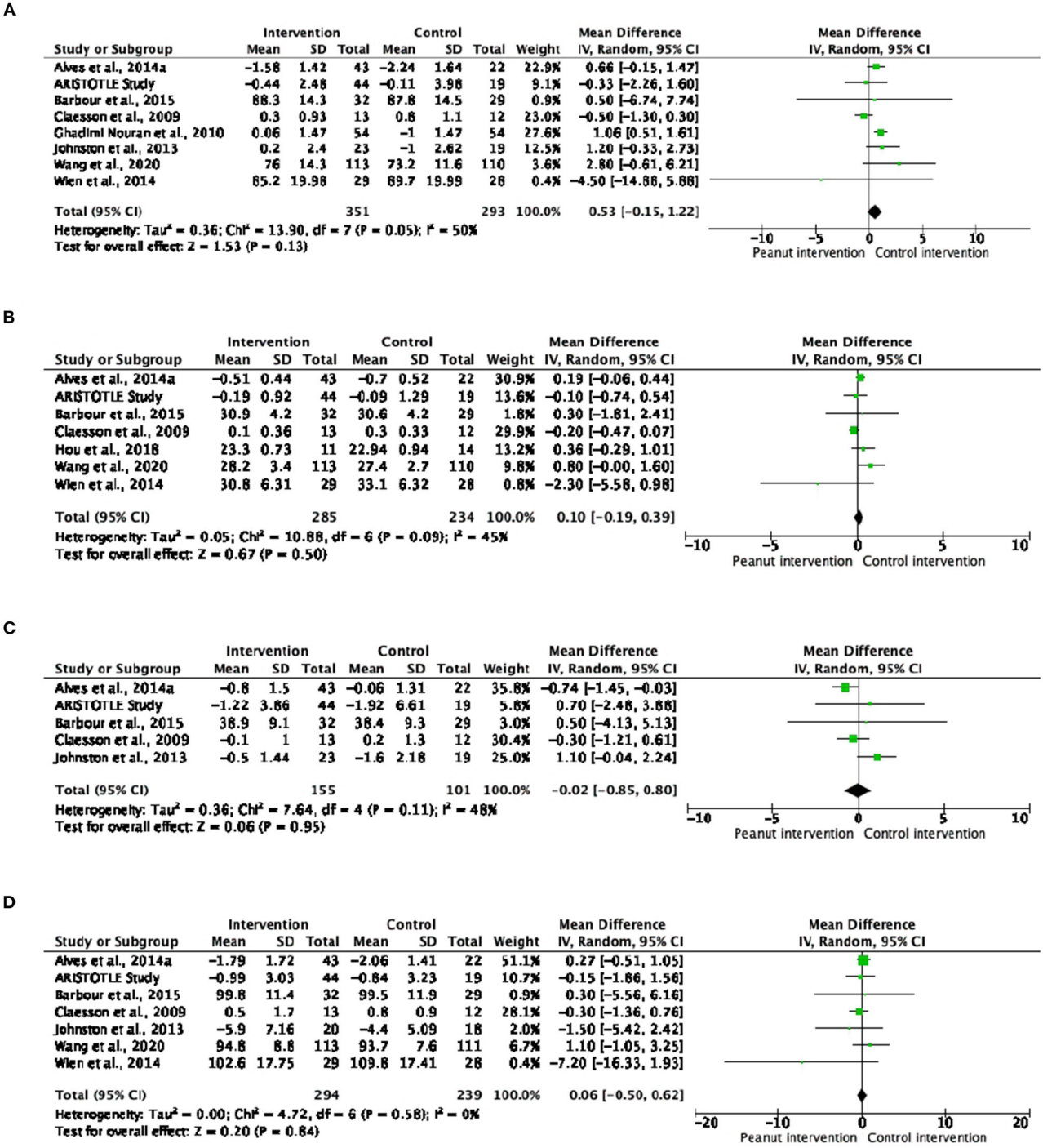

FIGURE 1 | Forest plot reporting mean differences for body weight **(A)**, body mass index **(B)**, body fat **(C)** and waist circumference **(D)** associated with peanut interventions compared to control interventions.

reduction in total cholesterol and LDL-cholesterol than those with normal cholesterol values. Similarly, subjects with a high triglyceride level underwent a more pronounced decrease in triglycerides (7). Consuming peanuts two or more times/week was associated with a 13% lower risk of total cardiovascular and coronary heart diseases in two large prospective cohorts of women from the Nurses' Health Study and men from the Health Professionals Follow-Up Study, but no significant associations were observed in those who consumed higher amounts of peanut butter (6). The lack of beneficial effects on the total cholesterol,

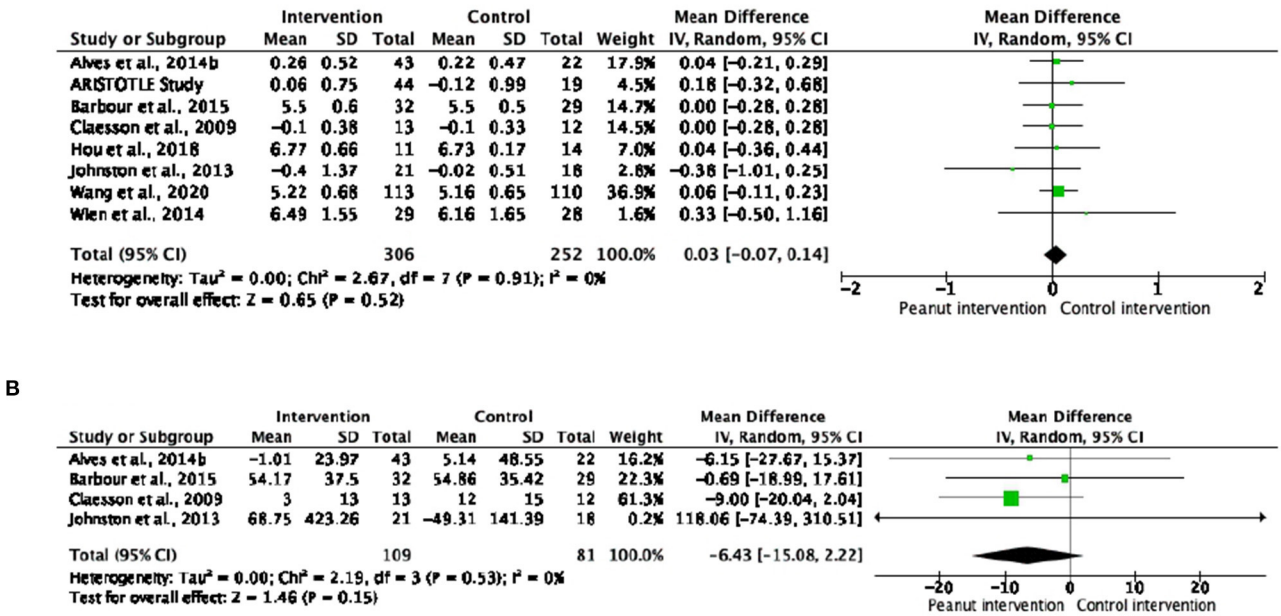

FIGURE 2 | Forest plot reporting mean differences for glucose **(A)** and insulin **(B)** associated with peanut interventions compared to control interventions.

LDL-cholesterol and HDL-cholesterol after peanut consumption observed in the ARISTOTLE study, highlights the importance of the analysis of atherogenic lipoproteins, and particularly sd-LDL-cholesterol, beyond lipid levels. Indeed, previous studies mention that sd-LDL-cholesterol are associated to cardiovascular diseases and closely linked to atherosclerosis formation and progression independently of LDL-cholesterol concentrations (31, 32). Therefore, to assure that peanut consumption may therefore have a positive impact on cardiovascular risk, beyond plasma lipid levels, sd-LDL-cholesterol levels must be addressed by future prospective studies.

More than half of the total lipid content in peanuts is composed of oleic acid, which is linked to better cardiovascular health (33, 34). In addition, peanuts contain specific very-long-chain saturated fatty acids (arachidic, behenic and lignoceric acids) that have been inversely associated with the risk of cardiovascular diseases and diabetes (35, 36) and we have previously found that participants from the ARISTOTLE study significantly increased the levels of these fatty acids in plasma after 6 months consuming peanut products (37, 38). Moreover, peanuts are also a good source of bioactive compounds known to be protective against cardiovascular diseases, including magnesium, folate and phytochemicals such as polyphenols and phytosterols (2).

No changes in body composition (body weight, BMI, body fat and/or waist circumference) were observed in healthy subjects in the ARISTOTLE study or meta-analysis. A slight but significant increase in body weight has been described in individuals at cardiometabolic risk. A slight increase on body weight was observed in those consuming higher amounts of peanut products, although studies report contradictory results for this effect. Similar to our findings, in a crossover randomized controlled trial, a higher body weight was observed in 54 hypercholesterolemic men consuming 60–93 g/day of peanuts for 4 weeks (20). Conversely, Alves et al. found that body fat decreased in overweight and obese subjects who consumed 56 g/day of conventional or high-oleic peanuts for 4 weeks compared to those who followed a hypocaloric diet (25). In a prospective cohort of women from the Nurses' Health Study, a marginally significant mean weight loss of 0.37 kg was found during 8 years of follow-up in those who consumed peanuts more frequently, but this trend was not associated with peanut butter intake. Similar weight loss was observed in normal weight, overweight and obese subjects (39). McKiernan et al. reported similar effects of peanut consumption on body weight independently of whether they were processed or not (7).

The incomplete absorption of fat from peanuts, which leads to less available energy, may be one of the elements protecting consumers against weight gain and body composition changes (40). Traoret et al. found that the intake of whole peanuts was associated with a higher excretion of fecal fat and energy compared to peanut butter, oil or flour (41). This loss, consistently reported by many studies, is attributed to inefficient mastication coupled with the resistance of peanut cell walls, which act as a physical barrier against the action of lipase and limit the bioaccessibility of peanut lipids and energy (42). Several authors describe a greater sensation of fullness and satiety after peanut intake (19, 43). A study even observed that peanuts consumed as a snack had a greater compensatory effect on

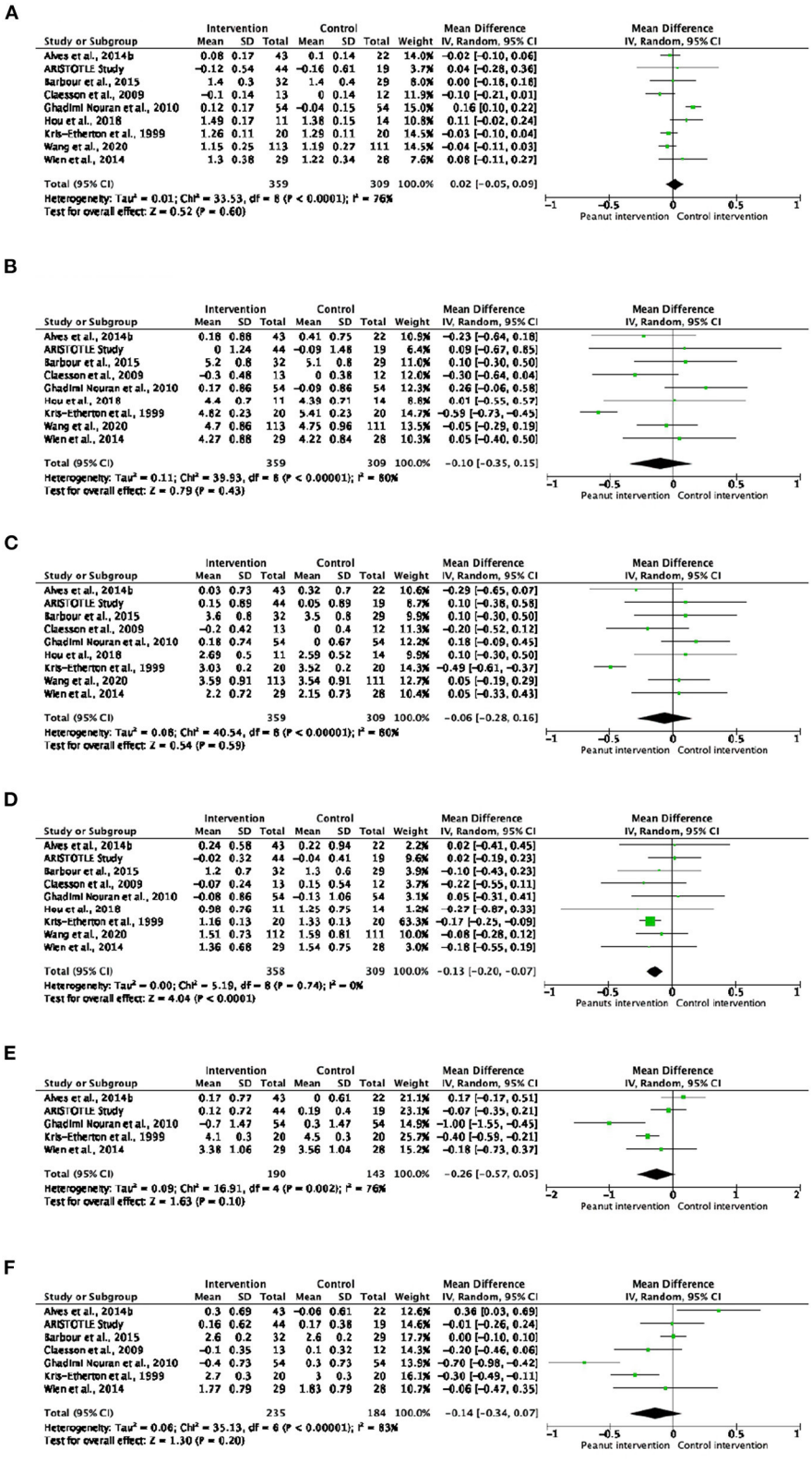

FIGURE 3 | Forest plot reporting mean differences for total cholesterol **(A)**, HDL-cholesterol **(B)**, LDL-cholesterol **(C)**, triglycerides **(D)**, total cholesterol/HDL-cholesterol ratio **(E)** and LDL-cholesterol/HDL-cholesterol ratio **(F)** associated with peanut interventions compared to control interventions. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

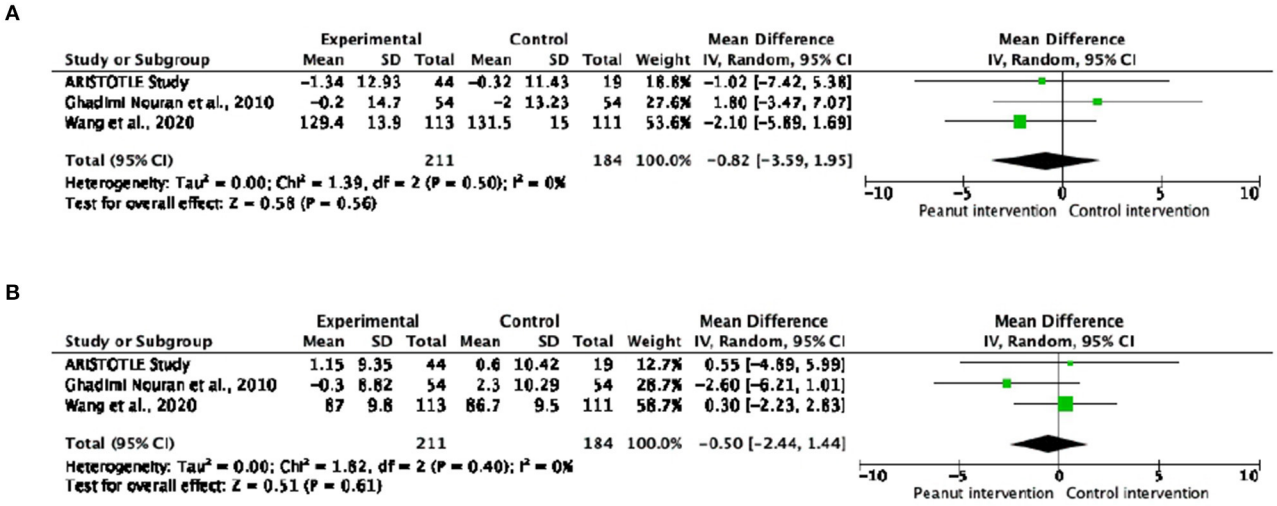

FIGURE 4 | Forest plot reporting mean differences for systolic blood pressure **(A)** and diastolic blood pressure **(B)** in relation to peanut interventions compared with control interventions.

energy intake than when consumed with a meal (44). In addition, regularly consumed peanut products could be replacing sugary or processed snacks (45).

Regarding glucose metabolism, no significant effects on glucose or insulin were observed in this research, in accordance with previous studies (5, 9, 28, 46, 47). However, the consumption of peanut butter five times or more per week reduced the incidence of diabetes by 21% in a prospective cohort of women from the Nurses' Health Study (48). The addition of 32 g of peanut butter to a high-glycemic index meal reduced the fasting blood glucose and overall glycemic response in 16 healthy adults (49). Reis et al. also showed a reduced glycemic response, depending on the processing and form of the consumed peanuts (4, 50). The fat in peanut products could delay gastric emptying and reduce the rate of glucose uptake into the circulation and the insulin response (51). Moreover, due to their high concentration of fiber, peanuts may be considered as prebiotics, which can reduce the glycemic index and glycemic load (52).

The three selected studies evaluating blood pressure after a peanut intervention did not report any differences from the control group. Supporting these findings, other studies indicate that peanut consumption has no significant effect on SBP or DBP (53). In contrast, a randomized clinical trial observed that daily peanut consumption significantly reduced DBP, but did not alter SBP (47). Peanuts are a rich source of polyphenol compounds that can affect blood pressure (54). A study administering peanut sprout extract, which has a higher resveratrol content than peanuts, observed a significant reduction in SBP (55). In addition, peanuts are a rich source of protein, predominantly arginine, which is reported to improve endothelial function through nitric oxide release (56).

The ARISTOTLE study has several strengths, including its randomized and controlled design and its focus on the impact of peanut and peanut butter intake on the health of young healthy adults. Moreover, the peanut butter used in the study consisted exclusively of peanuts and sea salt, unlike other peanut butters that contain saturated fats as added ingredients. The main strong point of the systematic review and meta-analysis is their concentration on randomized controlled trials, including a new clinical trial, that have studied the effect of peanut consumption on metabolic syndrome.

The limitations of this research include the relatively small sample size of the ARISTOTLE study (19 to 23 individuals in each intervention) and although the sample size was calculated to assure 80% of statistical power, this value decreased to 70% due to dropouts and secondary outcomes analyzed in this manuscript. Also, the control group was based on peanut oil, as the hypothesis of the study was that the health benefits of peanuts are due to prebiotic substances, namely, polyphenols and fiber. On the other hand, the major limitation of the systematic review and meta-analysis is the heterogeneity of participants, comparator groups and follow-up periods in the included studies, which reduces evidence quality. The evidence for our major finding, that peanut product consumption improves the lipid profile, was rated as moderately strong in the case of triglycerides. However, the evidence for the impact of peanut consumption on the other outcomes (body composition, blood lipids, glucose metabolism and blood pressure) ranged between moderate and very low. Intervention effects can vary depending on the participant health status, so a strong point of the analysis is that it was also conducted on subgroups (healthy subjects vs. those at a high risk of or suffering cardiometabolic conditions). Moreover, interventions with peanuts/peanut butter and high-oleic peanuts were analyzed separately to identify possible differences. Other factors that may have influenced the results include peanut processing and/or the use of additives (i.e., salted *vs.* unsalted, roasted *vs.* raw, skinned *vs.* Non-skinned peanuts). The results may also be inconclusive due to the variability of control groups

among the studies. Another potential limitation is the unclear risk of bias reported in studies, associated with the randomization process, outcome measurement and deviation from the intended intervention domain.

CONCLUSIONS

In conclusion, this meta-analysis of randomized controlled trials, including novel results from the ARISTOTLE study, provides moderate evidence that peanut consumption has beneficial effects on triglycerides and tends to improve blood lipid values in general. However, no changes in body weight, glucose metabolism and blood pressure were observed. Although peanuts are energy-dense, their consumption does not promote weight gain in healthy subjects, and they can be incorporated into a dietary pattern to improve health. To gain more knowledge about the effects of peanut products on cardiometabolic risk factors, more carefully designed studies in larger populations are needed.

DATA AVAILABILITY STATEMENT

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

AUTHOR CONTRIBUTIONS

RL-R and SH-B designed the study. IP-M and SH-B collected data, performed statistical analysis, interpreted results, and drafted the manuscript. RL-R and MG-F interpreted the study results and performed the writing review of the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by the funding from the Peanut Institute 2019, CICYT under Grant PID2020-114022RB-I00, AGL2016- 75329-R, CIBEROBN from the Instituto de Salud Carlos III, ISCIII from the Ministerio de Ciencia, Innovación y Universidades (AEI/FEDER, UE), and Generalitat de Catalunya (GC) under Grant 2017SGR 196. None of the funders had a role in the study design, implementation, analysis or interpretation of the data, or the writing of the manuscript.

ACKNOWLEDGMENTS

We would like to thank all the volunteers who participated in the ARISTOTLE study, and IP-M is grateful for the predoctoral scholarship FI-SDUR (EMC/2703/2019) from the Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) research fellowship program.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.853378/full#supplementary-material

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Conflict of Interest: RL-R reports receiving lecture fees from Cerveceros de España; and receiving lecture fees and travel support from Adventia. Nevertheless, these foundations were not involved in the study design, the collection, analysis and interpretation of data, the writing of the manuscript or the decision to submit the manuscript for publication.

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.

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frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 06 May 2022
doi: 10.3389/fnut.2022.836115

Associations of Plasma Fatty Acid Patterns During Pregnancy With Gestational Diabetes Mellitus

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

Received: 15 December 2021
Accepted: 15 February 2022
Published: 06 May 2022

Citation:
Li P, Hu S, Zhu Y, Sun T, Huang Y, Xu Z, Liu H, Luo C, Zhou S, Tan A and Liu L (2022) Associations of Plasma Fatty Acid Patterns During Pregnancy With Gestational Diabetes Mellitus. Front. Nutr. 9:836115. doi: 10.3389/fnut.2022.836115

Background: Limited studies have explored the difference of fatty acid profile between women with and without gestational diabetes mellitus (GDM), and the results were inconsistent. Individual fatty acids tend to be interrelated because of the shared food sources and metabolic pathways. Thus, whether fatty acid patters during pregnancy were related to GDM odds needs further exploration.

Objective: To identify plasma fatty acid patters during pregnancy and their associations with odds of GDM.

Methods: A hospital-based case-control study including 217 GDM cases and 217 matched controls was carried out in urban Wuhan, China from August 2012 to April 2015. All the participants were enrolled at the time of GDM screening and provided fasting blood samples with informed consent. We measured plasma concentrations of fatty acids by gas chromatography–mass spectrometry, and derived potential fatty acid patterns (FAPs) through principal components analysis. Conditional logistic regression and restricted cubic spline model were used to evaluate the associations between individual fatty acids or FAPs and odds of GDM.

Results: Twenty individual fatty acids with relative concentrations ≥0.05% were included in the analyses. Compared with control group, GDM group had significantly higher concentrations of total fatty acids, 24:1n-9, and relatively lower levels of 14:0, 15:0, 17:0, 18:0, 24:0, 16:1n-7, 20:1n-9,18:3n-6, 20:2n-6, 18:3n-3, 20:3n-3, 22:5n-3. Two novel patterns of fatty acids were identified to be associated with lower odds of GDM: (1) relatively higher odd-chain fatty acids, 14:0, 18:0, 18:3n-3, 20:2n-6, 20:3n-6 and lower 24:1n-9 and 18:2n-6 [adjusted odds ratio (OR) (95% confidence interval) (CI) for quartiles 4 vs. 1: 0.42 (0.23–0.76), *P*-trend = 0.002], (2) relatively higher n-3

polyunsaturated fatty acids, 24:0, 18:3n-6 and lower 16:0 and 20:4n-6 [adjusted OR (95% CI) for quartiles 4 vs. 1: 0.48 (0.26–0.90), *P*-trend = 0.018].

Conclusion: Our findings suggested that two novel FAPs were inversely associated with GDM odds. The combination of circulating fatty acids could be a more significant marker of GDM development than individual fatty acids or their subgroups.

Keywords: fatty acid, gestational diabetes mellitus, pattern, case-control study, pregnancy

INTRODUCTION

Gestational diabetes mellitus (GDM) refers to hyperglycemia diagnosed for the first time during pregnancy and is one of the most common complications of pregnancy in the world. According to the 10th edition of the *Diabetes Atlas* published by the *International Diabetes Federation*, an estimated 16.7% (21.1 million) of live births were affected by hyperglycemia in pregnancy in 2021. Of which, 80.3% were due to GDM (1). Although GDM usually resolves once the delivery ends, its impact on maternal and child health cannot be ignored. Women with GDM during pregnancy are at higher risk of adverse pregnancy outcomes and have an increased risk of developing GDM in subsequent pregnancies or type 2 diabetes later in life (2, 3). Babies exposed to hyperglycemia *in utero* also have a life-long higher risk of obesity and glucose intolerance (4, 5). The prevention of GDM could have far-reaching effects on the short-term and long-term health of mothers and offsprings. Hence, identifying potentially modifiable risk factors and evaluating their impact on GDM is of high priority.

Recent years, dietary fat intake has been shown to be associated with the development of insulin resistance and diabetes (6, 7). As the important composition of fat, fatty acids in tissues can reflect both the quantity and quality of dietary fat intake without being affected by recall bias and have been considered as reliable biomarkers in epidemiologic studies (8). However, up to date, limited studies have explored the difference of fatty acid profile between women with and without GDM (9). Some of them have suggested that certain fatty acids might be associated with the development of GDM, but the results were inconsistent and partly depends on the fatty acids involved (10–17). Furthermore, all the related studies only focused on traditional groups of fatty acids or a few selected individual fatty acids, whereas many circulating fatty acids of which the concentrations tend to be interrelated because of the shared food sources and metabolic pathways (18). Otherwise, fatty acid composition in previous studies were often expressed as relative amounts like mole percent or weight percent (19), which lead to the fact that a change in the level of one fatty acid might influence the amounts of others. Thus, the potential synergistic and additive effects among different fatty acids may be ignored when separately investigating the potential influence of targeted individual fatty acids or fatty acid groups on GDM risk.

To take the above complexity into account, using statistical pattern-recognition approach might be worth trying. Principal components analysis (PCA), a widely used statistical method of dimension reduction, has been applied to explore novel informative patterns such as dietary patterns or genetic patterns in complex data in the field of epidemiology (20, 21). As to biomarker panels like circulating fatty acids, PCA is also applicable and has a main effect on maximizing the information of both major and minor fatty acids. Several epidemiologic studies have identified associations of circulating or tissue fatty acid patterns (FAPs) with obesity, hypertension, cardiovascular diseases, type 2 diabetes, and cancers (22–26), which indicated that potential clinical and biological implications were existed and over that of individual fatty acids or fatty acid subgroups. However, whether FAPs during pregnancy were related to GDM odds remains unknown. We therefore conducted a matched case-control study among Chinese women to investigate the association of plasma fatty acid subgroups and individual fatty acids during pregnancy with odds of GDM initially, and further applied PCA to identify the novel FAPs associated with GDM.

MATERIALS AND METHODS

Study Population

The hospital-based case-control study was carried out in urban Wuhan, China from August 2012 to April 2015. Pregnant women who screened for GDM at the outpatient clinics of the Department of Endocrinology, Tongji Hospital were invited to participant in the study. The inclusion criteria were as follows: (1) age ≥20 years; (2) gestational age at GDM screening ≥24 weeks; (3) singleton pregnancy. We excluded women who met any of the following items: history of diabetes (including but not limited to GDM), cardiovascular disease, cancer or other systemic diseases; pharmacologic treatment or dietary supplements use (e.g., fish oil, cod liver oil, pure docosahexaenoic acid supplements, albumen power, etc.) that might influence glucose or lipids metabolism; accompanied by other pregnancy complications; blood sample hemolysis or insufficiency; and incomplete basic information. Fasting blood samples (5 ml) were collected at the time of GDM screening using anticoagulant tubes and centrifuged at 3,000 rpm for 5 min. Plasma were separated from blood cells and stored at −80°C for further assay. The study was approved by the Ethics Committee of Tongji

Abbreviations: BMI, body mass index; FAME, fatty acid methyl ester; FAP, fatty acid pattern; FPG, fasting plasma glucose; FPI, fasting plasma insulin; GC–MS, gas chromatography–mass spectrometry; GDM, gestational diabetes mellitus; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low density lipoprotein cholesterol; MUFA, monounsaturated fatty acid; OGTT, oral glucose tolerance test; PCA, principal components analysis; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.

Medical College and was registered at www.clinicaltrials.gov as NCT05146401. All participants gave written informed consent before enrolling in the study.

Selection of Gestational Diabetes Mellitus Cases and Controls

According to the diagnostic criteria advocated by International Association of the Diabetes and Pregnancy Study Groups (IADPSG)/WHO (27), the diagnosis of GDM can be made if one or more glucose values are above the cut points of 5.1, 10.0, and 8.5 mmol/L at fasting, 1 and 2 h during a 75-gram oral glucose tolerance test (OGTT). Controls were randomly selected and individually matched to cases by age (± 2 years), gestational age (± 2 weeks) and parity.

Measurement of Plasma Fatty Acids

The derivatization of fatty acids in plasma was achieved by the modified direct transesterification method proposed by Lepage and Roy (28). According to the method, total plasma lipids were hydrolyzed and derived to fatty acid methyl esters (FAMEs) for further instrumental analysis. Fifty microliter of plasma was mixed with 3.6 ml of methanol-acetyl chloride (8:1; v/v) in a disposable glass tube, then 10 μl internal standard composed of non-adecanoic acid methyl ester (800 μg/ml) and 150 μl hexane were added. The sample mixture was incubated at 100°C for 1 h and cooled on ice. Afterward, 2.5 ml of 12% K_2CO_3 solution was slowly added to stop the reaction and neutralize the mixture. The tubes were vortexed, followed by centrifugation at 3,000 rpm for 5 min. Then, the organic layer was collected for the subsequent analysis. FAMEs were separated and analyzed by an Agilent 7890B gas chromatography (GC) coupled with an Agilent 5977A Series mass spectrometry (MS). The inlet temperature was set as 250°C. The temperature of oven was initially held at 50°C for 1 min and increased to 100°C at a rate of 25°C/min, then to 130°C at a rate of 10°C/min, and finally raised to 250°C at a rate of 5°C/min and held for 5 min. Total run time was 33 min. As to scan modes, the total ion count was used for compound identification while the selected ion monitoring was used for quantification. The dilution series of the standard mixture of 37 FAMEs and the individual standard of C22:5n-3 FAME (both from Sigma Aldrich, Poole, United Kingdom) were used for the establishment of calibration curves. The molecular weights of FAMEs, selected ions, and retention times for fatty acids detection are shown in **Supplementary Table 1**. The validation of analytical method was performed by reference to the European Medicines Agency Guideline on bioanalytical method validation (29). Blank samples spiked with three known concentration levels (low, medium, and high) of quality control samples were used for accuracy and precision analyses. The intra- and inter-day accuracy (expressed as recovery values) of all analytes ranged from 86.04 to 114.78% and 86.11 to 114.11%, while the intra- and inter-day precision (expressed as the coefficient of variation) of all analytes ranged from 0.63 to 7.58% and 1.15 to 12.28%, respectively. Validation results were provided in detail in the **Supplementary Tables 2–4**.

Identification of Fatty Acid Patterns

In the current study, 18 trace fatty acids were excluded in the analyses because they were undetectable in most samples or the relative concentrations were <0.05% on average. After exclusion, 20 individual fatty acids, including 6 saturated fatty acids (SFAs), 4 monounsaturated fatty acids (MUFAs), and 10 polyunsaturated fatty acids (PUFAs), were used to derive FAPs through PCA. Principal components were inferred as FAPs, and we determined the number of patterns to analyze based on scree plot and eigenvalues (>1). The factor loadings reflect the contributions of individual fatty acids to principal components, which means the closer the absolute value is to 1, the greater the influence of single fatty acid on the pattern. A score of each pattern was calculated by summing fatty acid concentrations weighted by the scoring coefficients of each fatty acid.

Covariates

Sociodemographic factors, lifestyle, and health information were collected by trained interviewers with standardized questionnaires. Height was measured using calibrated instrument. Prepregnancy body mass index (BMI) was calculated as self-reported prepregnancy weight divided by height squared (kg/m^2). Fasting concentrations of plasma glucose (FPG), total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured using the commercial assay kits (Biosino Bio-Technology and Science, Inc.). Fasting plasma insulin (FPI) was measured by enzyme-linked immunoassay (ELISA) kits (Mercodia Company), and insulin resistance was assessed by homeostasis model assessment (HOMA) index.

Statistical Analyses

The concentration of total fatty acids (μmol/L) was calculated as a sum of individual fatty acids (μmol/L). Individual fatty acids were expressed as mole percentage of total fatty acids. Differences of continuous variables between case and control groups were tested by the Student's *t*-test or Mann-Whitney *U*-test, when appropriate. For categorical variables, Chi-square (χ^2) test were adopted. Spearman partial correlation coefficients were used to estimate the relations between individual fatty acids and plasma glucose in OGTT, lipids and insulin resistance. To evaluate the associations between fatty acids and odds of GDM, we used conditional logistic regression. Odds ratios (ORs) with 95% confidence intervals (CIs) of GDM were showed in quartiles which were based on the distribution of fatty acids among controls. Tests for linear trend were conducted by treating median value for each quartile of individual fatty acids as continuous variables. False discovery rate (FDR) correction was applied in the multiple comparisons of fatty acids to reduce the false positive rate (30). Potential confounding factors including age, gestational age at blood collection, parity, prepregnancy BMI, family history of diabetes, smoking and alcohol use were adjusted in multivariable models.

To explore the association between combinations of plasma fatty acids and GDM, we did further analyses. We treated the scores of each pattern as continuous variables and evaluated

the association of FAP score with GDM odds by ORs and 95% CIs through conditional logistic regression, with potential confounders adjusted. In addition, a restricted cubic spline model with four knots was used to assess the possible non-linear associations. The relationships of FAP score with indexes of glucose and lipid metabolism were also evaluated by Spearman partial correlation coefficients. Data were analyzed with SPSS 20.0 software package (SPSS, Inc.) or Stata version 13.0 (StataCorp). All *P*-values were two-sided and the threshold for statistical significance was 0.05.

RESULTS

Characteristics of the Participants

Among the 683 confirmed participants who met the inclusion criteria, 15 women were excluded for previous diagnosis of diabetes, 17 women were excluded because of supplements use that might influence glucose or lipids metabolism, 3 women were excluded for incomplete basic information, 105 participants were excluded due to insufficient plasma samples (mainly consumed by previous studies). Hence, 253 women with GDM and 290 healthy pregnant women were eligible for further case-control matching by SPSS software. Finally, 217 GDM cases and 217 matched controls were selected in this study.

The demographic, anthropometric, reproductive, and metabolic characteristics of the participants were exhibited in **Table 1** by case-control status. The two groups were comparable for age, gestational age, and parity. Compared to controls, GDM cases had significantly higher levels of prepregnancy BMI, FPG, 1- and 2-h post-glucose load, FPI, triglycerides and insulin resistance values, and are prone to have a family history of diabetes. The concentrations of total fatty acids were significantly higher in cases than in controls [median: 2624.96 (interquartile range 2073.66–3472.03) vs. 2458.38 (interquartile range 1857.25–2985.84) μmol/L, $P = 0.001$].

Association of Individual Fatty Acids and Gestational Diabetes Mellitus

Plasma fatty acid composition was shown in **Table 2**. According to the methods, only fatty acids with relative concentrations (mol%) higher than 0.05% are displayed in the results. In both case and control groups, the most abundant fatty acids were 16:0 and 18:2n-6, whereas 24:0 and 15:0 were present in low relative concentrations. When compared GDM with control subjects, significant difference was found with several fatty acids, including five SFAs (14:0, 15:0, 17:0, 18:0, 24:0), three MUFAs (16:1n-7, 20:1n-9, 24:1n-9), and five PUFAs (18:3n-6, 20:2n-6, 18:3n-3, 20:3n-3, 22:5n-3).

Spearman partial correlations between individual fatty acids and the indexes of glucose and lipid metabolism were shown in **Table 3**. HOMA-IR, the index that reflects insulin resistance levels, was correlated positively with 14:0, 16:0, 16:1n-7, 20:3n-6 (β = 0.14, 0.30, 0.14, and 0.15, respectively) and negatively

TABLE 1 | Characteristics among women with GDM and their matched controls.

Characteristics	GDM (*N* = 217)	Non-GDM (*N* = 217)	*P*
Age (years)	30.06 ± 3.81	29.63 ± 3.76	0.239
Pre-pregnancy BMI (kg/m^2)	22.15 ± 3.23	20.80 ± 2.64	<0.001
Parity, n (%)			1.000
1	176 (81.1)	176 (81.1)	
2	40 (18.4)	40 (18.4)	
3	1 (0.5)	1 (0.5)	
Gestational age at blood sample collection (weeks)	28.00 (26.00–30.00)	28.00 (26.00–30.00)	0.811
Family history of diabetes, n (%)	56 (25.8)	30 (13.8)	0.002
Alcohol use, n (%)	10 (4.6)	11 (5.1)	0.823
Smoking, n (%)	3 (1.4)	5 (2.3)	0.721
FPG (mmol/L)	5.21 (4.99–5.44)	4.73 (4.58–4.93)	<0.001
OGTT-1h (mmol/L)	9.78 (8.62–10.94)	7.59 (6.63–8.44)	<0.001
OGTT-2h (mmol/L)	8.65 (7.59–9.44)	6.86 (6.19–7.60)	<0.001
FPI (μU/mL)	10.38 (7.67–13.81)	8.16 (5.94–10.59)	<0.001
HOMA-IR	2.47 (1.74–3.25)	1.68 (1.25–2.31)	<0.001
Total cholesterol (mmol/L)	5.48 (4.80–6.37)	5.54 (4.78–6.14)	0.526
Triglycerides (mmol/L)	2.83 (2.26–3.73)	2.40 (1.87–3.40)	<0.001
LDL-C (mmol/L)	3.22 (2.52–4.01)	3.15 (2.44–3.85)	0.326
HDL-C (mmol/L)	1.36 (1.17–1.60)	1.38 (1.12–1.61)	0.903
Plasma total fatty acids (μmol/L)	2624.96 (2073.66–3472.03)	2458.38 (1857.25–2985.84)	0.001

Continuous variables are shown as mean ± SDs when normally distributed, or median (IQRs) when skewed distributed. Categorical variables are shown as n (%). BMI, body mass index; FPG, fasting plasma glucose; FPI, fasting plasma insulin; GDM, gestational diabetes mellitus; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low density lipoprotein cholesterol; OGTT-1h, 1-h post-glucose load; OGTT-2h, 2-h post-glucose load.

TABLE 2 | Composition (mol% of total fatty acids) of plasma fatty acids among GDM cases and non-GDM controls.

	GDM cases (*N* = 217),%	Non-GDM controls (*N* = 217),%	*P*
SFAs			
Total SFAs	43.77 (41.01–45.94)	43.85 (41.96–45.59)	0.547
Even-chain SFAs			
Total even-chain SFAs	43.45 (40.65–45.48)	43.37 (41.66–45.04)	0.635
14:0	0.34 (0.22–0.47)	0.37 (0.27–0.54)	0.006
16:0	35.47 (33.78–37.86)	35.02 (33.50–37.30)	0.154
18:0	5.86 (4.41–8.66)	7.23 (5.37–8.91)	0.001
24:0	0.07 (0.05–0.09)	0.08 (0.06–0.10)	0.001
Odd-chain SFAs			
Total odd-chain SFAs	0.29 (0.22–0.42)	0.35 (0.28–0.47)	<0.001
15:0	0.09 (0.07–0.11)	0.10 (0.08–0.13)	<0.001
17:0	0.21 (0.15–0.31)	0.25 (0.19–0.33)	0.001
MUFAs			
Total MUFAs	13.62 (11.63–15.41)	13.52 (11.99–16.14)	0.513
16:1n-7	0.50 (0.33–0.83)	0.63 (0.43–0.93)	0.003
18:1n-9	12.19 (10.00–13.89)	12.19 (10.58–14.49)	0.260
20:1n-9	0.16 (0.13–0.20)	0.19 (0.15–0.24)	<0.001
24:1n-9	0.41 (0.13–1.39)	0.24 (0.12–1.06)	0.006
PUFAs			
Total PUFAs	42.37 (40.12–45.59)	42.20 (39.61–44.77)	0.158
n-6 PUFAs			
Total n-6 PUFAs	36.15 (33.87–39.10)	35.55 (33.53–38.25)	0.187
18:2n-6	27.71 (23.68–31.47)	26.88 (23.78–30.94)	0.366
18:3n-6	0.17 (0.13–0.20)	0.19 (0.14–0.22)	0.001
20:2n-6	0.36 (0.27–0.48)	0.43 (0.33–0.56)	<0.001
20:3n-6	1.49 (1.11–2.13)	1.60 (1.114–2.20)	0.363
20:4n-6	6.02 (4.99–7.93)	6.18 (4.74–7.68)	0.688
n-3 PUFAs			
Total n-3 PUFAs	6.20 (5.18–7.09)	6.23 (5.11–7.09)	0.737
18:3n-3	0.45 (0.35–0.59)	0.51 (0.40–0.69)	0.001
20:3n-3	0.16 (0.11–0.20)	0.19 (0.15–0.23)	<0.001
20:5n-3	0.40 (0.31–0.51)	0.42 (0.34–0.53)	0.079
22:5n-3	0.40 (0.26–0.52)	0.43 (0.30–0.58)	0.020
22:6n-3	4.59 (3.93–5.52)	4.48 (3.70–5.24)	0.066
n-6/n-3 PUFAs	5.91 (4.91–7.13)	5.85 (4.89–7.14)	0.857

GDM, gestational diabetes mellitus; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

with 15:0, 17:0, 24:0, 20:1n-9, 18:2n-6, 18:3n-6, 20:2n-6, 18:3n-3 (β =−0.13, −0.12, −0.21, −0.24, −0.21, −0.17, −0.17, and −0.12, respectively).

In the conditional logistic regression analyses, the adjusted ORs (95% CIs) of GDM across increasing quartiles of plasma total fatty acid levels were 1.00 (referent), 1.43 (0.73–2.81), 1.11 (0.56–2.22), and 2.35 (1.24–4.47), respectively. Results for associations between fatty acid subgroups, individual fatty acids and GDM odds were displayed in **Table 4** (more details could be found in **Supplementary Tables 5,6**). After adjustment for age, gestational age at blood collection, parity, prepregnancy BMI, family history of diabetes, smoking, and alcohol use, total SFAs, MUFAs, or PUFAs were not significantly associated with GDM odds. However, odd-chain SFAs were inversely associated with GDM odds, whether individually or combined. Among the 20 individual fatty acids included in the analyses, 13 fatty acids were considered to be negatively associated with odds of GDM. It is worth noting that the abundance of the 13 fatty acids were all relatively low, with a proportion range of 0.08% (24:0) to 6.72% (18:0). In addition, the OR (95% CI) for GDM compared the highest with lowest quartiles of 24:1n-9 was 2.05 (1.12–3.76).

Association of Novel Fatty Acid Patterns and Gestational Diabetes Mellitus

The correlation matrix of individual fatty acids was shown in **Figure 1**. Four major FAPs were identified and explained 31.41, 18.47, 13.56, and 9.68% of the overall variability, respectively. The scree plot starts to flatten from the fifth principal component (**Figure 2**), which was consistent with the above results.

Factor loadings of the 20 fatty acids for the four patterns were shown in **Figure 3**. The first pattern (FAP1) was characterized by higher relative concentrations of odd-chain fatty acids, 14:0, 18:0, 18:3n-3, 20:2n-6, 20:3n-6, and lower relative concentrations

TABLE 3 | Partial correlation coefficients between plasma individual fatty acids and variables of interest[a].

	14:0	15:0	16:0	17:0	18:0	24:0	16:1	18:1	20:1	24:1	18:2n-6	18:3n-6	20:2n-6	20:3n-6	20:4n-6	18:3n-3	20:3n-3	20:5n-3	22:5n-3	22:6n-3
FPG (mmol/L)	−0.08	−0.23*	0.08	−0.18*	−0.10*	−0.14*	−0.11*	−0.01	−0.21*	0.04	−0.01	−0.15*	−0.21*	0.01	0.07	−0.13*	−0.14*	−0.06	−0.06	0.05
OGTT-1h (mmol/L)	−0.14*	−0.19*	0.01	−0.12	−0.11*	−0.14*	−0.14*	−0.03	−0.18*	0.05	0.06	−0.16*	−0.18*	−0.07	0.03	−0.16*	−0.24*	−0.15*	−0.18*	−0.01
OGTT-2h (mmol/L)	−0.02	−0.11*	0.07	−0.06	−0.12*	−0.15*	−0.02	0.03	−0.14*	0.03	−0.03	−0.12*	−0.14*	−0.01	0.03	−0.12*	−0.17*	−0.05	−0.13*	−0.01
TC (mmol/L)	−0.14*	−0.24*	0.09	−0.21*	−0.13*	−0.36*	−0.13*	0.01	−0.24*	0.06	0.06	−0.20*	−0.22*	−0.13*	−0.02	−0.19*	−0.17*	−0.19*	−0.04	0.05
TG (mmol/L)	0.17*	−0.41*	0.34*	−0.35*	−0.25*	−0.34*	0.20*	0.35*	−0.30*	−0.05	−0.09	−0.35*	−0.35*	−0.09	−0.20*	−0.11*	−0.23*	−0.30*	−0.19*	−0.26*
HDL-C (mmol/L)	−0.02	0.01	0.05	0.01	0.08	−0.03	−0.02	−0.19*	−0.10*	0.01	−0.07	−0.03	−0.03	0.09	0.17*	−0.12*	−0.01	0.07	0.19*	0.16*
LDL-C (mmol/L)	−0.09	−0.28*	0.10*	−0.28*	−0.29*	−0.12*	−0.16*	0.07	−0.23*	0.13*	0.19*	−0.14*	−0.28*	−0.20*	−0.16*	−0.20*	−0.18*	−0.25*	−0.18*	−0.08
FPI (μU/mL)	0.16*	−0.10*	0.31*	−0.11	0.02	−0.19*	0.17*	−0.01	−0.22*	−0.03	−0.21*	−0.15*	−0.15*	0.15*	0.06	−0.12*	−0.04	0.02	0.01	−0.07
HOMA-IR	0.14*	−0.13*	0.30*	−0.12*	0.03	−0.21*	0.14*	0.02	−0.24*	−0.03	−0.21*	−0.17*	−0.17*	0.15*	0.08	−0.12*	−0.07	−0.04	−0.01	−0.05

[a]*Adjustment were made for age, gestational age at blood collection, parity, prepregnancy BMI, family history of diabetes, smoking and alcohol use. *P < 0.05. FPG, fasting plasma glucose; FPI, fasting plasma insulin; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low density lipoprotein cholesterol; OGTT-1h, 1-h post glucose load; OGTT-2h, 2-h post glucose load; TC, total cholesterol; TG, triglycerides.*

of 24:1n-9 and 18:2n-6. The second pattern (FAP2) was characterized by higher abundance of n-3 PUFAs, 24:0, 18:3n-6 and lower proportions of 16:0 and 20:4n-6. The third pattern (FAP3) was featured by higher relative concentrations of 22:6n-3, 22:4n-6, 22:5n-3 and lower relative concentrations of 18:1n-9. The fourth pattern (FAP4) had moderated high factor loadings for 16:0 and 16:1n-7.

According to the scoring coefficients matrix (**Supplementary Table 7**), the score of each pattern can be calculated for each participant. **Table 5** displays the partial correlation coefficients between FAP scores and metabolic factors. Higher FAP1 and FAP2 scores were modestly correlated with lower FPG, 1-h post glucose load, total cholesterol, triglycerides and LDL-C, and FAP2 score was further negatively correlated with 2-h post-glucose load, FPI, and HOMA-IR. Moreover, FAP3 score was positively correlated with triglycerides and HDL-C, while FAP4 was positively correlated with triglycerides, FPI, and HOMA-IR.

After multivariable adjustment, the FAP1 and FAP2 were inversely associated with GDM odds (**Table 6**). Compared with women in the lowest quartile of FAP1 score, women in the highest quartile experienced a 58% lower odds of GDM (OR, 0.42; 95% CI, 0.23–0.76). The OR (95% CI) of GDM comparing extreme quartiles of FAP2 score was 0.48 (0.26–0.90). Potential non-linear associations of FAP1 and FAP2 with odds of GDM were also found in the restricted cubic spline model (**Figure 4**). Nevertheless, little evidence of an association was found between quartiles of FAP3 or FAP4 scores and odds of GDM.

DISCUSSION

In this matched case-control study, we initially found that 13 individual fatty acids including 5 SFAs (14:0, 15:0, 17:0, 18:0, 24:0), 2 MUFAs (16:1n-7, 20:1n-9), and 6 PUFAs (18:3n-6, 20:2n-6, 18:3n-3, 20:3n-3, 20:5n-3, 22:5n-3) were negatively associated with odds of GDM, while 24:1n-9 was positively associated with GDM odds. Afterward, we identified two certain patterns of circulating fatty acids which were associated with lower odds of GDM. The first pattern was characterized by higher relative concentrations of odd-chain fatty acids, 14:0, 18:0, 18:3n-3, 20:2n-6, 20:3n-6 and lower relative concentrations of 24:1n-9 and 18:2n-6. The second pattern was characterized by higher abundance of n-3 PUFAs, 24:0, 18:3n-6, and lower proportions of 16:0 and 20:4n-6. To our knowledge, this is the first study focused on circulating FAPs related to odds of GDM. The above results suggested that the combinations of circulating fatty acids could be a more significant marker of GDM development than individual fatty acids or their subgroups.

For fatty acids with relatively high concentrations, small changes in the levels of other fatty acids may have little impact on their relative amounts or the relationships with diseases. However, for fatty acids with relatively low concentrations, the influence of others might be more considerable. In our study, individual fatty acids which were significantly associated with GDM odds were mostly of the second type. Thus, investigation of the FAPs with PCA may confer benefits over investigating

TABLE 4 | Association between fatty acid subgroups, individual fatty acids and GDM[a].

	Quartiles of fatty acids (%)				P_{trend} [b]	P_{FDR} [c]
	Q 1	Q 2	Q 3	Q 4		
SFAs	1	0.51 (0.27–0.94)	0.57 (0.31–1.04)	0.74 (0.41–1.34)	0.132	0.198
Even-chain SFAs	1	0.39 (0.20–0.75)	0.62 (0.34–1.12)	0.83 (0.45–1.53)	0.241	0.325
14:0	1	0.54 (0.29–1.01)	0.77 (0.44–1.35)	0.55 (0.31–0.99)	0.101	0.170
16:0	1	0.89 (0.46–1.74)	1.64 (0.84–3.20)	1.71 (0.85–3.45)	0.054	0.097
18:0	1	0.20 (0.09–0.44)	0.21 (0.11–0.40)	0.32 (0.16–0.63)	<0.001	<0.001
24:0	1	0.51 (0.28–0.92)	0.26 (0.12–0.54)	0.41 (0.22–0.79)	0.002	0.009
Odd-chain SFAs	1	0.33 (0.17–0.63)	0.46 (0.26–0.81)	0.45 (0.25–0.83)	0.021	0.047
15:0	1	0.85 (0.47–1.54)	0.42 (0.23–0.78)	0.48 (0.25–0.91)	0.004	0.014
17:0	1	0.31 (0.17–0.59)	0.47 (0.26–0.84)	0.43 (0.23–0.78)	0.026	0.054
MUFAs	1	0.54 (0.29–1.01)	0.96 (0.53–1.75)	0.70 (0.37–1.33)	0.475	0.513
16:1n-7	1	0.48 (0.26–0.90)	0.31 (0.16–0.61)	0.49 (0.27–0.88)	0.007	0.019
18:1n-9	1	0.61 (0.34–1.11)	0.94 (0.51–1.73)	0.65 (0.33–1.25)	0.326	0.400
20:1n-9	1	0.50 (0.26–0.96)	0.29 (0.15–0.59)	0.25 (0.12–0.52)	<0.001	<0.001
24:1n-9	1	0.95 (0.51–1.80)	0.98 (0.51–1.87)	2.05 (1.12–3.76)	0.003	0.012
PUFAs	1	1.13 (0.61–2.07)	1.07 (0.58–1.98)	1.32 (0.73–2.37)	0.381	0.429
n-6 PUFAs	1	0.86 (0.46–1.60)	0.94 (0.51–1.74)	1.43 (0.78–2.61)	0.158	0.225
18:2n-6	1	0.54 (0.29–1.03)	0.93 (0.52–1.67)	1.08 (0.60–1.97)	0.364	0.427
18:3n-6	1	0.70 (0.38–1.26)	0.34 (0.17–0.67)	0.48 (0.25–0.92)	0.008	0.020
20:2n-6	1	0.52 (0.29–0.90)	0.39 (0.21–0.73)	0.30 (0.15–0.59)	<0.001	<0.001
20:3n-6	1	0.83 (0.45–1.51)	0.64 (0.34–1.19)	0.66 (0.36–1.19)	0.123	0.195
20:4n-6	1	1.03 (0.55–1.93)	0.57 (0.30–1.09)	1.06 (0.56–1.99)	0.747	0.776
n-3 PUFAs	1	0.92 (0.51–1.67)	0.93 (0.50–1.73)	0.96 (0.53–1.72)	0.907	0.907
18:3n-3	1	0.51 (0.28–0.92)	0.54 (0.28–1.03)	0.37 (0.19–0.73)	0.007	0.019
20:3n-3	1	0.45 (0.25–0.83)	0.32 (0.17–0.64)	0.26 (0.13–0.50)	<0.001	<0.001
20:5n-3	1	0.35 (0.18–0.67)	0.49 (0.26–0.90)	0.47 (0.25–0.89)	0.030	0.058
22:5n-3	1	0.52 (0.27–0.98)	0.51 (0.27–0.98)	0.33 (0.17–0.66)	0.002	0.009
22:6n-3	1	1.01 (0.55–1.86)	0.95 (0.51–1.76)	1.34 (0.77–2.34)	0.297	0.382

[a] *Values are ORs (95% CIs). Adjustments were made for age, gestational age at blood collection, parity, prepregnancy BMI, family history of diabetes, smoking, and alcohol use.*

[b] P_{trend} *values were obtained from logistic regression by treating median value of each quartile of individual fatty acids as continuous variables.*

[c] P_{FDR} *values were P values of FDR corrections for multiple comparisons of fatty acids.*

FDR, False discovery rate; GDM, gestational diabetes mellitus; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

individual fatty acids or fatty acid groups, and provide new insight into the joint effect of circulating fatty acids on GDM development.

The FAP1 that we identified contained positive loadings for odd-chain fatty acids, 14:0, 18:0, 18:3n-3, 20:2n-6, 20:3n-6 and negative loadings for 24:1n-9 and 18:2n-6. Although the main fatty acids involved in this pattern were distributed in various subgroups, internal correlation existed. Linoleic acid (18:2n-6), the most abundant n-6 PUFA in human circulating, is the precursor for the endogenous synthesis of other long-chain n-6 PUFAs, including 20:2n-6, 20:3n-6, and 20:4n-6 (8). A recent study conducted by Kim et al. found that low 18:2n-6 diet and estrogen significantly increased the expression of peroxisome proliferator-activated receptor α (PPAR-α), fatty acid desaturase 2 (Δ6 desaturase), and elongases of vary long chain fatty acids in rats liver (31). The fatty acid desaturases and elongases play a major role in the levels of circulating long chain PUFAs (32). Linoleic acid can compete with α-linolenic acid (18:3n-3) for rate-limiting enzyme like Δ6 desaturase and affect circulating level of 18:3n-3 and its conversion into very long chain n-3 PUFAs eventually (33). As a ligand-dependent transcription factor, PPAR-α can be activated by fatty acids and their derivatives (34), and have been found to be able to downregulate the expression of Δ9-16 and Δ9-18 desaturases which are the rate-limiting enzymes in synthesis of 16:1n-7 and 18:1n-9. The above evidence may partly explain the inverse correlation of 18:2n-6 with 18:0, 18:3n-3, 20:2n-6 and 20:3n-6 in our study. Several potential mechanisms might contribute to the protective role of FAP1 in GDM development. Firstly, 18:2n-6 is the direct precursor of hydroxyl conjugated linoleic acid. Research evidence has suggested that lowering dietary 18:2n-6 from 6.7 to 2.4% of calories for 12 weeks could markedly reduce the abundance of human plasma hydroxyl conjugated linoleic acid which is the important component of oxidized low density lipoprotein and has the effect of promoting inflammatory (35). Inflammation and dyslipidemia are considered to be the major mechanisms of insulin resistance. Secondly, 18:2n-6 competes with 18:3n-3

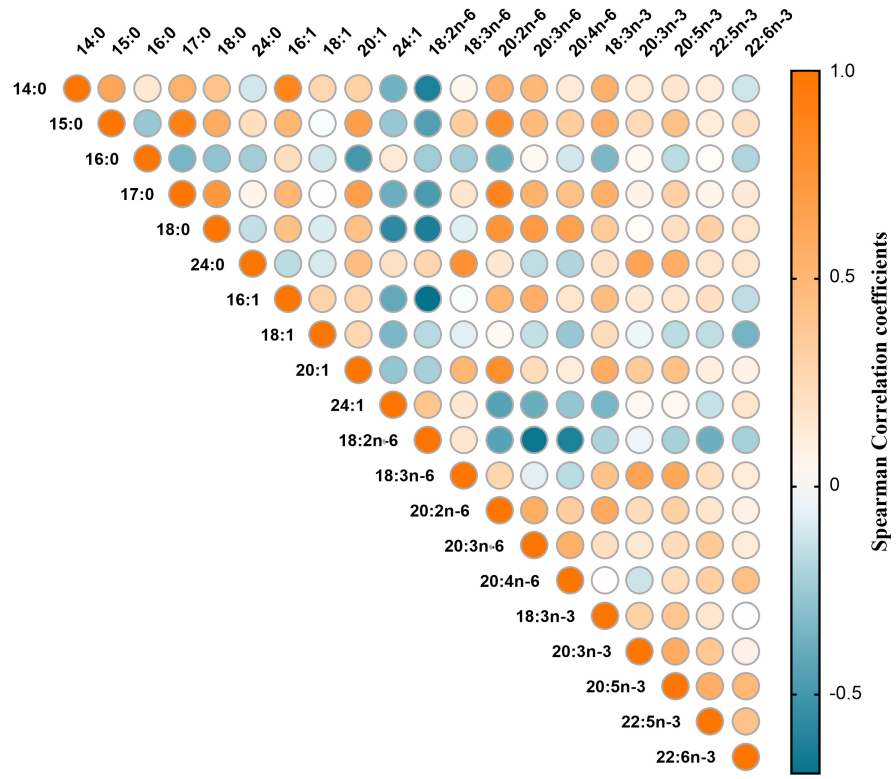

FIGURE 1 | Heat map for spearman correlation matrix of 20 fatty acids measured among 217 GDM cases and matched controls.

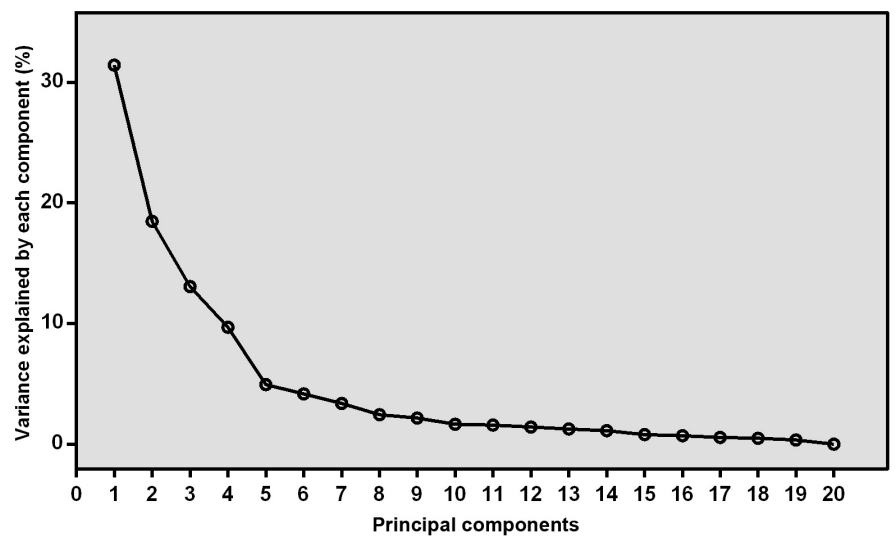

FIGURE 2 | The scree plot derived from principal component analysis. The variance that explained by each component (%) was exhibited.

for conversion process. Study conducted by Ghafoorunissa et al. found that the substitution of one-third of dietary 18:2n-6 with 18:3n-3 resulted in lowered blood lipid levels and increased insulin sensitivity in sucrose fed rats, possibly due to the resulting high long chain n-3 PUFA levels in target tissues of insulin action (36). Thirdly, odd-chain fatty acids, a subclass of SFA with very low abundance, are proved to be biomarkers of dairy fat and dietary fiber intake. A large meta-analysis that pooled findings from 16 prospective cohort studies indicated that higher levels of 15:0 and 17:0 were associated with a lower risk of type 2 diabetes (37). The inverse association between odd-chain fatty acids and diabetes was

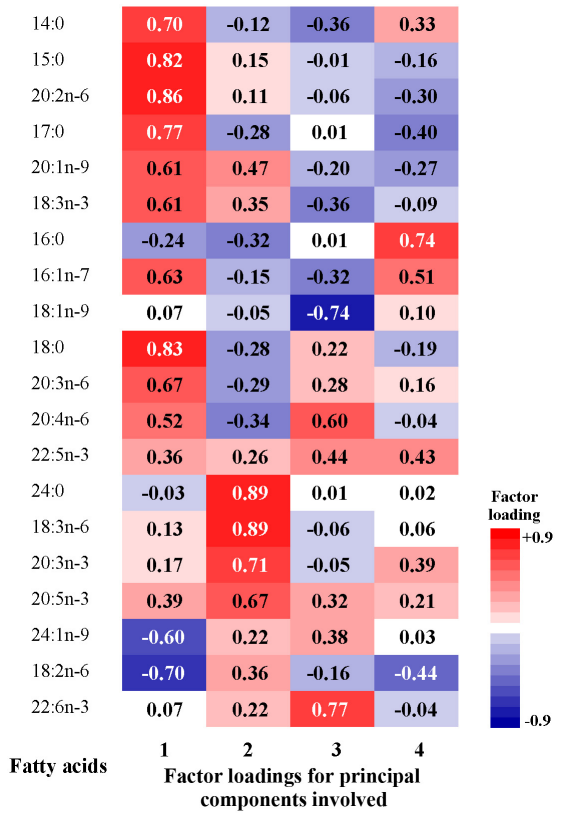

FIGURE 3 | Factor loadings of the 20 fatty acids for the 1st to 4th principal components. The closer the factor loading (absolute value) is to 1, the greater the influence of single fatty acid on the pattern.

considered to be partly related to dietary fiber and dairy fiber intake (38).

The FAP2 was characterized by higher abundance of n-3 PUFAs, 24:0, 18:3n-6 and lower proportions of 16:0 and 20:4n-6. As the most abundant fatty acid in circulation, 16:0 can be directly obtained from foods or synthesized from other fatty acids, amino acids or carbohydrates *via de novo* lipogenesis. The level of 16:0 in tissue was relatively stable, and changes of dietary intake seemed have little influence on its concentration because the exogenous source was counterbalanced by the endogenous biosynthesis (39). However, pathological state, chronic malnutrition, or unhealthy lifestyle factors like physical inactivity may have great impact on *de novo* lipogenesis and further affect 16:0 level (40, 41). Mounting evidence suggested that disruption in 16:0 homeostasis was associated with the development of diseases including cardiovascular diseases, metabolic diseases, neurodegenerative diseases and cancers (39). Higher tissue 16:0 could induce inflammatory responses and metabolic dysregulation which further result in dyslipidemia, hyperglycemia, and insulin resistance (42). These findings have also been validated in animal and cell researches (43, 44). In addition, an optional intake of 16:0 in a certain ratio with PUFA (including n-3 and n-6 families) was considered to have significant effect on human health, which was in line with our findings on FAP2 (40). The *in vitro* experiments showed that 22:6n-3 could reduce 16:0 induced endoplasmic reticulum stress in pancreatic β cells (45), and reverse atherosclerotic changes in human endothelial cells induced by 16:0 (46). Moreover, n-3 and n-6 PUFAs are precursors of eicosanoids which have effects in mediating inflammation and regulating immune response. Interestingly, the eicosanoids derived from n-6 PUFAs are prone to be pro-inflammatory, whereas those derived from n-3 PUFAs are anti-inflammatory (47). This suggested that n-3 PUFAs may, to some extent, improve insulin sensitivity through modulating inflammation.

Up to date, no studies have explored the potential fatty acid patters that associated with GDM odds through statistical method of dimension reduction like PCA. The results of the few studies that assessed the association of individual fatty acids with incident GDM showed some inconsistency with the present study's findings (9). Reasons for such variability include differences in gestational age at blood sample collection, biological specimen sampled, and the method of fatty acid measurement or expression. Pooled results from two nested case-control studies conducted in China indicated that higher levels of 14:0 and 16:0 were associated with higher odds of GDM, whereas higher 18:2n-6 was associated with lower odds of GDM (14). However, the fatty acids were measured for different plasma compartments in the two studies (one study used total plasma samples and another used plasma phospholipid contents), and significant associations found in one study may not be replicated in another one. Zhang et al. found that 18:3n-3 and 22:6n-3 in early pregnancy were associated with a higher risk of GDM subtype (17). One case-control study nested in a cohort of US women suggested a beneficial role of odd-chain fatty acids, 22:5n-3 and 22:4n-6 in prevention of GDM, while 16:0, 18:3n-6, and 20:3n-6 at 10–14 weeks of pregnancy were associated with increased risk of GDM (10, 11). In normal pregnancy, plasma phospholipids in maternal circulation would increase by nearly 50% as compared to non-pregnant circulation (9), and the composition of plasma fatty acids is expected to fluctuate throughout gestation, which may lead to the discrepancy in the association between individual fatty acids and GDM risk in different trimesters on pregnancy (15, 17). Ortega-Senovilla et al. found that in the serum of women with GDM, the concentrations of most fatty acids were lower than in control women, except for 20:4n-6 and 22:6n-3, which remained the same. However, when values were expressed as a percentage of total fatty acids, different results emerged, serum from women with GDM showed significantly higher proportions of 18:2n-6, 20:4n-6, 22:6n-3, and lower proportions of 16:0, 16:1n-7, 18:1n-9, 20:5n-3 (48). The discrepancy between the above results mainly due to the difference in fatty acid expression. Only 10 individual fatty acids were measured in this study, which may lead to the possibility of lacking of comparability with other studies when fatty acids were expressed as relative concentrations. It should also be noted that the placenta plays an essential role in determining how fatty acids are transferred from maternal to embryonic circulation (49). Multiple studies have evaluated placental preference of fatty acid transport and found that the placenta places a higher preference on docosahexaenoic acid transport (9). However, GDM appears

to influence the transfer of PUFAs from mother to fetus. The percentages of docosahexaenoic acid, arachidonic acid, and n-6 and n-3 PUFAs were found to be lower in the cord blood of mothers with GDM than in controls (50). Hence, the impaired transfer of some certain fatty acids through the placenta to the cord blood and fetus may be a possible factor in changing the patterns of fatty acids in GDM women.

Previous studies have explored the FAPs that related to a series of diseases such as cardiovascular disease (24), type 2 diabetes (25), metabolic syndrome (51), and cancer (52). A large nested case-cohort study indicated that a combination of fatty acids that characterized by high concentrations of 18:2n-6, odd-chain fatty acids, and very long-chain fatty acids, and low concentrations of 18:3n-6 and 16:0 was associated with lower risk of type 2 diabetes (25). Another previous study using PCA on 11 fatty acids in serum found that low 18:2n-6 factor and n-3 PUFA factor predicted metabolic syndrome development over 20 years, independent of lifestyle factors (51). Significant differences of the results on identified fatty acid patters were existed between our study and studies above, which might be attributable to several reasons. First, although metabolic syndrome, type 2 diabetes, and GDM are all metabolic diseases with insulin resistance, diverse pathogenesis, and potential risk factors still exist. Besides, circulating fatty acids could be selectively transported from pregnant women to the fetus through placenta (53), which results in the difference of fatty acid composition in the plasma of

TABLE 5 | Partial correlation coefficients between fatty acid pattern scores and variables of interest[a].

	FAP1	FAP2	FAP3	FAP4
FPG (mmol/L)	−0.15*	−0.14*	0.09	0.05
OGTT-1h (mmol/L)	−0.16*	−0.16*	0.05	−0.08
OGTT-2h (mmol/L)	−0.09	−0.15*	0.02	0.02
TC (mmol/L)	−0.21*	−0.17*	0.03	0.02
TG (mmol/L)	−0.21*	−0.37*	0.32*	0.33*
HDL-C (mmol/L)	0.02	−0.02	0.25*	0.06
LDL-C (mmol/L)	−0.31*	−0.14*	−0.10*	0.04
FPI (μU/mL)	−0.01	−0.22*	0.04	0.35*
HOMA-IR	−0.03	−0.24*	0.05	0.33*

[a]*Adjustments were made for age, gestational age at blood collection, parity, pre-pregnancy BMI, family history of diabetes, smoking and alcohol use. *P < 0.05. FAP, fatty acid pattern; FPG, fasting plasma glucose; FPI, fasting plasma insulin; HDL-C, high density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL-C, low density lipoprotein cholesterol; OGTT-1h, 1-h post glucose load; OGTT-2h, 2-h post glucose load; TC, total cholesterol; TG, triglycerides.*

TABLE 6 | ORs (95% CIs) for GDM according to quartiles of plasma fatty acid pattern scores.

	Quartiles of plasma fatty acid pattern score				P_{trend} [a]
	Q 1	Q 2	Q 3	Q 4	
FAP 1					
N (Case/control)	91/54	50/54	34/54	42/55	
Crude model	1	0.58 (0.35–0.97)	0.40 (0.23–0.68)	0.46 (0.27–0.79)	0.001
Model 1	1	0.54 (0.31–0.95)	0.40 (0.22–0.73)	0.45 (0.26–0.81)	0.002
Model 2	1	0.50 (0.28–0.89)	0.40 (0.21–0.74)	0.42 (0.23–0.76)	0.002
FAP 2					
N (Case/control)	87/55	48/54	39/54	43/54	
Crude model	1	0.55 (0.33–0.93)	0.44 (0.25–0.78)	0.48 (0.28–0.82)	0.005
Model 1	1	0.47 (0.26–0.84)	0.43 (0.23–0.80)	0.50 (0.27–0.91)	0.019
Model 2	1	0.48 (0.26–0.85)	0.40 (0.20–0.77)	0.48 (0.26–0.90)	0.018
FAP 3					
N (Case/control)	36/54	51/55	59/54	71/54	
Crude model	1	1.42 (0.81–2.51)	1.72 (0.96–3.09)	1.98 (1.13–3.45)	0.014
Model 1	1	1.12 (0.60–2.09)	1.55 (0.81–2.98)	1.82 (0.99–3.35)	0.035
Model 2	1	1.17 (0.61–2.23)	1.48 (0.76–2.89)	1.76 (0.94–3.33)	0.058
FAP 4					
N (Case/control)	49/54	62/55	57/54	49/54	
Crude model	1	1.25 (0.73–2.12)	1.18 (0.69–2.00)	0.97 (0.55–1.71)	0.848
Model 1	1	1.06 (0.60–1.89)	1.02 (0.56–1.83)	1.02 (0.54–1.91)	0.996
Model 2	1	1.06 (0.58–1.96)	0.98 (0.53–1.82)	1.01 (0.52–1.95)	0.937

[a] P_{trend} *values were obtained from logistic regression by treating median value of each quartile of FAP scores as continuous variables. FAP, fatty acid pattern; GDM, gestational diabetes mellitus.*

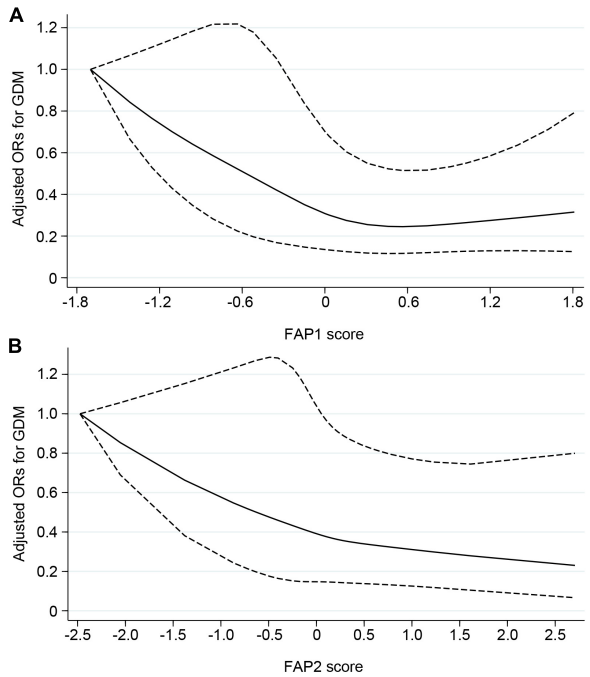

FIGURE 4 | Potential non-linear associations of FAP1 **(A)** and FAP2 **(B)** with odds of GDM assessed by restricted cubic spline model. Solid line indicated adjusted ORs for GDM, and dashed line indicated 95% CIs. Adjustments were made for age, gestational age at blood collection, parity, prepregnancy BMI, family history of diabetes, smoking and alcohol use. FAP, fatty acid pattern; GDM, gestational diabetes mellitus; ORs, odds ratios; CIs, confidence intervals.

pregnant women compared with non-pregnant adults (8, 54). Furthermore, differences in study design, such as the preference of biological samples, measurement of fatty acids, sample size, and the analytical method, were all the possible reasons for the discrepancy of the results. Thus, more prospective studies are needed to validate our findings.

The major strength of our study is the application of PCA on FAPs' exploring, making it possible to take into account the interaction of individual fatty acids. Additionally, plasma fatty acids, the objective biomarker of fatty acid status, were measured by GC–MS, which was independent of diet records and recall bias. Further, all the GDM cases included in this study were new cases and were not managed with lifestyle counseling or treated with medicine. However, several limitations should also be considered. Firstly, the sample size in the current study was relatively small. Secondly, the non-prospective design of this study disenabled us to infer the causal relations between FAPs and GDM development. The trajectory of fatty acid levels across the duration of gestation may be more informative than single-point measurement. Thus, large prospective study with longitudinal data collection is warrant in future. Thirdly, whether the FAPs identified in this study are related to certain dietary patterns or food preferences remains unclear. This could also be an important direction for future research. Fourthly, despite the adjustment for potential confounding factors in study design and statistical process, we cannot exclude the possibility that residual confounders existed.

In conclusion, in this matched case-control study, we identified two novel FAPs that were inversely associated with GDM odds. The combination of circulating fatty acids could be a more significant marker of GDM development than individual fatty acids or their subgroups. Prospective studies in other populations are needed to validate our findings, and explore how to optimize FAPs during pregnancy to achieve better health outcomes.

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 Ethics Committee of Tongji Medical College. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

PL, AT, and LL designed the research. PL, SH, YZ, TS, YH, ZX, HL, and SZ contributed to the data collection and analysis. PL wrote the manuscript. CL, AT, and LL edited the manuscript. LL and AT are the guarantors of this work and have full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors read and approved the final manuscript.

FUNDING

This work was supported by the Young Scientists Fund of the National Natural Science Foundation of China (82103822 and 81803226), the Natural Science Foundation of Hubei Science and Technology Commission (2020CFB360), and the Major International (Regional) Joint Research Project (NSFC 81820108027).

ACKNOWLEDGMENTS

We gratefully acknowledge the cooperation of all the pregnant women who took part in this study. We are also sincerely grateful to the staffs in Tongji Hospital for their considerable assistance with many aspects.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.836115/full#supplementary-material

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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.

The reviewer XL declared a shared affiliation with the authors PL, SH, YZ, TS, YH, ZX, HL, CL, SZ, and LL to the handling editor at the time of review.

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frontiers | Frontiers in Nutrition

SYSTEMATIC REVIEW
published: 06 June 2022
doi: 10.3389/fnut.2022.838762

Relationships Between Biological Heavy Metals and Breast Cancer: A Systematic Review and Meta-Analysis

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

Received: *18 December 2021*
Accepted: *26 April 2022*
Published: *06 June 2022*

Citation:
Liu L, Chen J, Liu C, Luo Y, Chen J, Fu Y, Xu Y, Wu H, Li X and Wang H (2022) Relationships Between Biological Heavy Metals and Breast Cancer: A Systematic Review and Meta-Analysis. Front. Nutr. 9:838762. doi: 10.3389/fnut.2022.838762

Introduction: Heavy metals were classified as essential, probably essential, and potentially toxic in the general population. Until now, it has been reported inconsistently on the association between heavy metals and BC. In this meta-analysis, we aimed to assess the association between heavy metals and BC and review the potential mechanisms systematically.

Methods: We searched for epidemiological studies in English about the association between heavy metals and BC published before September 2020 in PubMed, Web of Science, and Embase databases. In total 36 studies, comprising 4,151 individuals from five continents around the world were identified and included.

Results: In all biological specimens, Cu, Cd, and Pb concentrations were higher, but Zn and Mn concentrations were lower in patients with BC than in non-BC participants [SMD (95% CIs): 0.62 (0.12, 1.12); 1.64 (0.76, 2.52); 2.03 (0.11, 3.95); −1.40 (−1.96, −0.85); −2.26 (−3.39, −1.13); p = 0.01, 0.0003, 0.04, <0.0001, <0.0001]. Specifically, higher plasma or serum Cu and Cd, as well as lower Zn and Mn, were found in cases [SMD (95% CIs): 0.98 (0.36, 1.60); 2.55 (1.16, 3.94); −1.53 (−2.28, −0.78); −2.40 (−3.69, −1.10); p = 0.002, 0.0003, <0.0001, 0.0003]; in hair, only lower Zn was observed [SMD (95% CIs): −2.12 (−3.55, −0.68); p = 0.0004]. Furthermore, the status of trace elements probably needs to be re-explored, particularly in BC. More prospective studies, randomized clinical trials, and specific pathogenic studies are needed to prevent BC. The main mechanisms underlying above-mentioned findings are comprehensively reviewed.

Conclusion: For BC, this review identified the current knowledge gaps which we currently have in understanding the impact of different heavy metals on BC.

Systematic Review Registration: www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020176934, identifier: CRD42020176934.

Keywords: breast cancer, copper, cadmium, lead, manganese

INTRODUCTION

Heavy metals exist in the Earth's crust as the natural ingredients and are present in all aspects of the environment, including the air, water, soil, and plants (1). Human beings absorb heavy metals mainly from crops, vegetables, water, and sediments (1–4). Heavy metals are difficult to biodegrade *in vivo*, and an overload of these metals in the body can cause vomiting, stomach irritation, hair loss, cardiovascular disease, diabetes, leukemia, and other diseases (5–8). On the other hand, the lack of several special heavy metals leads to cardiovascular diseases, central nervous system diseases, and others (9, 10). The impact of heavy metals on human health has become an important public health problem; either excess or deficient content of heavy metals leads to a variety of potential health hazards.

As the most universal malignant disease of women worldwide, the incidence of cases of breast cancer (BC) is expected to increase by more than 46% by 2040 (11). In addition to genetic factors, smoking, and lifestyle factors, which are known to be related to raising the BC risk (12), in recent years, *in vivo* metabolism of several heavy metals has been found to have something to do with BC (13). For example, cadmium (Cd) has been reported to mimic estrogenic effects to promote the development of BC (14). The ROS pathway mediated the effects of copper (Cu), zinc (Zn), and manganese (Mn) on the occurrence and development of BC (15). However, the published results are varied in different biological samples. Thus, it is not clear which heavy metals and which kind of biological samples can be used as predictors of BC incidence. To this end, it is necessary to clarify the reliable relationships and underlying mechanisms of heavy metals on BC.

In the human body, the influence of the environment, nutritional status, or metabolism of heavy metals could be reflected by detecting the biomarkers present in the blood, tissue, skin, and nails (16–20). In this review, population, exposure, comparator, and outcome ("PECO") approach has been used to analyze the relationship between different heavy metals and BC (21). The question below was answered through PECO: What are the relationships between heavy metal concentration in plasma or serum, tissue, skin, and nails and risk of BC? We then systematically summarized the existing relevant mechanisms. The systematic review and meta-analysis were conducted in accordance with the COSTER recommendations (22).

METHODS

PubMed, Web of Science, and Embase databases were searched to confirm studies published up until September 2020 on the relationship between heavy metal concentration and BC. The research question was generated by merging keywords representing the exposure and outcome components according to the PECO guideline (21). Detailed study selection and data analysis: refer to **Supplementary File 1**. The following search keywords were used in the search strategy: "heavy metals" or "trace elements" combined with "breast cancer" or "mammary carcinoma." Besides, we searched all results in the reported reviews and all relevant meta-analyses. A protocol of the systematic review was registered a priori in the PROSPERO register (International Prospective Register of Systematic Reviews), number CRD42020176934 https://www.crd.york.ac.uk/prospero/. The quality of included studies was assessed by Newcastle–Ottawa scale (NOS) and Critical Appraisal Skills Program (CASP) checklist.

Study Selection

Qualified studies had to accord with the following standards: (1) the population (P) was restricted to the general population; (2) exposure (E) to heavy metals was estimated by long-term exposure biomarkers, i.e., evaluation of plasma/serum/tissue/hair/nail concentration; (3) the comparator (C) was specified for including individuals without BC; (4) the outcome (O) was BC prevalence; (5) studies of human beings were included; and (6) the studies that were available in the English language. The exclusive criteria were as follows: (1) animal studies; (2) *in vitro* or laboratory studies; (3) studies that did not present original data; (4) studies that evaluated heavy metals not in the human body; (5) studies conducted in the nuclear radiation areas; and (6) studies that did not report the heavy metal content in the BC cases and healthy participants.

Data Collection

Data were collected from the confirmed studies according to a standardized procedure. The information picked up included first author, study design, year of publication, geographic area, sample size, age, and heavy metals concentration.

Data Analysis

The quality of included studies was assessed by NOS and CASP checklist (23, 24) (**Supplementary Tables 1, 2**). The study quality assessed by NOS was as follows: low quality: 0–3; moderate quality: 4–6; high quality: 7–9 (25). A number of two authors double-checked and completed all the above process independently. The extracted data were used for meta-analyses to acquire the standardized mean differences (SMDs) and 95% confidence intervals (95% CIs). The Q-test and I^2 tests were used to examined the heterogeneity among studies. In case the $I^2 > 50\%$ or the $p < 0.05$, heterogeneity was considered in the meta-analysis. The SMDs were computed by random-effects model with heterogeneity. Subgroup analyses were also conducted stratified by geographic background and source of the biological samples including plasma or serum, hair, tissue, and toenails. Potential publication bias of studies was estimated by funnel plots and Egger test (26). Sensitivity analysis was carried out to investigate the constancy of the results; for this, each included study was excluded, one at a time, and the meta-analysis was re-run with the exclusion of each study. All the statistical analyses were conducted by Review Manager 5.3. The world maps on the association between heavy metals and BC were conducted by PyCharm 2021.3.3 (Community Edition).

RESULTS

The literature search produced 12,824 initial studies, containing 12,128 studies from PubMed, 557 studies from Web of Science,

and 139 studies from Embase (**Figure 1**). After a detailed review of records, 36 case–control studies (including two nested case–control studies) with a total of 4,151 individuals (1,996 cases and 2,155 non-BC participants) were selected for this meta-analysis. **Supplementary Tables 1, 2** show the quality assessment of included articles using the NOS and CASP checklist. Based on NOS, all the studies were evaluated with 5 or more points, which means that no studies with low quality were included. Among them, the quality scores of two studies were 7. The others' quality scores were between 5 and 7. **Supplementary Table 3** gives detailed characteristics of the studies used for the meta-analysis. A total of 19 studies were conducted in Asia (27–45), 12 in Europe (46–57), 2 in Africa (19, 58), 2 in South America (59, 60), and 1 in North America (61). There are nine heavy metals that are categorized into three groups according to the WHO classification (62): essential trace elements — Cu, chromium (Cr), cobalt (Co), iron (Fe), Zn; probably essential trace elements — Mn, nickel (Ni); potentially toxic trace elements — Cd, lead/plumbum (Pb).

Meta-Analysis of the Associations Between Essential Trace Elements According to the WHO Definition and BC

Copper and Breast Cancer

A total of 30 studies investigated the differences in Cu concentration between patients with BC and non-BC participants (**Figure 2A**). In plasma/serum and tissue, Cu concentration in patients with BC was significantly higher than those in non-BC participants. In hair and toenails, the relationship was non-significant ($p > 0.05$). In Africa and Europe, patients with BC have a higher Cu concentration in plasma/serum than in non-BC participants [SMD (95% CIs): 2.44 (1.80, 3.09), 1.66 (0.84, 2.48); ($I^2 = 11\%$, $I^2 = 96\%$)]. In Asia, it was found non-significance in plasma/serum [SMD (95% CIs): 0.16 (−0.89, 1.21), $I^2 = 98\%$] (**Figure 3A**). No significant difference was found in hair Cu concentration between patients with BC and non-BC in Asia and Europe. The total number of studies on toenail and tissue was limited to be analyzed by region further. No significant publication bias was found for all the studies ($p_{\text{Egger's test}} = 0.21$).

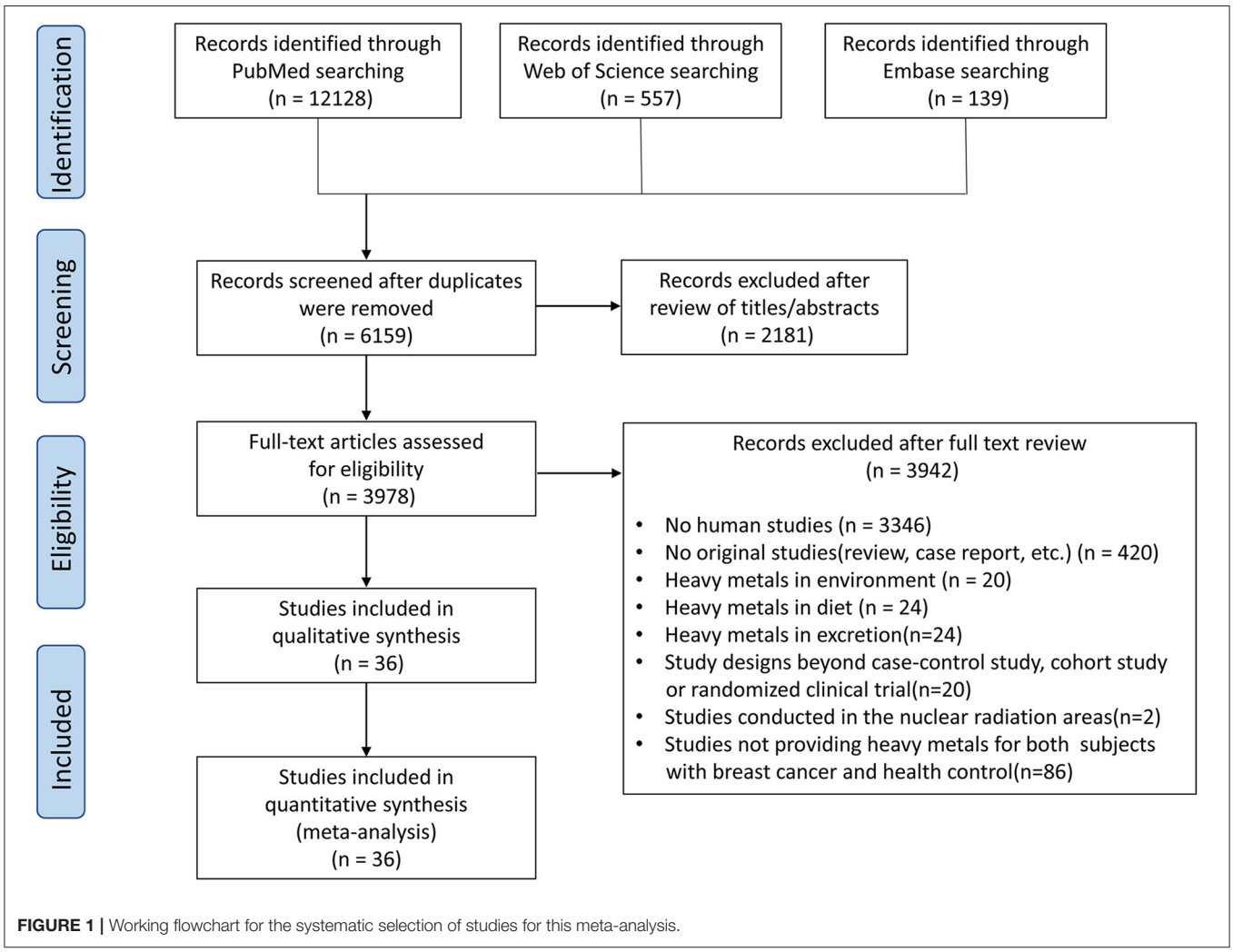

FIGURE 1 | Working flowchart for the systematic selection of studies for this meta-analysis.

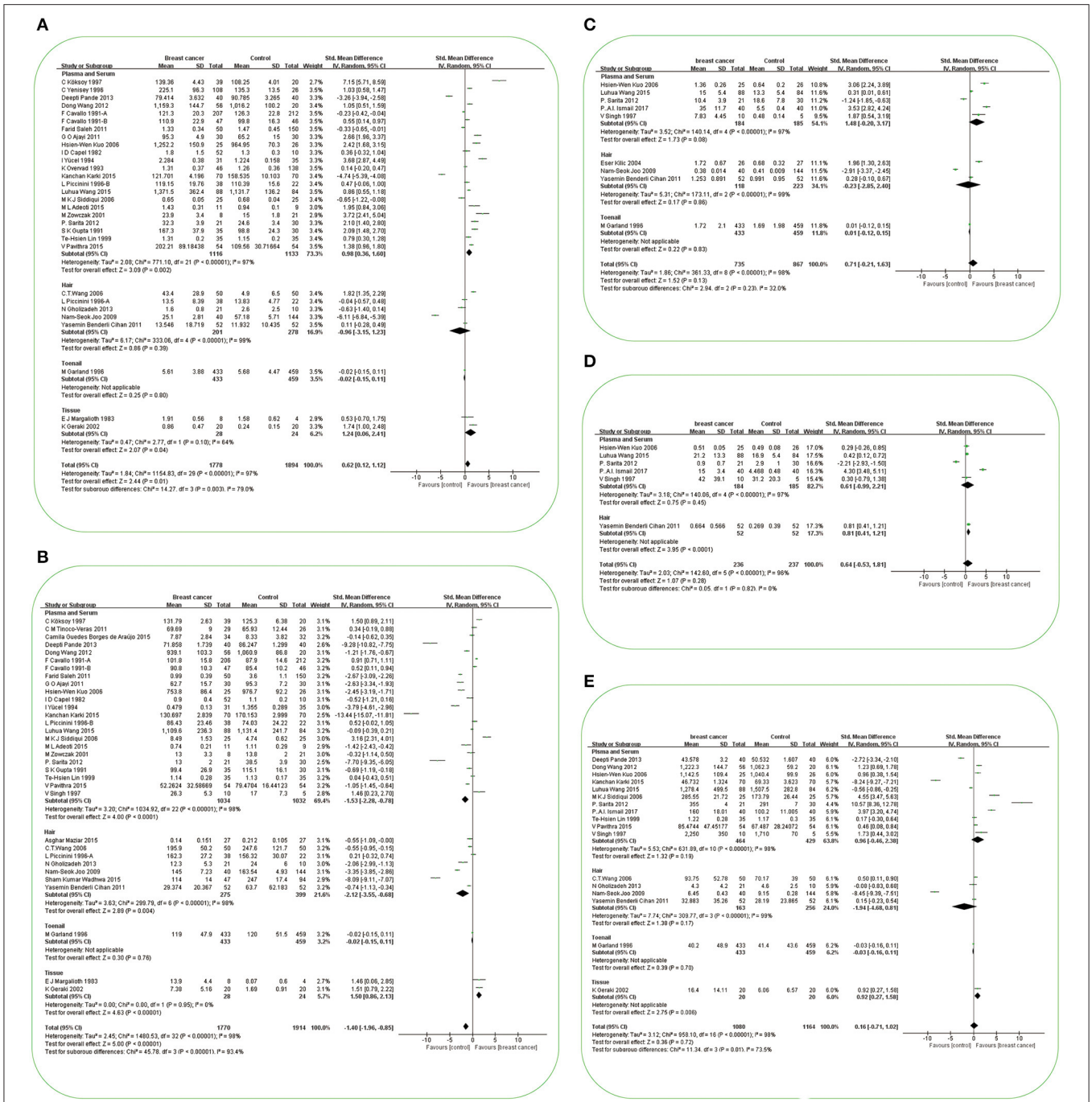

FIGURE 2 | Forest plot of studies of essential trace elements in subjects with breast cancer vs. non-breast cancer controls. **(A)** Forest plot of studies of Cu levels in subjects with breast cancer vs. non-breast cancer controls. **(B)** Forest plot of studies of Zn levels in subjects with breast cancer vs. non-breast cancer controls. **(C)** Forest plot of studies of Cr levels in subjects with breast cancer vs. non-breast cancer controls. **(D)** Forest plot of studies of Co levels in subjects with breast cancer vs. non-breast cancer controls. **(E)** Forest plot of studies of Fe levels in subjects with breast cancer vs. non-breast cancer controls. The standard mean differences (SMD) and 95% confidence intervals (CIs) were calculated using the random-effects model. Cu, copper; Zn, zinc; Cr, chromium; Co, cobalt; Fe, iron.

Zinc and Breast Cancer

A total of 33 studies investigated the differences in Zn concentration between patients with BC and non-BC participants (**Figure 2B**). In plasma/serum and hair, Zn concentration in patients with BC was significantly lower than in non-BC participants, but it was reversed in tissue. In toenails, no significant difference was found between BC and non-BC participants ($p > 0.05$). In Africa and Asia, BC

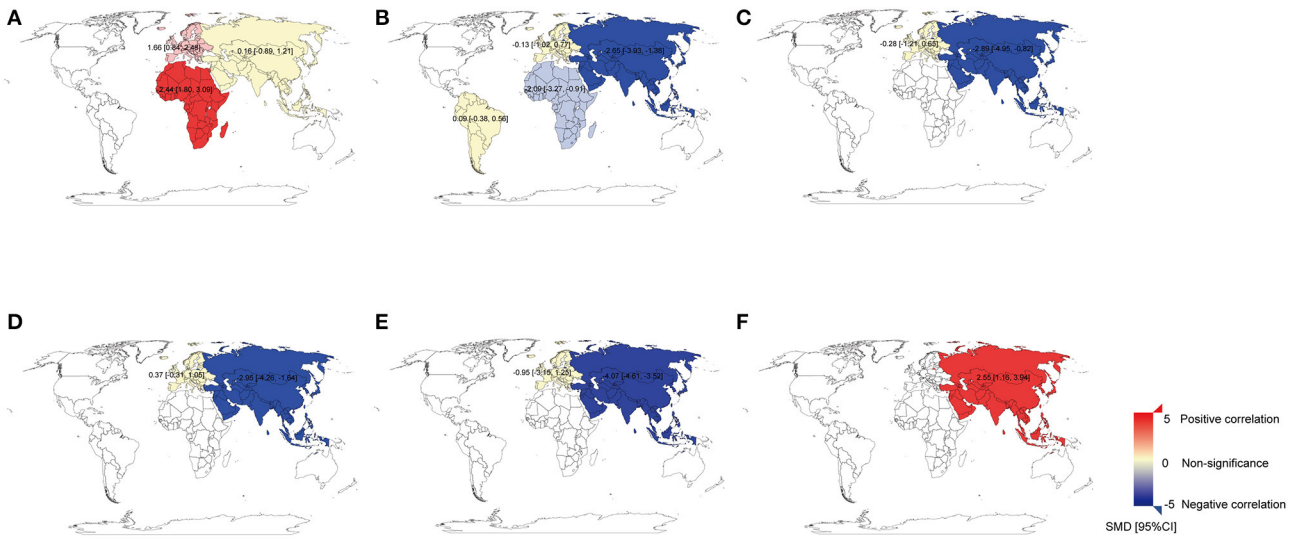

FIGURE 3 | The world map for the associations between heavy metals and breast cancer. **(A)** The world map for the association between Cu and breast cancer in plasma/serum. **(B)** The world map for the association between Zn and breast cancer in plasma/serum. **(C)** The world map for the association between Zn and breast cancer in hair. **(D)** The world map for the association between Mn and breast cancer in plasma/serum. **(E)** The world map for the association between Mn and breast cancer in hair. **(F)** The world map for the association between Cd and breast cancer in plasma/serum. The standard mean differences (SMD) and 95% confidence intervals (CIs) were calculated using the random-effects model and remarked in the maps by regions. Cu, copper; Zn, zinc; Mn, manganese; Cd, cadmium.

patients have a lower Zn concentration in plasma/serum than in non-BC participants [SMD (95% CIs): −2.09 (−3.27, −0.91); −2.65 (−3.93, −1.38); I^2 = 73%, 98%] (**Figure 3B**). In Asia, it was also found to be significant in hair [SMD (95% CIs):−2.89 (−4.95, −0.82); I^2 = 98%] (**Figure 3C**). But it was non-significant for the results on plasma/serum in European and South American and hair in Europe (**Figures 3B,C**) (all $P > 0.05$). The total number of studies on toenails was limited to be analyzed by region further. Publication bias was found in all the studies ($p_{\text{Egger'stest}} = 0.0053$).

Chromium and Breast Cancer

A total of nine studies investigated the differences in Cr concentration between patients with BC and non-BC participants (**Figure 2C**). In plasma/serum, hair, and toenails, there were no significant differences in Cr concentration between BC and non-BC participants. In Asia, non-significance of Cr concentration in plasma/serum between the BC and non-BC participants was found. But patients with BC in Asia had a lower Cr concentration in hair than non-BC participants [SMD (95% CIs): −2.91 (−3.37, −2.45)]. In Europe, Cr concentration was found to be non-significant in hair [SMD (95% CIs): 1.10 (−0.55, 1.75), I^2 = 95%]. The total number of studies on toenails was limited to be analyzed by region further. No significant publication bias was found for all the studies ($p_{\text{Egger'stest}} = 0.46$).

Cobalt and Breast Cancer

A total of six studies investigated the differences in Co concentration between patients with BC and non-BC participants (**Figure 2D**). In plasma/serum, there was no significant difference in Co concentration between the two groups of participants. In hair, Co concentration in patients with BC was significantly higher than those in non-BC participants. In Asia, Co concentration was found to be non-significant in plasma/serum. The total number of studies on hair was limited to be analyzed by region further. No significant publication bias was found for all the studies ($p_{\text{Egger'stest}} = 0.87$).

Iron and Breast Cancer

A total of 17 studies investigated the differences in Fe concentration between patients with BC and non-BC participants (**Figure 2E**). In plasma/serum, hair, and toenails, there was no significant difference in Fe concentration between BC and non-BC participants. In tissue, Fe concentration in patients with BC was significantly higher than those in non-BC participants. In Asia, Fe concentration was found to be non-significant in plasma/serum and hair. In Europe, Fe concentration was non-significant in hair. The total number of studies on toenails was limited to be analyzed by region further. No significant publication bias was found for all the studies ($p_{\text{Egger'stest}} = 0.92$).

Meta-Analysis of the Associations Between Probably Essential Trace Elements According to the WHO Definition and BC

Nickel and Breast Cancer

A total of six studies investigated the differences in Ni concentration between patients with BC and non-BC

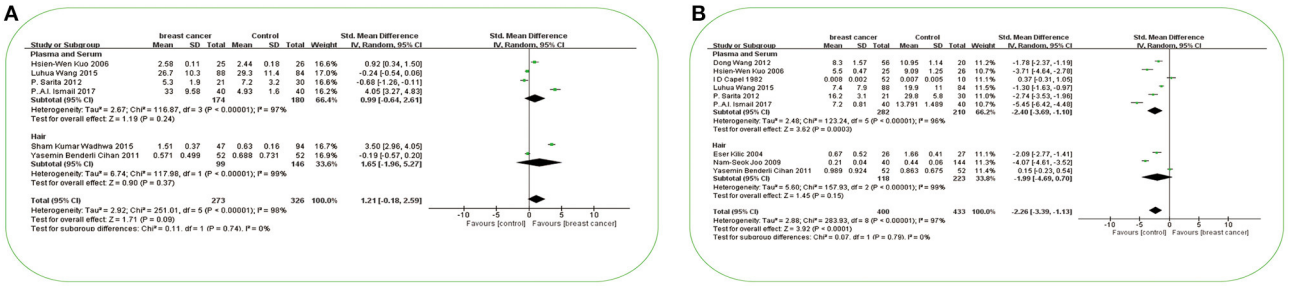

FIGURE 4 | Forest plot of studies of possible essential trace elements in subjects with breast cancer vs. non-breast cancer controls. **(A)** Forest plot of studies of Ni levels in subjects with breast cancer vs. non-breast cancer controls. **(B)** Forest plot of studies of Mn levels in subjects with breast cancer vs. non-breast cancer controls. The standard mean differences (SMD) and 95% confidence intervals (CIs) were calculated using the random-effects model. Ni, nickel; Mn, manganese.

participants (**Figure 4A**). In plasma/serum and hair, there was no significant difference in Ni concentration between BC and non-BC participants. In Asia, Ni concentration was non-significant in plasma/serum. The total number of studies on hair was limited to be analyzed by region further. No significant publication bias was found for all the studies ($p_{\text{Egger'stest}} = 0.14$).

Manganese and Breast Cancer

A total of nine studies investigated the differences in Mn concentration between patients with BC and non-BC participants (**Figure 4B**). In plasma/serum, Mn concentration in patients with BC was significantly lower than those in non-BC participants. In hair, there was no significant difference in Mn concentration between BC and non-BC participants. In Asia, Mn concentration in plasma/serum and hair in patients with BC was significantly lower than those in non-BC participants [SMD (95% CIs): −2.95 (−4.26, −1.64), −4.07 (−4.61, −3.52); $I^2 = 95\%$]. In Europe, Mn concentration was non-significant in plasma/serum and hair (**Figures 3D,E**) (all $p > 0.05$). No significant publication bias was found for all the studies ($p_{\text{Egger'stest}} = 0.10$).

Meta-Analysis of Associations Between Potentially Toxic Trace Elements According to the WHO Definition and BC

Cadmium and Breast Cancer

A total of eight studies investigated the differences in Cd concentration between patients with BC and non-BC participants (**Figure 5A**). In plasma/serum and hair, Cd concentration in patients with BC was significantly higher than those in non-BC participants. In Asia, Cd concentration in plasma or serum in patients with BC was significantly higher than it in non-BC participants [SMD (95% CIs): 2.55(1.16, 3.94); $I^2 = 97\%$] (**Figure 3F**). In Europe, it was found to be significant in hair [SMD (95% CIs): 0.71(0.31, 1.10)]. The total number of studies on hair and tissue was limited to be analyzed by region further. In the publication bias test, a slight publication bias was found using Egger's test ($p = 0.06$).

Lead and Breast Cancer

A total of four studies investigated the differences in Pb concentration between patients with BC and non-BC participants (**Figure 5B**). In plasma/serum, there was no significant difference in Pb concentration between BC and non-BC participants. In hair, Pb concentration in patients with BC was significantly lower than those in non-BC participants. In Asia, Pb concentration was non-significant in plasma and serum. The total number of studies on hair was limited to be analyzed by region further. No significant publication bias was found for all the studies ($p_{\text{Egger'stest}} = 0.70$).

In addition, the symmetry of the funnel plots also indicates less evidence of publication bias. However, this bias could not be completely ruled out due to the limited number of publications. **Figure 6** shows the details of the mechanisms underlying the associations between BC and Cu, Cd, Pb, Zn, and Mn. Finally, the evidence for the association between heavy metals and BC was low, due to the case–control studies included and the publication bias of studies about Zn and Cd (**Supplementary Table 4**).

DISCUSSION

Across all biological specimens, we discovered that Cu, Cd, and Pb concentrations in patients with BC were significantly higher than those in non-BC participants, but Zn and Mn concentrations were significantly lower than them (**Supplementary Table 4**). In plasma/serum, Cu and Cd concentration in patients with BC was significantly higher than those in non-BC participants, but Zn and Mn concentrations were significantly lower than them. In hair specimens, only Zn concentration in patients with BC was significantly lower than those in non-BC participants. Furthermore, the subgroup analyses indicated variation in findings across different ethnic groups. The detailed mechanisms have been described and suggest that Cu, Cd, Pb, Zn, and Mn may eventually lead to BC through different molecular modifications.

Associations Between Essential Trace Elements According to the WHO Definition and BC

Copper and Breast Cancer

Cu is one of the essential trace elements for the general population. It exists in two forms, Cu^{+} and Cu^{2+}. Cu in the diet is in the bivalent form, which is restored to monovalent

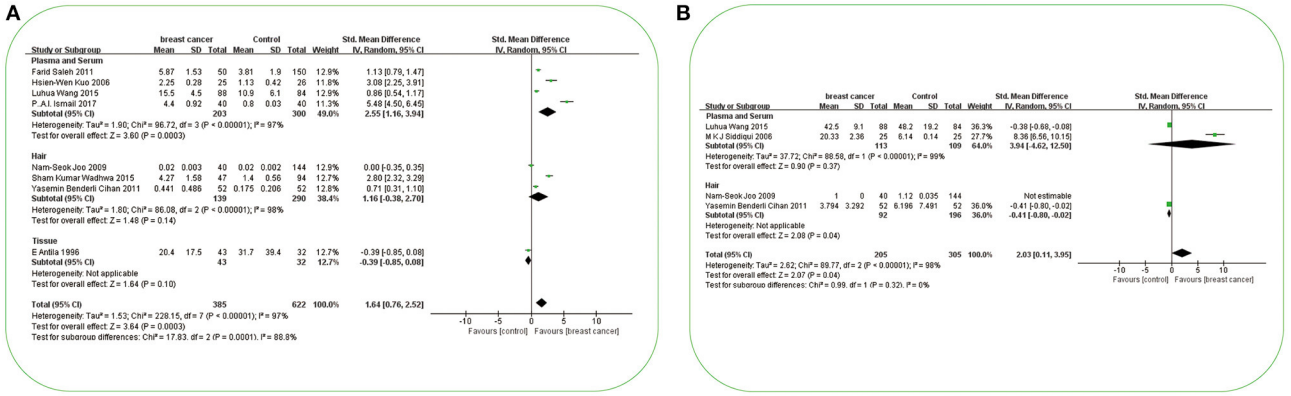

FIGURE 5 | Forest plot of studies of potential toxic trace elements in subjects with breast cancer vs. non-breast cancer controls. **(A)** Forest plot of studies of Cd levels in subjects with breast cancer vs. non-breast cancer controls. **(B)** Forest plot of studies of Pb levels in subjects with breast cancer vs. non-breast cancer controls. The standard mean differences (SMD) and 95% confidence intervals (CIs) were calculated using the random-effects model. Cd, cadmium; Pb, lead.

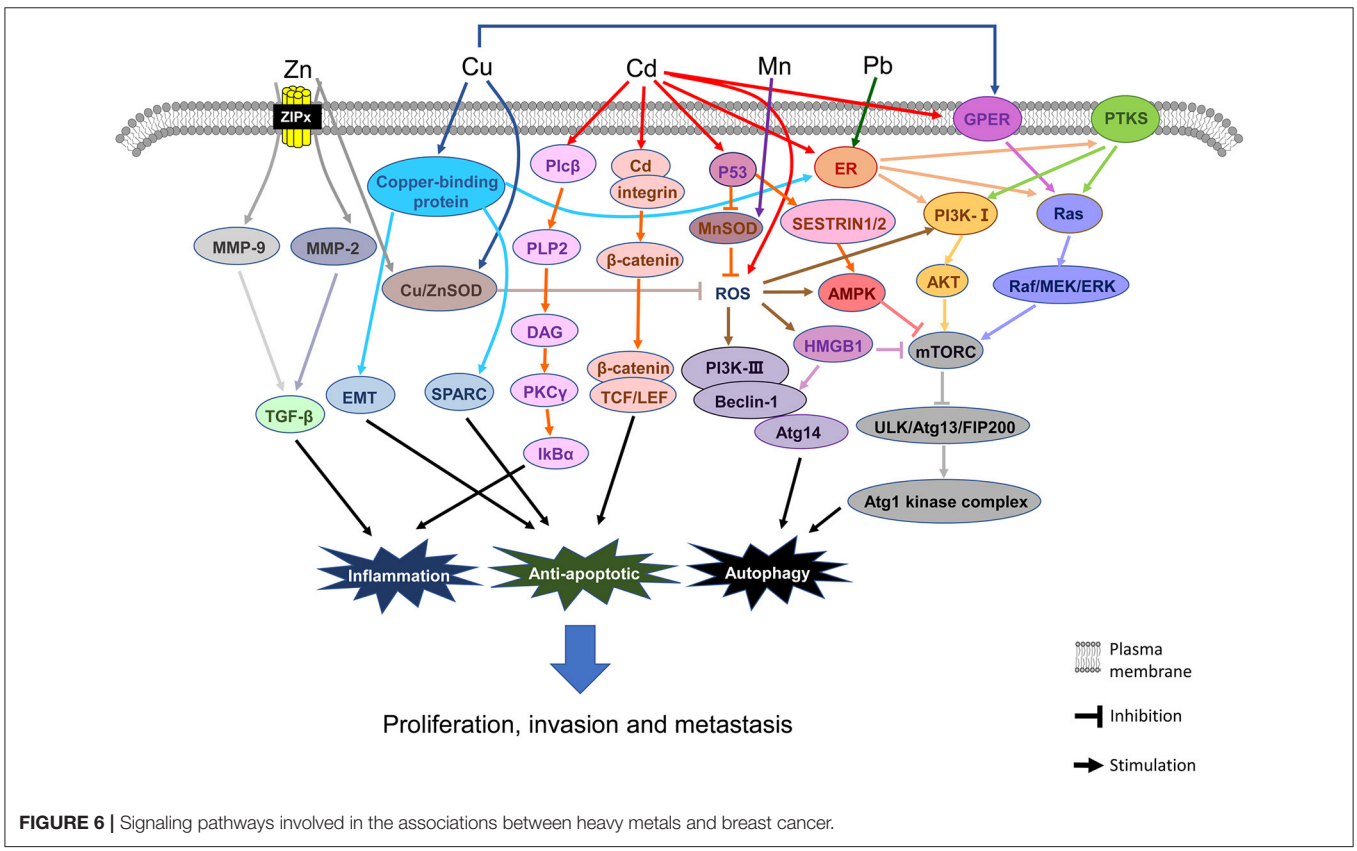

FIGURE 6 | Signaling pathways involved in the associations between heavy metals and breast cancer.

state by related enzymes before being transported into cells (63). The delicate *in vivo* balance of Cu is preserved by ATP7A and ATP7B as two Cu-transporting adenosine triphosphatases (ATPases) bound to the membrane (63). Cu in the reduced state is bound to its chaperone and it is transferred to ATP7A and ATP7B through antioxidant-1 (ATOX1) as a chaperone (64). The small intestine is the site where most dietary Cu is absorbed, and Cu flows out in the bile from the liver (65).

In this meta-analysis, across various specimens from human beings, we found a statistically significant difference in Cu concentration between patients with BC and non-BC participants. In plasma/serum and tissue, Cu concentration in patients with BC was significantly higher than those in non-BC participants; however, no significant differences were obtained between cases and non-BC participants in hair and toenail specimens. There are several possible reasons for the variation in findings as a function of the sample source. First, of the

human specimens, blood and plasma/serum are the strongest proof specimens for diagnosis of deficiency or exposure to Cu (66), but hair Cu content can be influenced by dyeing, bleaching, shampoos, and the gap to the scalp; thus, hair is not appropriate for evaluating Cu concentration (67). The small quantity of studies included in this meta-analysis on Cu in tissue and toenails is another possible reason. In the subgroup analysis by region, the result showed that the Cu concentration in plasma/serum of patients with BC in Africa and Europe was significantly higher than those in non-BC participants; however, there was no significant difference in Cu concentration between Asian cases and non-BC participants. The reason for this difference as a function of ethnicity may be that the obesity rate is lower in Asia than in Africa and Europe (68). The content of Cu in serum is known to be elevated in obesities (69, 70). Along with serum Cu concentration, the ceruloplasmin, as the body's main Cu carrier, is overexpressed in adipose tissue and obesity-related cancer cells (71). It should be noted that there is currently no data on Cu from patients with BC in Australia and America. With respect to the findings from Africa, enrichment of Cu has been observed in the natural environment in Africa, such as in plants, soil, and rivers (72–74), which is partly caused by mining activity, disposal of E-waste, and so on (75–78).

Figure 6 shows the main mechanistic pathways from *in vivo/vitro* experiments. The most important factors and pathways involved in Cu elevation and the pathogenesis of BC are copper-binding protein, G-protein estrogen receptor (GPER), and reactive oxygen species (ROS). ① For copper-binding protein, the LOX-like (LOXL) family of proteins, mediator of cell motility 1 (MEMO1), and ATOX1 are the three major proteins related to BC (79). It has been shown that the LOXL family of proteins promotes the invasion or metastasis phenotype of BC cells *via* the epithelial-mesenchymal transition (EMT) and secreted protein acidic and rich in cysteine (SPARC) pathways (80, 81). MEMO1 promotes tumor cell migration and metastasis *via* EMT-related pathways, which are obtained from BC cells (82). MEMO1 also controls the subcellular localization and phosphorylation of the estrogen receptor (ERα) and downstream function of ErbB2/ER or IGFIR/ER, thus activating the MAPK and PI3K signaling pathways and promoting the migration and/or proliferation of BC cells (83, 84). ATOX1 may also act on the migration of BC cells unknowingly (85). ②Cu also stimulates estrogenic GPER signaling transduction, inducing expression of the Raf/MEK/ERK signaling pathways and activating the downstream pathway of mTOR, finally leading to angiogenesis and tumor growth in BC cells (86). ③ Cu is an accessory factor of copper-zinc superoxide dismutase (CuZnSOD), but its activity is influenced by the concentration of Zn (87). Furthermore, Cu chaperone (CCS) (88) and vascular endothelial growth factor receptor 2^+ ($VEGFR2^+$) endothelial progenitor cells (EPCs) are the signaling pathways for Cu to promote BC *in vivo* (89).

Zinc and Breast Cancer

Zn is one of the essential trace elements for the general population. It exists in organisms in the form of redox-inert Zn^{2+} (90). Zn is mainly distributed in the liver, and the gastrointestinal tract is the main organ for Zn absorption and excretion (90). The homeostasis of Zn in cells is monitored by Zn transporters (ZnT) regulating the outflow of Zn and by Zn importers (ZIP) regulating the inflow of Zn (91, 92).

In this meta-analysis, Zn concentration in plasma/serum and hair from patients with BC was significantly lower than those in non-BC participants; however, in tissue, Zn concentration in patients with BC was significantly higher than those in non-BC participants, but the total number of the studies for the analysis is not large enough. There are several possible reasons for the similar results for plasma/serum and hair specimens. First, plasma Zn is the most widely applied and widely accepted biomarker of Zn status (20). Furthermore, Zn is a structural component of the hair matrix formed in hair follicles, and the Zn concentration in hair reflects, to some extent, the availability of Zn from the blood supply during hair growth (93). Besides, there are also several possible reasons for the different results between tissue and plasma/serum or hair. Plasma Zn can be used as a biomarker of Zn deficiency to assess the Zn nutritional conditions of a population (94). Metallothionein (MT)/thionein (T) couple is a homeostatic system of Zn. When the nutrition of Zn in the body is sufficient, Zn is used to guide the T synthesis and result in the MT formation; when the amount of available Zn is low, Zn is released from MT (95, 96). Therefore, the relationship between Zn and BC may be U-shaped; excess or deficiency of Zn may have adverse effects (97). On the one hand, when the body has too little Zn, the absorption and utilization of Zn are reduced, CuZnSOD synthesis is reduced, and ROS is increased (98). On the other hand, if there is too much Zn, it will accumulate in the body.

Figure 6 shows the main mechanistic pathways from *in vivo/in vitro* experiments. The most important factors and pathways involved in Zn deficiency and the pathogenesis of BC are MMPs and ROS, respectively. ①Once Zn enters a cell, there is a decrease in Zn in the blood and the Zn compartmentalization promotes invasion, metastasis, and angiogenesis of tumors, which is mediated by matrix metalloproteinases, especially MMP-2 and MMP-9 (99). ②Zn keeps the structure of the effective zone of CuZnSOD, which can effectively suppress cancer cell growth (98). If the Zn concentration in the blood is low, there may not be enough Zn to maintain the structure of the active site of CuZnSOD to slow down the cancer cell growth, thus boosting cancer cell growth rapidly.

In the subgroup analysis by region, we found that Zn concentration in the plasma/serum of patients with BC in Africa and Asia was significantly lower than those in non-BC participants; it was also found to be significant in hair in Asia; no significant differences between cases and non-BC participants were found in samples from Europe and South America. The reason for these differences may attribute to the dietary shortage of Zn in Africa and Asia, especially in South Asia, South East Asia, and sub-Saharan Africa (100, 101). In addition, rs10822013 on the chromosome at 10q21.2 in the zinc finger protein 365 (ZNF365) gene is a genetic risk variant for all four stages of BC among East-Asian women (102). In this study, there were too few studies of Zn in hair specimens from European samples and in toenail specimens to enable further analysis.

Chromium and Breast Cancer

Cr is one of the essential trace elements for the general population. In the environment, Cr has a variety of oxidation valence states: Cr^0 (elemental chromium), Cr^{1+}, Cr^{2+}, Cr^{3+}, Cr^{4+}, Cr^{5+}, and Cr^{6+}, among which Cr^{3+} is the most stable, followed by Cr^{6+} (103). The main source of oral Cr in non-occupational populations is oral intake (103). *In vivo*, Cr^{6+} can be conveyed into cells *via* anion transporters and reduced to Cr^{3+} through a series of metabolic reductions (104, 105). Cr^{3+} is mainly excreted through the kidneys (106).

In this meta-analysis, we only found a positive result in hair specimens from one Asian study, but due to the small sample size, this result requires further verification. The studies of hair, toenail, and plasma/serum specimens showed no significant differences between cases and non-BC participants. In this meta-analysis, the form and valence state of Cr were not distinguished, but different valence states lead to different pathogenic effects and mechanisms.

In relation to the mechanism, Cr^{3+} is mainly related to blood glucose homeostasis and is an active component factor of glucose tolerance (GTF), which regulates blood glucose concentration, including carbohydrate and lipid metabolism (107, 108). In addition, Cr^{3+} complexes have been used to treat type 2 diabetes as insulin amplifiers (109). However, for cancer, the carcinogenicity of Cr depends on its valence state. Cr^{6+} was categorized as a class I carcinogen by the International Agency for Research on Cancer (IARC) (110). Cr^{6+} compounds can be introduced into cells through sulfonamides and can then be reduced by a variety of cellular reducers, for example, glutathione (GSH) and ascorbic acid (111). In the process, a spectrum of ROS is generated, which can interact with intermediates and may result in oxidative stress and DNA damage, which are the unstable factors causing mutagenesis (112). The relationships between the concentration of Cr in different valence states and the BC risk and other nutrients need to be explored in detail in various biological specimens.

Cobalt and Breast Cancer

Co is one of the essential trace elements for the general population. It mainly exists in two valence states: Co^{2+} and Co^{3+} (113). The most likely source of Co exposure is contaminated food or water (114). Co plays a role in physiological functions in the only known form of Vitamin B_{12} (115). After being taken up by the digestive or respiratory system, some Co is quickly excreted in feces (114). The rest is dispersed throughout the tissues *via* the blood, especially into the liver, kidneys, and bones (114). The absorbed Co slowly leaves the body through the urine (114).

In this meta-analysis, we only found a positive result in hair from one study, but this requires further verification because of the insufficient sample size. We found no differences between cases and non-BC participants in plasma/serum concentration of Co. However, it has been reported that Co induces breast tumors by interfering with signaling, such as estrogen receptor α (ERα) signaling, and simulating hypoxia in angiogenesis and apoptosis, both internally and externally (116, 117). Therefore, Co concentration in BC requires further study.

Iron and Breast Cancer

Fe is one of the essential trace elements for the general population. Fe exists as Fe^{2+}, Fe^{3+}, and Fe^{4+} (90). The main inorganic Fe in the diet is Fe^{3+}, which is reduced by ferrireductase duodenal cytochrome b (DCYTB) on the surface of duodenal intestinal cells (90). The resulting Fe^{2+} enters cells through proton-coupled divalent metal transporters (118). In the body, hemoglobin is the main form of Fe; the rest of the Fe in the body is in the form of non-heme enzymes or ferritin in cells. Fe cannot be actively excreted from the human body and is stored in the body for about 10 years (90).

In this meta-analysis, we only found a positive result in tissue from one study, but this result requires replication because of the insufficient sample size. We found no differences in Fe concentration between cases and non-BC participants in plasma/serum, hair, and toenail specimens. However, it has been proved that Fe not only works fundamentally in many pathophysiological functions but also participates in the occurrence of breast tumors by interfering with signalings, such as VEGF, ROS, MAPK, and IL-6/JAK2/STAT3 signaling in animal models (119, 120). Therefore, further research on the effect of Fe concentration in BC is demanded before firm conclusions can be researched.

Associations Between Probably Essential Trace Elements According to the WHO Definition and BC

Manganese and Breast Cancer

Mn is one of the probably essential trace elements for the general population. It is easily oxidized and exists in the form of oxides, carbonates, and silicates in nature (121). In living beings, Mn^{2+} and Mn^{3+} are the most common oxidized states (122). $Mn^{2+/3+}$ is in the effective zone of manganese superoxide dismutase (MnSOD), which is in charge of the ROS detoxification in mitochondria (123). Replacing C with T in the MnSOD gene leads to a change from Val to Ala at the - 9 position of the mitochondrial target sequence (Val-9Ala), thus causing changes to the substructure of MnSOD (124) and affecting the transport of MnSOD into mitochondria (125). The main Mn exposure routes are through dietary intake, skin absorption, and inhalation (126). Once in the bloodstream, Mn is rapidly distributed to various tissues throughout the body *via* blood circulation (121). Most Mn in the body is combined with bile by the liver and excreted in the feces (127).

In this meta-analysis, in plasma/serum, Mn concentration in patients with BC was significantly lower than those in non-BC participants; in hair, there was no significant difference in Mn concentration between the two groups of participants. The possible reason for the different results in the different specimen types is as follows. Mn metabolism and state could be universally evaluated in whole blood or plasma/serum (128), and the Mn concentration in the hair can be confounded by several factors (129). In the subgroup analysis by region, we found that Mn concentration in hair and plasma/serum specimens of patients with BC in Asia was significantly lower than those in non-BC participants; no significant differences were observed in samples

from Europe. The reason for this difference may be due to the MnSOD gene polymorphism among Asian women, especially those who eat foods containing less selenium and/or vitamins (130, 131).

Figure 6 shows the major mechanistic pathways described in *in vivo/in vitro* experiments. The most important factor and pathway for Mn elevation and the pathogenesis of BC is ROS signaling. High Mn can make up for the loss of SOD and defend against oxidative stress (15). Like CuZnSOD, when the Mn concentration in blood is low, there is not enough Mn to maintain the structure of the active site of MnSOD, thus promoting rapid cancer cell growth. Moreover, the activity of MnSOD is repressed by p53 at the early stage of BC (132).

Nickel and Breast Cancer

Ni is another one of the probably essential trace elements for the general population. It exists in many valence states, Ni^{-}-Ni^{4+}, among which Ni^{2+} accounts for the largest proportion in the environment and biological systems (133). Ni can get into the human body in three ways – respiratory tract, digestive tract, and skin (134). Once in the body, the absorbed Ni is eliminated from the blood *via* the urinary system, whereas the unabsorbed Ni is excreted *via* the feces (135).

In this meta-analysis, there were no differences between cases and non-BC participants in the plasma/serum and hair specimens nor in the subgroup analyses. Nevertheless, there are some *in vitro* studies describing the mechanism by which Ni could induce BC, particularly in Martin et al., exhibiting that Ni binds to ERα in BC cells and induces cell proliferation *via* mimicking estradiol (116). Thus, future large-scale cohort studies are required in different populations to further investigate the effect of Ni in BC.

Associations Between Potentially Toxic Trace Elements According to the WHO Definition and BC

Cadmium and Breast Cancer

Cd, as one of the potentially toxic trace elements for the general population, is on the list of class 1 carcinogens by the IARC (110). It exists uniquely in the inorganic and divalent state (Cd^{2+}) (136). Following exposure, Cd concentration is first highest in the liver. Cd then accumulates in the kidneys and is discharged slowly into the urinary system (137).

In this meta-analysis, in plasma/serum, Cd concentration in patients with BC was found to be significantly higher than those in non-BC participants. In the subgroup analysis by region, we found that Cd concentration in the plasma/serum of patients with BC was significantly higher than those in non-BC participants in Asia. In this meta-analysis, sample test data from China, Iraq, and Kuwait have been included (29, 30, 43, 45). Meanwhile, serious Cd contamination has been found in rice (138), the dominating staple food for people mainly in Asia (139–141). These concomitant findings are of concern for BC prevention and treatment in the future. To be sure, we only found a positive result in hair specimens from one European study, but due to the small sample size, this result requires further verification. **Figure 6** shows the main mechanistic pathways described in *in vivo /in vitro* experiments. The most important factors and pathways for Cd elevation and the pathogenesis of BC are as follows: ER, GPER, ROS, p53, PLP2, and β-catenin, respectively. ① Cd can increase BC cell proliferation by binding with ERα, which can then activate Akt, ERK, and PTK (PDGFRα/Src) kinases (142, 143). ② In ER-negative BC cells, Cd induces the activation of MEK/ERK through GPER, leading to the breed of BC cells (144). ③ Cd induces an increase in ROS concentration, and if persistent, this can lead to changes in oxide-reducing signaling pathways, DNA damages, and methylation and chromatin remodeling patterns (145). ④ Cd induces the increase of p53 in the cytoplasm by downregulating the expression of the p53 inhibitor, Ube2d, and ubiquitin-binding enzyme (146). ⑤ Cd, as a transcription regulator, upregulates the expression of PLP2, which encodes proteolipid protein 2 independently (147). ⑥ Cd also stimulates metastasis-related phenotypes of triple-negative BC cells through the activation of β-catenin signaling transduction (148). In hair and tissue, Cd concentration was not significantly different between cases and non-BC participants. Among the human specimens, blood Cd measurement could reflect not only long-term exposure but also short-term exposure (149), whereas hair content of Cd can be altered by dyeing, bleaching, and shampoos; further, the exposure concentration detected in hair depends on the distance from the scalp (129). The insufficient number of studies on Cd in tissue is another possible reason for the variation in findings.

Lead and Breast Cancer

Pb is another one of the potentially toxic trace elements for the general population. It gets into the human body mainly *via* the respiratory and digestive tract (150), but organic Pb compounds can also be absorbed by the skin in rare cases (151). More than 90% of Pb binds to erythrocytes in the form of Pb^{2+}, once it enters the bloodstream (152). It can interplay with proteins in plasma and cellular, mostly the ones with thiol and sulfhydryl-containing (153). Pb is mostly discharged in the feces and urine (154). In this meta-analysis, there was no significant difference in plasma or serum Pb concentration between cases and non-BC participants; in hair, the concentration of Pb in patients with BC was significantly inferior to that in the control group. However, the number of studies included and the total sample size were comparatively small; thus, larger sample studies are required to confirm the relevance between Pb and BC.

In addition, studies on the mechanism concerning Pb in BC are limited, but the main research results show that Pb is tightly correlated with the pathogenesis of BC. **Figure 6** shows the major mechanistic pathways from *in vivo / in vitro* experiments. The most important factor and pathway for Pb elevation and the pathogenesis of BC is ER signaling. Pb can activate ERα to direct the estrogen target genes expression and the BC cell reproduction (116). Moreover, Pb is a nonessential metal that can imitate or obstruct the function of essential metals to induce toxicity associated with BC (155, 156).

This study has several advantages. This is the first study to analyze the probable categories of heavy metals—essential or probably essential or potentially toxic—in patients with BC. It summarized the correlation using population studies data and mechanisms in detail. In addition, the conclusions were derived from different samples and regions. Besides, the publication bias

was not found in the studies, meaning that these with obverse and the reverse results have been published, except the ones about Zn and Cd. However, there are also several limitations. First, terms of SMD were calculated to present the final pooled outcomes, which can eliminate the effect of multiple dimensions for lack of data measured in the uniformed dimension. However, the results in terms of SMD can only show whether there is a difference in heavy metal concentration between abnormal patients and non-BC participants. In the future, WMD can be used in analysis to get more information in clinical applications, if more original studies are conducted using data with the uniformed dimension. Second, this meta-analysis has included the studies on the relationship between heavy metals and BC comprehensively up to now. Although the quality of the studies has been evaluated with 5 or more points, several unmatched case–control studies included could partly lead to bias. Third, the results should be interpreted cautiously for possible publication bias about Zn and Cd. Fourth, it still cannot be analyzed about the effect of heavy metals on different degrees and molecular types of BC for the lack of related data, which are still needed to be studied further. Fifth, we summarized how Cr, Co, and Fe play roles in the mechanism of BC, but until now, there is only one population study on hair and tissue. Therefore, it remains to be verified in more population-based studies. Sixth, although the review and meta-analysis predicted the trends and also found the relative mechanism of effect of heavy metals in BC, heterogeneity in this meta-analysis was still not lowered after subgroup analysis, which may affect the stability of the results, but the mechanisms of heavy metals in BC had been found by signal pathways. Besides, although the differences in heavy metals in different biological specimens between patients with BC and patients with non-breast cancer are reviewed, whether heavy metals can directly lead to the incidence rate of BC cannot be verified. Therefore, the causal relationship requires more evidence in the future, and more prospective studies are still needed. In addition, the concentration of heavy metals in various biological samples has many factors (157). For example, hair Zn concentration is influenced by age, gender, season, hair growth rate, severity of malnutrition, and possibly hair color and other hair cosmetics (158). The concentrations of Cd, Pb in blood, and Pb in the hair seem to increase with smoking (159). In clinical application, the discovery of biomarkers of heavy metal contents with sensitivity and specificity in the prevention and diagnosis of diseases in the future is still needed.

The transport process of heavy metals is regulated by specific transporters. Divalent metal transporter 1 (DMT1), known as natural resistance-associated macrophage protein 2 (NRAMP2), divalent cation transporter 1 (DCT1), or solute carrier family 11, member 2 (SLC11A2), is a divalent metal transporter belonging to the proton-coupled metal-ion transporter family (160, 161). It regulates the transportation of bivalent metals including Fe, Zn, Mn, Cu, Co, Ni, Cd, and Pb (160). The increased expression of DMT1 could raise the uptake of Fe, Pb, and Cd in the duodenum (162, 163). Besides, DMT1 IVS4 + 44 C/A polymorphism impacts the individual differences in blood Fe, Pb, and Cd concentrations (161). However, few pieces of evidence have been reported relating to the ethnic and genetic differences in the transport, absorption, and elimination of heavy metals, which are required to be further studied.

CONCLUSION

On the whole, the findings of this study made clear that heavy metals may be related to BC. In terms of the essential trace elements, a higher concentration of Cu and a lower concentration of Zn in plasma/serum were observed in patients with BC as compared to patients with non-BC. For the probably essential trace elements, higher Mn in plasma/serum may help to reduce the BC risk. For the potentially toxic trace elements, higher Cd may be associated with the BC risk. In hair, we only observed a beneficial effect of Zn on BC development. No significant differences were indicated in plasma or serum samples of Cr, Co, and Ni between cases and non-BC participants. For the higher elements, studies with larger sample sizes and in various populations are needed to further verify the roles of these heavy metals in BC. To sum up, different from the ordinary population, the role of trace elements probably needs to be re-explored, particularly in BC.

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.

AUTHOR CONTRIBUTIONS

XL designed, supervised the study, guided research methods, and revised the manuscript. LL and JieC extracted the data, collected, analyzed, and double checked the data. LL wrote and revised the manuscript. LL, JieC, CL, YL, JiaC, YF, YX, HWu, and HWa reviewed and edited the manuscript. All authors have read and approved the final draft.

FUNDING

This study was supported by the grants from the Scientific research project of the Shanghai Health Committee (201940188); the Three-Year Action Program of the Shanghai Municipality for Strengthening the Construction of Public Health System (GWV-10.2-YQ34, GWV-10.1-XK05); the National Key R&D Program of China (2018YFC2000700); the SJTU Public Health School Local High-Level University Achievement-oriented Top-Notch Cultivation Program for Undergraduate Students (18ZYGW06); the innovative research team of high-level local universities in Shanghai.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.838762/full#supplementary-material

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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.

The reviewer JL declared a shared affiliation with the authors at the time of review.

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ORIGINAL RESEARCH
published: 16 June 2022
doi: 10.3389/fnut.2022.872773

A Follow-Up Study of Ovarian Cancer (OOPS): A Study Protocol

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The ovarian cancer (OC) follow-up study (OOPS) is an on-going hospital-based large prospective longitudinal cohort study aimed to explore the relationship between pre/post-diagnostic biological, clinical, environmental, and lifestyle factors with focus on the diet and OC prognosis (including drug resistance, relapse, and mortality). Patients recruited during the baseline survey were between 18 and 79 years old, with histologically confirmed OC diagnosis. Their follow-up and medical treatment were conducted at the gynecological oncology ward at Shengjing Hospital of China Medical University, Shenyang, China after 2015. A total of 703 OC patients made up the final OOPS study population. The follow-up stage was conducted in both passive and active modes. In the passive mode, the follow-up was performed by linkage to the Liaoning Providence Center for Disease Control and Prevention every 6 months to obtain health outcome results. The status of lifestyle factors was re-estimated using the same measurements as those in the baseline survey. OC participants in the OOPS study completed a questionnaire and anthropometric examinations. In addition, biological specimens were collected during the baseline survey, which included blood, urine, and stool samples that were stored for further use. This article is intended to serve as an introduction to this project and to provide details for investigators who may be carry out related analysis.

Keywords: ovarian cancer, follow-up, cohort study, survival, diet

OPEN ACCESS

Edited by:
Letizia Bresciani,
University of Parma, Italy

Reviewed by:
Ling Yang,
University of Oxford, United Kingdom
Yongjie Chen,
Tianjin Medical University, China

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

Received: *09 February 2022*
Accepted: *09 May 2022*
Published: *16 June 2022*

Citation:
Gong T-T, Liu F-H, Liu Y-S, Yan S, Xu H-L, He X-H, Wei Y-F, Qin X, Gao S, Zhao Y-H and Wu Q-J (2022) A Follow-Up Study of Ovarian Cancer (OOPS): A Study Protocol. Front. Nutr. 9:872773. doi: 10.3389/fnut.2022.872773

INTRODUCTION

Ovarian cancer (OC) is the third most common gynecologic malignancy with a high incidence and about 239,000 new cases reported annually worldwide (1, 2). Its mortality rate has always ranked first in gynecological malignancies and the 5-year survival rate is still <45% in most countries (3, 4). Not surprisingly, the current state of OC in China is not optimistic, with 55,342 new cases and 37,519 deaths in 2020, which have been increasing each year (5, 6). Three main types of OC have been identified, including epithelial, germ cell, and sex cord-stromal, with epithelial tumors comprising ∼95% of all OC cases (7).

Despite remarkable advances in surgery and chemotherapy, prognosis still requires considerable improvement (8). Thus, identifying prognostic factors for OC is critical for reducing its high mortality rates. Over the past decade, a growing number of studies have focused on prognostic

factors associated with OC. Some studies have reported that clinical characteristics (e.g., stage at detection, success in optimal debulking, histologic subtype, and chemotherapy) may play critical roles in prognosis (9). Additionally, several demographical and modifiable lifestyle factors (e.g., menopausal hormone therapy use, breastfeeding, psychosocial stress, and diet) can also influence survival in OC (10–17). However, high-quality prognostic studies focusing on these issues have been limited. Furthermore, inconsistent findings have been reported over the course of the recent decade. To gain deeper insight into prognostic factors and to further address inconsistencies related to OC patient survival, a prospective cohort study was conducted at Shengjing Hospital of China Medical University in Shenyang, China.

This prospective cohort study included eligible OC patients who provided informed consent for long-term prospective follow-up. The aim of the present study was to investigate the prognostic factors of OC, including patients' clinical information [such as pathological grade, imaging, and International Federation of Gynecology and Obstetrics (FIGO) stage], biomarkers (from the patient's tissue, blood, urine, and stool), and pre/post-diagnostic environmental exposure information (such as personal habits, sleep and mental state, fertility history, diet, physical activity, history of disease and surgery, and family history of chronic diseases) (**Figure 1**). The cohort was designed to support comprehensive research of the biological, clinical, environmental, and lifestyle factors determining prognosis in OC patients, as well as their inter-relationships, making the present study superior to prior research that only explored the impact of a single factor on the prognosis of OC. The present report describes the study design, provides a cohort description, and outlines preliminary results for factors affecting OC prognosis.

METHODS AND ANALYSIS

Design Overview

The ovarian cancer follow-up study (OOPS) is an on-going hospital-based large prospective longitudinal cohort study aimed to explore the relationship between pre/post-diagnostic biological, clinical, environmental, and lifestyle factors with focus on the diet and OC prognosis (including drug resistance, relapse, and mortality). Patients recruited during the baseline survey were between 18 and 79 years old, with histologically confirmed OC diagnosis. Their follow-up and medical treatment were conducted at the gynecological oncology ward at Shengjing Hospital of China Medical University, Shenyang, China after 2015. All patients were able to answer the epidemiological questionnaire. By December 31, 2020, 744 (93%) out of 853 distributed questionnaires were filled out and returned. Of these, 17 OC participants reported a significantly abnormal caloric intake (<500 or >3,500 calories/day) and 24 participants left 11 (10%) or more food items blank, which were excluded. A total of 703 OC patients made up the final OOPS study population. Median recruitment was achieved by the beginning of 2018, with 90% of participants recruited between the beginning of 2016 and the beginning of 2019 (**Figure 2**).

Follow-Up Schedules

The follow-up stage was conducted in both passive and active modes. In the passive mode, the follow-up was performed by linkage to the Liaoning Providence Center for Disease Control

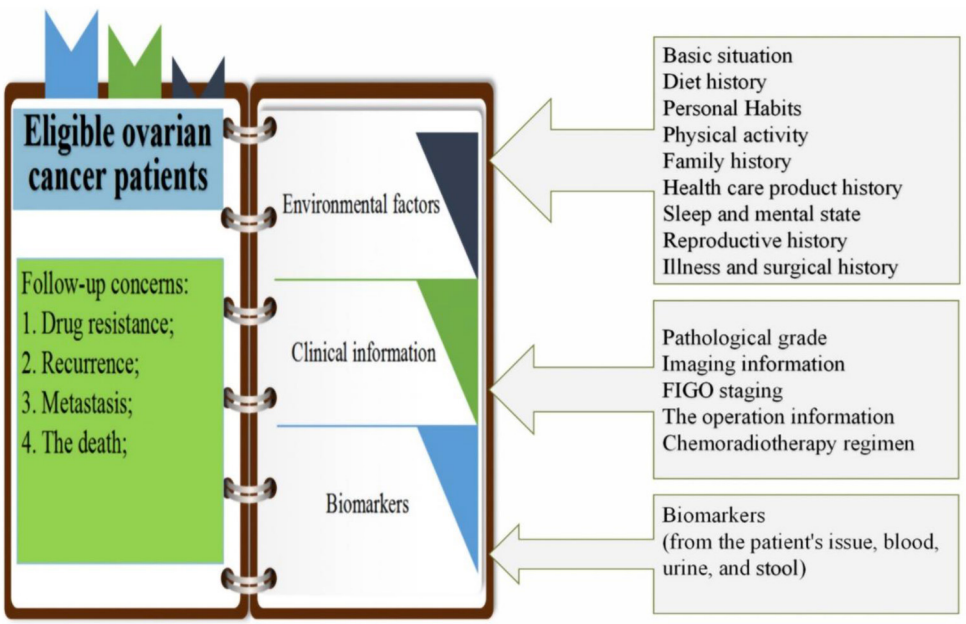

FIGURE 1 | Cohort design for prognosis study of patients with ovarian cancer.

and Prevention every 6 months to obtain health outcome results. In addition, clinical specialists extracted patient medical data from the information system at Shengjing Hospital every 6 months after the patient finished the baseline survey. This time lapse allowed for definitive staging, pathology evaluation, diagnosis determination, and initial treatment to be completed. In the active mode, all included and surviving OC patients were invited for a face-to-face interview every 6 months. The status of lifestyle factors was re-estimated using the same measurements as those in the baseline survey.

Data Collection

Ovarian cancer participants in the OOPS study completed a questionnaire and anthropometric examinations. In addition, biological specimens were collected during the baseline survey, which included blood, urine, and stool samples that were stored for further use. Developed written protocols for questionnaires, anthropometric measurements, and biospecimen collection, delivery, and storage were strictly enforced during the baseline survey.

Questionnaires

Well-trained interviewers conducted face-to-face interviews using a structured questionnaire to gather information on demographics and socio-economic characteristics, health care product use, health status, reproductive history, diet, individual living habits, passive smoking and indoor air pollution status, physical activity, past medical history, and family history of chronic diseases (**Table 1**). An average of 45–60 minutes was required to complete a questionnaire. Unqualified questionnaires were excluded from the final analysis. For example, more food items were blank ($\geq$11 items), the same frequency was chosen in most food items.

Anthropometric Measurements

Well-trained personnel used standard methods to measure patients' height, weight, waist circumference, hip circumference, and blood pressure. Body weight was measured using a digital scale to the nearest 0.1 kg while wearing light clothing and without shoes, after emptying of the bladder. Height was measured without shoes, with stadiometer to the nearest 0.1 cm. Body mass index was calculated using the height and weight measurements. Waist circumference was measured to the nearest 0.1 cm, using plastic tape measure at midpoint between the costal margin and iliac crest in the mid-axillary line, with the subject standing and at the end of a gentle expiration. Hip circumference was measured to the nearest 0.1 cm, at the level of greater trochanters, with the legs close together. Blood pressure after a 5-min rest was measured by a well-trained staff. Two consecutive blood pressure measurements were taken and the arithmetic mean of both measurements were calculated (**Table 1**).

A clinical nurse was in charge of quality control. He or she checked whether the measurement instruments were in good condition, inspected whether the measurement was in accordance with standard operating procedures, and randomly assigned participants for re-testing.

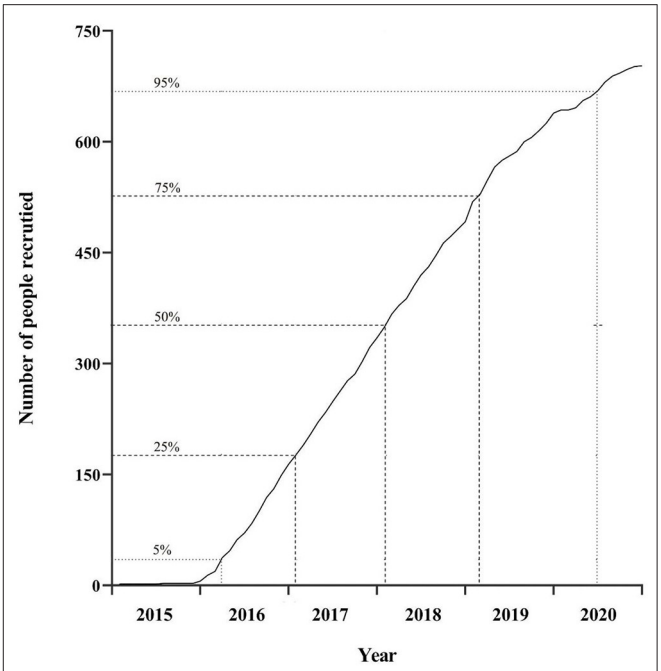

FIGURE 2 | Cumulative recruitment into the cohort.

Biorepository

Participants in the OOPS study provided blood, urine, and fecal samples on-site at the time of the baseline survey. Venous blood was collected after overnight fasting (at least 8 h) and mid-stream urine samples were collected. Fecal samples were collected from the middle portion of the feces using a sterile feces collector. The blood samples were aliquoted into 1-ml straws (two straws of each blood and plasma). The urine samples were aliquoted into two 4.5-ml straws. All samples were stored at −80°C in cryogenic refrigerators. Computer systems were used for sample entry, access, and temperature monitoring.

Ethics and Dissemination

All cohort participants provided written informed consent before the baseline survey. The study was approved by the Institutional Review Board of the Ethics Committee of Shengjing Hospital of China Medical University (2015PS38K). The data would be accessed just by the researchers, who associated with the study and the Ethics Committee. The results of this study will be shown at national and international conferences and peer-reviewed scientific journals. All the results shown in our study will be of group data; thus, individual participants will not be identifiable.

Patient and Public Involvement

No patients have been involved in the development of the plan for designing, conducting, reporting or implementing this study.

Statistics

The results are presented as means with standard deviation (SD) for continuous variables and as frequency with percentage for categorical variables. Cox proportional hazards regression was

TABLE 1 | Summary of investigations at the baseline survey.

Investigations	No. of variables	Variables
Questionnaire		
Demographics and socio-economic characteristics	13	Name, sex, race, national ID number, present address, contact information, occupation, company name, household income, family number, education, marital status, and type of medical insurance
Health care products	12	Vitamin A, vitamin B, vitamin C, vitamin D, vitamin E, multivitamins, calcium, iron, zinc, fish oil/DHA, ginseng, and other dietary supplements
Health status	49	Sleep quality, stress life events, Patient Health Questionnaire (PHQ-9), Generalized Anxiety Disorder scale (GAD-7)
Reproductive history	10	Age of menarche, menopause status, age of menopause, history of menopausal drug use, experience of pregnancy, history of breast feeding, age at parturition of each live birth, duration of breast feeding, experience of assisted reproductive technology, history of intrauterine contraceptive device, history of contraceptive pills use
Diet	131	A 110-item food frequency questionnaire (FFQ), cooking methods of meat, vegetables, and seafoods, dietary habits
Individual living habits	36	Smoking status, age at starting smoking, number of cigarettes per day, depth of smoking, current smoking status, smoking situation today, changes in smoking, drinking status, age at starting drinking, symptoms of drinking, current drinking status, changes in drinking, status of tea drinking, age at starting tea drinking, frequency of changing tea per day, changes in tea drinking, status of carbonated drinks drinking, age at starting carbonated drinks drinking, current status of carbonated drinks drinking, changes in carbonated drinks drinking, status of coffee drinking, age at starting coffee drinking, current status of coffee drinking, changes in coffee drinking
Passive smoking and indoor air pollution	28	Passive smoking exposure, indoor air pollution, pesticide exposure
Physical activity	25	Occupational physical activity, transportation physical activity, leisure-time physical activity, high intensive physical activity, housework, weight changes during last 12 months, weight and height at 25 years old
Past medical history	8	History of disease and history of surgery
Family history	2	Cancers diagnoses and age/dates for first-degree relatives
Anthropometric measurement	4	Height, weight, waist circumference, hip circumference
Clinical information	5	Histological type, histopathologic grade, FIGO stage, residual lesions, comorbidities

used to calculate the hazard ratio (HR) and 95% confidence interval (CI) for the association of baseline clinical information with overall survival. The proportional hazards assumption was evaluated by including an interaction term between each activity variable and log survival time. No violations were observed (all $P > 0.05$). All analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC, United States). Two-sided *P*-Values of < 0.05 were considered statistically significant.

RESULTS

Demographics

Table 2 shows the baseline characteristics of OC patients. Participants in this cohort were older, with an average age of 53.63 ± 9.45 years. The Pittsburg Sleep Quality Index was 6.31 ± 3.76. OC patients were assessed using the Patient Health Questionnaire-9 and showed mild depression (6.26 ± 4.72 score). Anxiety in OC patients was assessed using the Generalized Anxiety Disorder scale, which presented normal at 4.16 ± 3.67. More than half of participants had a lower level of education. Similar results were observed for income, with 59.88% (421) with a lower income per month. Almost all participants were insured. Only 7.11% (50) had a family history of cancer. Half of the participants had normal weight. However, above three quarters of them (76.53%) were central obese. And we will (68) have a history of smoking, 21.49% (21.19) have a history of drinking, 32.15% (226) have a history of tea drinking, and over 20% have changed dietary habits or taken dietary supplements. A total of 72.26% (508) went through menopause, and 71.83% (505) had one child or less.

Diet Information

Diet information for the OC patients is presented in **Table 3**. Total energy intake for OC patients was 1,455.75 ± 552.64 kcal/day. Staple food consumption was 615.46 ± 233.11 g/day. The intake of meat and eggs was similar at about 37 g/day. The intake amounts of fish and seafood and beans and bean products were 28.52 ± 30.31 and 85.27 ± 78.45 g/day, respectively. Vegetables intake was 214.22 ± 121.72 g/day, which reached the level recommended by the Dietary Guidelines for Chinese residents. However, the intake of fruits was relatively low at 194.64 ± 157.81 g/day. The intake of carbohydrate, fat and protein were 226.90 ± 78.37, 35.04 ± 18.27, and 58.45 ± 24.78 g/day. For vitamins, the highest intake is vitamin C (103.10 ± 62.40 mg/day), the lowest intake is vitamin B12 (0.14 ± 0.21 mg/day). The total fatty acid intake was 9.00 ± 5.72 g/day.

TABLE 2 | Demographic information of OOPS from 2015 to June 2020.

Variables	Consented (n =703)	%
Age at diagnosis (years)		
<40	43	6.12
40–49	159	22.62
50–59	288	40.96
60–69	169	24.04
≥69	44	6.26
Age at diagnosis (years; Mean ± SD)	53.63 ± 9.45	
Pittsburg sleep quality index (Mean ± SD)	6.31 ± 3.76	
Depression scale scores (Mean ± SD)	6.26 ± 4.72	
Anxiety scale scores (Mean ± SD)	4.16 ± 3.67	
Age at menopause (Mean ± SD)	49.73 ± 3.33	
Educational level		
Junior secondary or below	375	53.34
Senior high school/technical secondary school	147	20.91
Junior college/university or above	181	25.75
Income per month (Yuan)		
<5,000	421	59.88
5,000 to <10,000	194	27.60
≥10,000	88	12.52
Ever insurance	681	96.87
Ever family history of cancer	50	7.11
Body mass index (kg/m^2)		
<18.5	58	8.25
18.5–23.9	391	55.62
24–27.9	190	27.03
≥28	64	9.10
Waist-hip ratio		
<0.85	165	23.47
≥0.85	538	76.53
Physical activity (MET/h/days)		
<8	207	29.45
8–15.9	193	27.45
16–23.9	163	23.19
≥24	140	19.91
Ever diet change	168	23.90
Ever cigarette smoking	68	9.67
Ever alcohol drinking	149	21.19
Ever tea drinking	226	32.15
Ever dietary supplement	150	21.34
Ever menopause	508	72.26
Parity		
0	5	0.71
1	500	71.12
≥2	198	28.17
Ever oral contraceptive	70	9.96

SD, standard deviation.

TABLE 3 | Diet information of OOPS from 2015 to June 2020.

Variables	Mean	Standard
Total energy intake (kcal/day)	1,455.75	552.64
Staple food (g/day)	615.46	233.11
Meat (g/day)	36.38	29.471
Eggs (g/day)	37.76	27.131
Fish and seafood (g/day)	28.52	30.31
Beans and bean products (g/day)	85.27	78.45
Vegetables (g/day)	214.22	121.72
Fruits (g/day)	194.64	157.81
Carbohydrate (g/day)	226.90	78.37
Fat (g/day)	35.04	18.27
Protein (g/day)	58.45	24.78
Fiber (g/day)	17.51	8.61
Vitamin A (μg/day)	459.93	374.65
Thiamine (mg/day)	0.54	0.27
Riboflavin (mg/day)	0.89	0.41
Niacin (mg/day)	13.66	4.46
Vitamin B6 (mg/day)	0.44	0.22
Vitamin B12 (mg/day)	0.14	0.21
Vitamin C (mg/day)	103.10	62.40
Vitamin E (mg/day)	14.11	9.24
Total fatty acid (g/day)	9.00	5.72

Clinical Information

Diagnostic information included histological type, histopathologic grade, FIGO stage, residual lesions, and comorbidities (**Table 4**). A total of 68.14% patients in the cohort had serous OC. In terms of histopathologic grade, 85.21% (n = 599) of patients had poorly differentiated OC. There were no residual lesions after surgery in 78.66% (n = 553) of patients. A total of 130 (18.49%) deaths occurred before March 31, 2021 during a median follow-up of 37.17 months (interquartile: 24.73–50.17 months).

Clinical Information and Associations With All-Cause Mortality

Non-serous histological subtype, later-stage disease, and greater residual disease were statistically significantly associated with worse survival in this cohort (**Table 5**). However, an association between histopathologic grade and comorbidities and OC survival was not observed.

Key Publications

The OOPS study has provided results for diet and OC survival (**Table 6**). For instance, pre-diagnosis healthy pattern was related to better survival, whereas animal foods pattern was associated with worse survival (18); pre-diagnosis dairy product intake was associated with worse survival (19); pre-diagnosis total cruciferous vegetables and isothiocyanates intake was associated with better survival (20); pre-diagnosis consumption of vitamin B was associated with worse survival (21).

TABLE 4 | Clinical information of OOPS from 2015 to June 2020.

Clinical variables	Consented ($n = 703$)	%
Histological type		
Serous	479	68.14
Non-serous	224	31.86
Histopathologic grade		
Well-differentiated	56	7.97
Moderately differentiated	48	6.82
Poorly differentiated	599	85.21
FIGO stage		
I-II	342	48.65
III-IV	338	48.08
Unknown	23	3.27
Residual lesions		
No	553	78.66
<1 cm	106	15.08
≥1 cm	44	6.26
Comorbidities	310	44.10
Vital status		
Alive	573	81.51
Died	130	18.49

TABLE 5 | Clinical information and associations with all-cause mortality among OOPS participants.

Clinical variable	No. of deaths/ total (%)	Crude HR (95% CI)	Adjusted HR[a] (95% CI)
Histological type			
Serous	92/479 (19.21)	1.00 (ref)	1.00 (ref)
Non-serous	38/224 (16. 96)	0.87 (0.59–1.27)	1.71 (1.11–2.66)
Histopathologic grade			
Well-differentiated	5/56 (8.93)	1.00 (ref)	1.00 (ref)
Moderately differentiated	7/48 (14.58)	1.44 (0.46–4.57)	1.12 (0.35–3.57)
Poorly differentiated	118/599 (19.70)	2.32 (0.95–5.67)	1.76 (0.70–4.43)
FIGO stage			
I-II	41/342 (11.99)	1.00 (ref)	1.00 (ref)
III-IV	89/338 (26.33)	2.75 (1.89–4.00)	2.54 (1.65–3.91)
Residual lesions			
No	82/553 (14.83)	1.00 (ref)	1.00 (ref)
<1 cm	31/106 (29.25)	2.22 (1.47–3.36)	1.73 (1.11–2.68)
≥1 cm	17/44 (38.64)	3.18 (1.89–5.37)	2.41 (1.39–4.16)
Comorbidities			
No	74/393 (18.83)	1.00 (ref)	1.00 (ref)
Yes	56/310 (18.06)	0.82 (0.58–1.16)	0.97 (0.68–1.38)

CI, confidence interval; HR, hazard ratio; Ref, reference.
[a]*Mutually adjusted for all other variables listed in the table.*

TABLE 6 | Previous results for ovarian cancer survival in the OOPS study.

Reference	Journal	Year of publication	Exposure
(18)	Clinical nutrition	2022	Dietary pattern
(19)	Frontiers in nutrition	2021	Dairy product
(20)	Frontiers in nutrition	2021	Cruciferous vegetables and isothiocyanates
(21)	Frontiers in nutrition	2021	Dietary supplements

DISCUSSION

As the cohort continues to age, further analyses will be performed to explore the importance of lifestyle and biological characteristics in OC prognosis. This will include studying not only the traditional risk factors, such as cancer characteristics and treatment, but also genetics and other omics, lifestyle (pre/post–prognosis physical activity, diet, passive smoking, and sleep), environmental factors (air pollution and living area conditions), and medical history. And we will apply for funding to ensure the above research proceed. Furthermore, collaborations will be established with nationwide and international institutions to create a multicenter network that includes different types and levels of hospitals. This broad range of participants enables us to generalize results for all patients and thus provides an evaluation of a much wider range of personal health behaviors. Moreover, an in-depth study investigating genetic and molecular profiles will also be conducted. Genome-wide association studies will be carried out to identify novel genetic variants associated with disease development and related phenotypes. Metabolomics (a mass spectroscopy-based technology) techniques will also be available to study the associations between disease status and metabolome. Proteomics will be used to investigate proteins and their post-translational modifications and interactions, providing an opportunity to elucidate complex biological processes and conditions. These studies can lead to a discovery of underlying mechanisms of metastasis, recurrence, and fatality among OC patients. The studies of OC mechanisms will broadly advance the goal of personalized medicine, evidence-based survivorship care, and translation into practical applications, ultimately leading to improvements in public health and healthcare. Finally, artificial intelligence as well as machine and deep learning will be used in future studies to develop integrative risk prediction algorithms for clinical outcome and survivorship endpoints.

The present study collected detailed and comprehensive clinical, demographic, and biological characteristics data. The resulting well-annotated biorepository is rapidly growing, adding opportunities for genetic and molecular profiling to better explore the linkage between biospecimens and clinical data throughout the patient treatment journey. In addition, various administrative register data and electronic medical records independent of the study hypothesis were accessed in the present investigation. This type of data almost completely avoids the possibility that selection, information, and recall bias influence the results, ensuring observation validity and minimizing participant loss at follow-up. Furthermore, the present prospective cohort study was performed in collaboration with a Clinical Research Center and Department of Obstetrics and Gynecology within the Shengjing Hospital of China Medical University. Thus, a large patient population from a single institution minimized treatment and clinical data collection heterogeneity. In addition, the quality of study design,

measurement, and evaluation was guaranteed with the help of staff from the clinical research center. Of note, the study included OC patients who were treated using different measures, which means that the present study was able to provide an opportunity to clarify the progression of OC and explore different therapeutic methods.

The potential weaknesses of the OOPS include the fact that the self-reported data for some variables, such as lifestyle factors and occupational exposure, may introduce recall bias. Apart from that, some information on clinical outcome prognoses relies on inpatient medical, readmission, or outpatient records. As a result, some complications and other related events may be underreported and underestimated. In addition, the sample size is small in current analysis. However, the OOPS study is an ongoing cohort, more and more OC patients will be recruited in our cohort. Finally, a single-institution patient population and its results may not be generalizable to all patients. Nevertheless, collaborations with a wider range of institutions are planned in order to create a cooperation network.

CONCLUSION

In conclusion, the OOPS study collected detailed and comprehensive clinical, demographic, and biological characteristics data, which added opportunities to better explore the linkage between biospecimens and clinical data throughout the patient treatment journey. More research will be conducted on the prognosis of ovarian cancer.

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/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Shengjing hospital of China Medical University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

T-TG, Y-HZ, and Q-JW contributed to the study design. T-TG, SY, X-HH, XQ, and SG collection of data. F-HL and Y-FW analysis of data. T-TG, F-HL, Y-SL, H-LX, Y-FW, and Q-JW wrote the first draft of the manuscript and edited the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by the National Key R&D Program of China (No. 2017YFC0907404 to Y-HZ), the Natural Science Foundation of China (No. 82073647 to Q-JW, No. 81602918 to Q-JW, and No.82103914 to T-TG), LiaoNing Revitalization Talents Program (No. XLYC1907102 to Q-JW), Shenyang high level innovative talents support program (No. RC190484 to Q-JW), 345 Talent Project of Shengjing Hospital of China Medical University (Q-JW and T-TG), and Clinical Research Cultivation Project of Shengjing hospital to SG.

ACKNOWLEDGMENTS

We thank the research team for their daily efforts in material collection and manuscript writing.

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frontiers | Frontiers in Nutrition

ORIGINAL RESEARCH
published: 06 July 2022
doi: 10.3389/fnut.2022.931171

Flavonoid Intake From Cocoa-Based Products and Adiposity Parameters in Adolescents in Spain

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Nutritional Epidemiology, a section of the journal Frontiers in Nutrition

Received: 28 April 2022
Accepted: 08 June 2022
Published: 06 July 2022

Citation:
Laveriano-Santos EP, Arancibia-Riveros C, Tresserra-Rimbau A, Castro-Barquero S, Ruiz-León AM, Estruch R, Casas R, Bodega P, de Miguel M, de Cos-Gandoy A, Martínez-Gómez J, Rodríguez C, Santos-Beneit G, Fernández-Alvira JM, Fernández-Jiménez R and Lamuela-Raventós RM (2022) Flavonoid Intake From Cocoa-Based Products and Adiposity Parameters in Adolescents in Spain. Front. Nutr. 9:931171. doi: 10.3389/fnut.2022.931171

Background: Cocoa-based products are a good source of flavonoids, which may have beneficial effects on metabolic health.

Objective: The aim of this study is to assess the relationship between flavonoids from cocoa-based products and adiposity parameters in adolescents.

Methods: A cross-sectional study was conducted involving 944 adolescents aged 11–14 years enrolled in the SI! Program for Secondary Schools trial in Spain with available baseline data from food frequency questionnaires and anthropometric measurements [weight, height, waist circumference (WC), and fat mass percentage (% FM) by bioimpedance analysis]. Fat mass index (FMI) and waist-to-height ratio (WHtR) were obtained by dividing fat mass by height and WC by height, respectively. Body mass index (BMI), WC, and FMI for age and gender z-score were calculated. Overweight/obesity was defined as BMI $\geq$ 85th percentile and excess adiposity as %FM or FMI $\geq$ 75th percentile. WC $\geq$ 90th percentile and WHtR with a 0.5 threshold were considered as criteria of abdominal obesity. Multilevel mixed-effect regressions were used to evaluate the association between flavonoids from cocoa-based products and adiposity parameters. Municipalities and schools were considered random effects.

Results: Participants with a higher flavonoid intake from cocoa-based products had lower WC z-score [B = −0.04, 95% CI (−0.07; −0.01), *P-for trend* = 0.045] and WHtR [B = −0.01, 95% CI (−0.02; −0.01), *P- for trend* < 0.001]. They also had lower probability of having abdominal obesity [OR 0.66, 95% CI (0.52; 0.85), *P- for trend* = 0.001]. Inverse associations were observed between flavonoids from cocoa powder and BMI z-score [B = −0.08, 95% CI (−0.12; −0.05), $P < 0.001$], WC z-score [B = −0.06, 95% CI (−0.11; −0.02), $P = 0.003$], WHtR [B = −0.01, 95% CI (−0.01; −0.00), $P < 0.001$],

%FM [B = −1.11, 95% CI (−1.48; −0.75), $P < 0.001$], and FMI z-score [B = −0.18, 95% CI (−0.20; −0.17), $P < 0.001$]. Regarding dark chocolate, an inverse association only with WC z-score [B = −0.06, 95% CI (−0.08; −0.05), $P < 0.001$] was found. However, no association was observed between flavonoids from milk chocolate intake and anthropometric parameters.

Conclusions: A higher intake of flavonoids from cocoa-based products was associated with lower adiposity parameters and a lower probability of presenting abdominal obesity.

Keywords: (poly)phenols, catechin, epicatechin, proanthocyanidins, cardiometabolic, obesity

INTRODUCTION

Obesity, which is characterized by abnormal or excessive body fat accumulation, is a serious public health problem worldwide (1, 2). Excessive adiposity in children and adolescents leads to metabolic disorders such as vascular dysfunction and subclinical indicators of atherosclerosis, increasing the risk of cardiovascular disease and mortality in adulthood (2, 3).

Marked by physiological and emotional changes, adolescence is a critical period for managing obesity. Behavioral modifications, particularly fomenting physical activity and healthy dietary patterns are one of the best strategies used in primary health care settings to reduce obesity among adolescents (2, 4). A diet based on polyphenol-rich foods is of interest because of the antioxidant and anti-inflammatory effect and their influence on physiological and molecular pathways related to body weight maintenance (5–8). The positive impact of cocoa-based products on obesity has been attributed to their content of flavonoids (a large class of phenolic compounds), specifically flavanols (catechins and procyanidins) (9, 10). Systematic reviews and meta-analyses support the beneficial effect of cocoa flavonoids on cardiovascular risk factors since they are reported to favorably improve blood pressure, lipid profile, inflammation, and adiposity parameters (11–13). However, as most of the research in this field has been performed in adults, there is a need for studies on adolescents to establish dietary recommendations for the consumption of cocoa-based products in this target population, always within the framework of a healthy lifestyle. Therefore, this study aimed to investigate the association between flavonoid intake from cocoa-based products and adiposity parameters in a large sample of adolescents in Spain.

MATERIALS AND METHODS

Study Population

The SI! (*Salud Integral-Comprehensive Health*) Program for Secondary Schools trial (NCT03504059) is a cluster-randomized controlled intervention trial conducted in adolescents from 24 secondary schools in Spain and conducted from 2017 to 2021. The main objective of this trial was to evaluate the effectiveness of an educational intervention to promote cardiovascular health at schools. A detailed description of the original study design and recruitment procedures has been previously published (14). Parents or caregivers provided assent and written informed consent before entering the study.

The present cross-sectional study derived from the SI! Program for Secondary Schools trial was carried out using baseline data (2017) collected from 944 participants with available information on food consumption frequency, and whose total energy intake ranged from 803 to 4,013 kcal/day in boys and 502 to 3,511 kcal/day in girls (15) (**Figure 1**).

Assessment of Flavonoids From Cocoa-Based Products

Dietary intake was assessed by a validated semi-quantitative food frequency questionnaire (15, 16). Cocoa-based product intake was expressed in grams and included cocoa powder (25% of pure cocoa), dark (more than 70% pure cocoa), and milk chocolate (about 30% pure cocoa). Cookies, pastries, and beverages made of cocoa-based products were not considered due to the lack of information on their content of flavonoids. Flavonoids from cocoa-based products were estimated using the Phenol-Explorer database (http://www.phenol-explorer.eu), which included flavanols, such as catechin, epicatechin, cinnamtannins, and proanthocyanidins (dimers, trimers, 4–6 mers, 7–10 mers, polymers, and monomers), and flavonol-like quercetin (17, 18). In brief, flavonoids from cocoa-based products were estimated (mg/100 g fresh food weight) for each food and then multiplied by intake of the respective foods (g/day). Total flavonoids from cocoa-based products were estimated as the sum of intakes of the individual flavonoids (catechin, epicatechin, cinnamtannins, proanthocyanidins, and quercetin). Energy-adjusted flavonoid intake was calculated by the residual method established by Willet et al. (19).

Assessment of Adiposity Parameters

All participants were evaluated by trained staff, who performed the anthropometric measurements of weight, height, and waist circumference (WC) according to standard procedures (14). Weight was obtained to the nearest 0.1 kg using a digital scale (OMRON BF511) and height to the nearest 0.1 cm with a portable SECA 213 stadiometer. WC was measured to the nearest 0.1 cm. To minimize measurement errors, WC was measured three times and a mean value was calculated. The percentage of fat

Abbreviations: BMI, body mass index; FDR, false discovery rate; FM, fat mass; FMI, fat mass index; SI, *Salud Integral;* WC, waist circumference; WHtR, waist-to-height ratio.

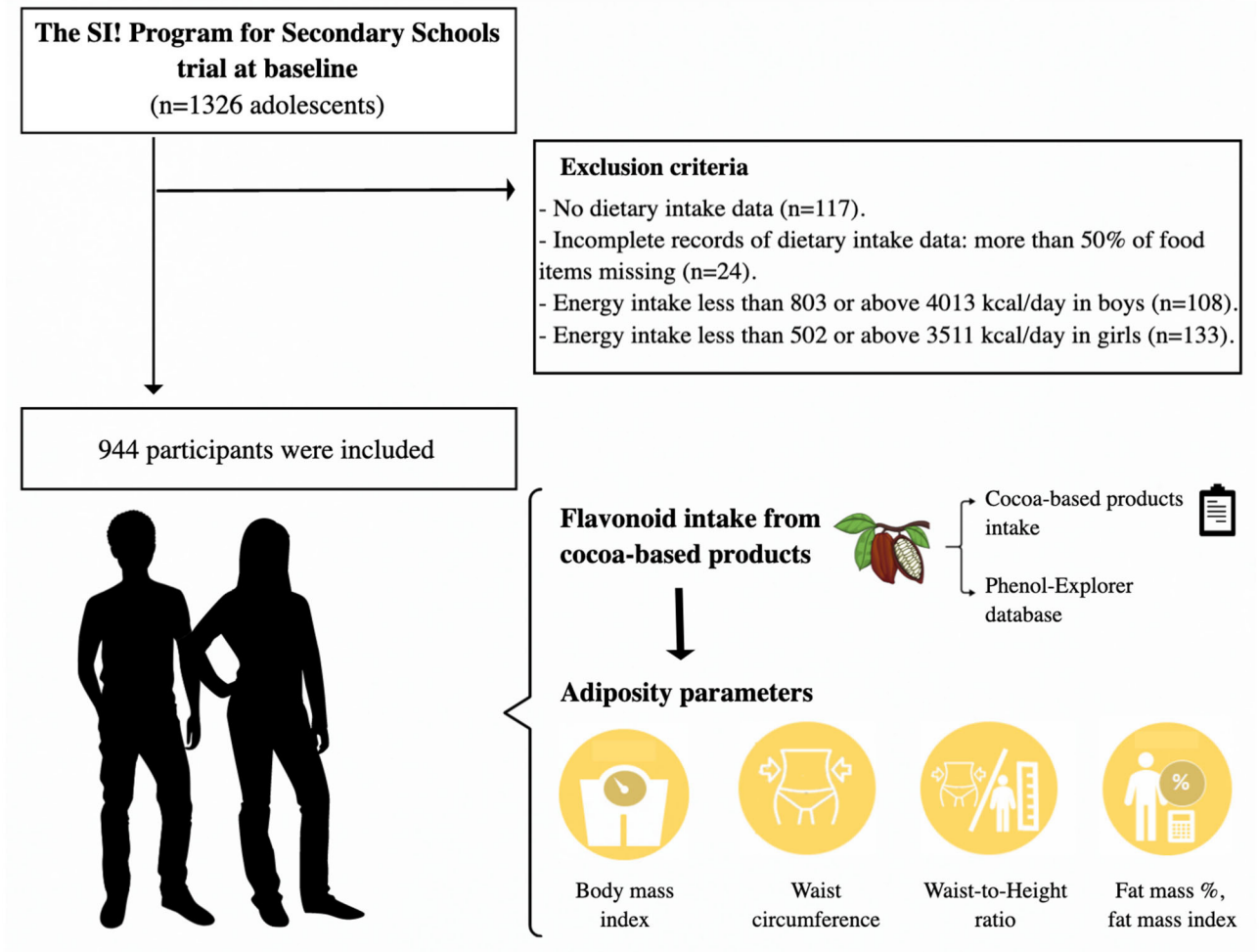

FIGURE 1 | Study design of the study.

mass (%FM) was estimated by bioelectrical impedance using a tetrapolar OMRON BF511 and fat mass weight was calculated as the product of fat percentage and body weight.

Body mass index (BMI) was calculated as body weight in kilograms divided by height squared in meters (kg/m^2). The fat mass index (FMI) and waist-to-height ratio (WHtR) were obtained by dividing fat weight (kg) by height squared (m^2) and WC (cm) by height (cm), respectively. Age- and gender-specific BMI, WC z-scores, and FMI z-scores (standard deviation score) were calculated according to the Center for Disease Control growth references and the National Health and Nutrition Examination Survey data (20–23).

Overweight was defined as BMI at or above the 85th percentile to less than the 95th percentile, and obesity as equal to or greater than the BMI 95th percentile (21, 22). Participants with a BMI percentile equal to or above the 85th percentile were classified as overweight/obese (21, 22). Abdominal obesity was defined by WC at or above the 90th percentile and/or WHtR equal to or above the 0.5 threshold (20, 24, 25). Finally, participants with a %FM and/or FMI greater than or equal to the age- and gender-specific 75th percentile were classified as excess adiposity, according to published reference data for %FM and FMI (23, 26).

Assessment of Covariates

Intake of energy, foods, and nutrients was determined from the semi-quantitative food frequency questionnaire that was previously described, with the use of values from Spanish food composition tables (27, 28).

Information on physical activity was obtained with the use of accelerometers and a standardized questionnaire. Physical activity was measured using accelerometers (Actigraph wGT3X-BT, ActiGraph, Pensacola, USA) worn on the non-dominant wrist for 7 days, except during water-based activities (14). Moderate-to-vigorous physical activity was estimated according to the cut points of Chandler et al. and is presented as the average minutes of moderate-to-vigorous physical activity per day (29). In participants with missing accelerometer data (n = 48), the information from the QAPACE survey (*Quantification*

TABLE 1 | Characteristics of participants according to quintiles of flavonoid intake from cocoa-based products (mg/day).

	Overall (n = 944)	Q1 (<12.1) (n = 189)	Q2 (12.1–32.0) (n = 189)	Q3 (32.1–53.2) (n = 189)	Q4 (53.3–83.8) (n = 189)	Q5 (>83.8) (n = 188)	*P-for trend*
Girls, *n* (%)	455 (48)	76 (40)	101 (53)	89 (47)	90 (48)	99 (53)	0.098
Age, years	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	12.0 (0.4)	0.740
Anthropometric measurements							
BMI, kg/m^2	20.2 (3.7)	20.2 (3.6)	20.4 (3.9)	20.5 (4.1)	20.4 (3.7)	19.7 (3.3)	0.286
WC, cm	71.9 (10.1)	72.2 (10.1)	71.7 (10.1)	72.7 (11.6)	72.5 (9.9)	70.5 (8.7)	0.232
WHtR	0.4 (0.1)	0.5 (0.1)	0.4 (0.1)	0.5 (0.1)	0.5 (0.1)	0.5 (0.1)	0.082
%FM	23.3 (8.3)	22.7 (8.2)	23.5 (8.1)	23.7 (9.0)	23.9 (8.2)	22.7 (8.0)	0.980
FMI, kg/m^2	5.0 (2.7)	4.9 (2.6)	5.1 (2.8)	5.2 (2.9)	5.1 (2.6)	4.7 (2.4)	0.656
Adiposity parameters, *n* (%)							
BMI ≥ 85th to <95th percentile	172 (18)	39 (21)	33 (18)	32 (17)	41 (22)	27 (14)	0.334
BMI ≥ 95th percentile	89 (9)	14 (7)	18 (10)	24 (13)	19 (10)	14 (7)	0.943
WC ≥ 90th percentile	153 (16)	34 (18)	25 (13)	36 (19)	33 (17)	25 (13)	0.546
WHtR ≥ 0.5	213 (23)	48 (25)	39 (21)	44 (23)	50 (26)	32 (17)	0.258
%FM ≥ 75th percentile	79 (8)	14 (7)	14 (7)	19 (10)	17 (9)	15 (8)	0.646
FMI ≥ 75th percentile	146 (16)	28 (15)	26 (14)	37 (20)	31 (16)	24 (13)	0.888
Physical activity, *n* (%)							
≥60 min/day MVPA	310 (33)	71 (38)	64 (34)	57 (30)	61 (32)	57 (30)	0.137
Parental education, *n* (%)							
University level	240 (26)	58 (31)	44 (24)	49 (27)	46 (25)	43 (24)	0.165
Municipality, *n* (%)							**0.021**
Barcelona	644 (68)	138 (73)	141 (75)	121 (64)	119 (63)	125 (66)	
Madrid	300 (32)	51 (27)	48 (25)	68 (36)	70 (37)	63 (34)	

Data are expressed as mean (SD) or frequency (percentage).

Q, quintiles of flavonoids from cocoa-based products; n, number; SD, standard deviation; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; %FM, body fat percentage; FMI, fat mass index; MVPA, moderate-to-vigorous physical activity.

Statistical analyses were conducted using one-way ANOVA for continuous variables and the chi-square test for categorical variables. P-for trend were obtained using orthogonal contrasts test. $P < 0.05$ are considered statistically significant.

Significant differences are bolded.

de L'Activité Physique en Altitude chez les Enfants) was used to estimate moderate-to-vigorous physical activity according to the frequency and duration of recreational physical activity and competitive sports performed inside or outside schools, on school days, and at weekends (14, 30). A conversion factor was used to calculate moderate-to-vigorous physical activity in terms of minutes per day. For the analysis, physical activity was categorized as below or equal to/above 60 min/day of moderate-to-vigorous physical activity based on physical activity recommendations for adolescents by the World Health Organization (31).

Information about parental education was obtained from a general questionnaire answered by the parents (14). A high level of parental education corresponded to university studies according to the International Standard Classification of Education (32). Puberty development was categorized according to the Tanner maturation stages using pictograms (33).

Statistical Analysis

A descriptive analysis of the population was carried out using mean (SD) and frequency distribution. Participants were categorized into quintiles of energy-adjusted flavonoids from cocoa-based products (Q1: <12.1, Q2: 12.1–32.0, Q3: 32.1–53.2, Q4: 53.3–83.8, and Q5 >83.8 mg/day). One-way analysis of variance, including Bonferroni *post-hoc* test, and chi-square analysis were performed to assess the differences in means and frequencies across quintiles of flavonoids from cocoa-based products, respectively.

Multilevel mixed-effects linear regression models with robust error variance were used to evaluate the association between quintiles of flavonoids from cocoa-based products with the anthropometric measurements (BMI *z*-score, WC *z*-score, WHtR, %FM, and FMI *z*-score) as continuous variables. Multilevel generalized logistic regression was performed to study the association between quintiles of flavonoids from cocoa-based products and adiposity parameters (BMI ≥ 80th to <95th percentile, BMI ≥ 95th percentile, WC ≥ 90th percentile, and WHtR ≥ 0.5). The fixed effects were gender (girls/boys), age (continuous, year), Tanner maturation stage (from I to V), physical activity (≥60 min/<60 min moderate-to-vigorous physical activity), parental education (university studies/lower than university studies), intake of energy (continuous, Kcal/day), sweetened products like breakfast cereals (continuous, g/day), pastries (continuous, g/day), sugar-sweetened beverages (continuous, g/day), meat, and processed meat (continuous,

TABLE 2 | Dietary food intake of participants according to quintiles of flavonoids from cocoa-based products (mg/day).

	Overall ($n = 944$)	Q1 (<12.1) ($n = 189$)	Q2 (12.1–32.0) ($n = 189$)	Q3 (32.1–53.2) ($n = 189$)	Q4 (53.3–83.8) ($n = 189$)	Q5 (>83.8) ($n = 188$)	*P-for trend*
Fish products, g/day	86.6 (55)	103.3 (67.2)[a]	89.2 (52.6)[a,b]	81.6 (47.0)[b]	76.1 (49.6)[b]	82.9 (53.1)[b]	**<0.001**
Meat, g/day	172.6 (90.4)	207.4 (107.9)[a]	161.2 (82.8)[b]	172.5 (87.5)[b]	155.8 (75.2)[b]	166.0 (86.6)[b]	**<0.001**
Processed meat, g/day	7.0 (6.9)	8.7 (8.5)[a]	7.1 (7.1)[a,b,c]	6.6 (6.3)[b,c]	6.7 (6.3)[a,b,c]	6.1 (5.4)[c]	**0.001**
Dairy products, g/day	406.6 (253.9)	448.4 (275.8)[a]	404.9 (254.3)[a,b]	382.4 (231.5)[a,b]	357.7 (193.6)[b]	439.9 (293.3)[a]	0.269
Refined grains, g/day	113.2 (69.4)	139.8 (85.8)[a]	115.6 (70.1)[b]	107.2 (59.1)[b]	105.7 (62.9)[b]	97.6 (58.7)[b]	**<0.001**
Wholegrains, g/day	18.5 (32.2)	19.2 (33.6)	18.9 (32.0)	15.1 (25.9)	19.7 (34.1)	19.7 (34.8)	0.814
Breakfast cereals, g/day	14.6 (19.7)	17.8 (29.1)[a]	15.3 (18.8)[a]	13.4 (15.2)[a]	14.6 (16.9)[a]	12.1 (14.7)[a]	**0.008**
Legumes, g/day	60.6 (44.2)	72.0 (64.7)[a]	59.6 (37.8)[a,b]	58.4 (36.2)[b]	57.2 (37.3)[b]	55.6 (36.4)[b]	**0.001**
Vegetables, g/day	205.3 (150.6)	246.4 (199.8)[a]	200.9 (137.9)[b]	194.9 (134.7)[b]	190.4 (129.7)[b]	193.5 (133.1)[b]	**0.001**
Fruits, g/day	334.7 (250.7)	430.6 (290.0)[a]	338.6 (216.2)[b]	310.3 (243.1)[b]	292.4 (208.5)[b]	301.1 (263.3)[b]	**<0.001**
Nuts, g/day	11.3 (14.5)	16.1 (19.7)[a]	10.8 (13.7)[b]	9.4 (10.6)[b]	9.7 (11.7)[b]	10.6 (14.1)[b]	**<0.001**
Olive oil, g/day	16.6 (14.5)	17.6 (13.6)	17.2 (17.2)	16.9 (13.6)	15.3 (12.6)	16.1 (15.0)	0.141
Cocoa-based products, g/day	7.4 (7.6)	2.6 (2.5)[a]	3.9 (3.5)[a]	5.7 (4.1)[b]	8.0 (5.3)[c]	16.6 (10.3)[d]	**<0.001**
Sugar-sweetened beverages, g/day	54.8 (95.1)	73.2 (123.2)[a]	52.4 (96.5)[a,b]	50.6 (94.5)[a,b]	55.3 (81.3)[a,b]	42.3 (69.4)[b]	**0.007**
Pastry products, g/day	69.1 (52.5)	74.2 (54.6)	67.2 (53.9)	68.4 (53.5)	66.5 (46.6)	73.0 (53.2)	0.561

Data are expressed as mean (SD).

Q, quintiles of flavonoids from cocoa-based products; n, number; SD, standard deviation.

Statistical analyses were conducted using one-way ANOVA for continuous variables and the chi-square test for categorical variables.

[a,b,c,d]Data sharing the different letters are statistically different after Bonferroni post-hoc test. P-for trend were obtained using orthogonal contrasts test. $P < 0.05$ are considered statistically significant.

Significant differences are bolded.

g/day), and other polyphenol-rich food intakes like fruits (continuous, g/day), vegetables (continuous, g/day), legumes (continuous, g/day), nuts (continuous, g/day), and extra olive oil (continuous, g/day). Municipalities (Barcelona/Madrid) and schools were included as random effects. Gender interaction was considered to evaluate potential effect modification in the association between flavonoids from cocoa-based products and adiposity parameters. Orthogonal polynomial contrast was used to determine linear trends.

In addition, a multilevel mixed-effects linear regression analysis was conducted to explore associations between flavonoids of each cocoa-based product (cocoa powder, dark chocolate, and milk chocolate) and adiposity parameters, all of them as continuous variables. For this analysis, data from participants who reported daily intake of at least one cocoa-based product were considered (700 participants reported cocoa powder intake, 294 reported dark chocolate intake, and 644 reported milk chocolate intake). The model included the same fixed and random effects variables as described earlier. Moreover, Pearson correlation coefficients were used to explore the relationship between individual flavonoids from cocoa-based products and adiposity parameters. Finally, the false discovery rate (FDR) by the Benjamini-Hochberg procedure was applied to adjust *p*-values for multiple correlations (34). Before these analyses, values of flavonoids were normalized and scaled in 1-SD with the inverse normal transformation (35).

All statistical analyses were conducted using Stata statistical software package version 16.0 (StataCorp., College Station, TX, USA) and R 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria). Statistical tests were two-sided and statistical significance was set as 0.05.

RESULTS

General Characteristics of the Study Participants

The characteristics of the cohort stratified by quintiles of flavonoid intake from cocoa-based products are shown in **Table 1**. Based on BMI z-score, 18% of adolescents presented overweight and 9% obesity. Regarding the abdominal obesity parameters, 16% of participants had a WC greater than the 90th percentile, and 23% had a high WHtR ($\geq$0.5 threshold). Finally, regarding the excess of adiposity, 8 and 16% of adolescents had %FM and FMI equal to or greater than the 75th percentile, respectively. Compared to the lowest quintile, participants in the highest quintile tended to have slightly lower BMI, WC, and WC *z*-score, although the differences were not significant in the univariate analysis.

The mean cocoa-based product intake was 7.4 (7.6) g/d, equivalent to one tablespoon of cocoa powder or one square piece of a chocolate bar. More than 90% of the participants reported daily intake of at least one cocoa-based product, from them, 75% ($N = 700$) reported intake of cocoa powder, 31% ($N = 294$) dark chocolate, and 68% ($N = 644$) milk chocolate. The mean flavonoid intake from cocoa-based products was 57.4 (74.5) mg/day, where 26.6 (35.3) mg/day were from cocoa powder, 24.0 (62.9 mg/day) from dark chocolate, and 6.7 (11.3) mg/day from milk chocolate (Data not shown). Participants with a

TABLE 3 | Nutrients and (poly)phenols intake of participants according to quintiles of flavonoids from cocoa-based products (mg/day).

	Overall (*n* = 944)	Q1 (<12.1) (*n* = 189)	Q2 (12.1–32.0) (*n* = 189)	Q3 (32.1–53.2) (*n* = 189)	Q4 (53.3–83.8) (*n* = 189)	Q5 (>83.8) (*n* = 188)	*P-for trend*
Nutrients intake							
Energy, Kcal/day	2,539.2 (601.8)	3,013.4 (435.9)[a]	2,510.9 (490.9)[b]	2,402.6 (562.7)[b,c]	2,307.3 (614.7)[c]	2,461.3 (622.6)[b,c]	**<0.001**
Carbohydrates, g/day	256.5 (72.2)	303.2 (65.8)[a]	255.8 (65.7)[b]	240.6 (64.3)[b]	236.3 (71.3)[b]	246.7 (73.3)[b]	**<0.001**
Fiber, g/day	29.4 (10.7)	35.5 (11.4)[a]	29.1 (8.5)[b]	27.6 (9.9)[b]	27.2 (10.3)[b]	27.6 (10.7)[b]	**<0.001**
Proteins, g/day	120.9 (33.3)	144.2 (30.1)[a]	118.9 (27.8)[b]	115.9 (30.8)[b,c]	108.3 (30.3)[c]	117.5 (35.5)[b]	**<0.001**
SFA, g/day	36.8 (11.5)	44.2 (11.3)[a]	35.8 (9.5)[b]	34.6 (10.6)[b,c]	32.7 (10.8)[c]	36.8 (11.7)[b]	**<0.001**
MUFA, g/day	48.5 (16.2)	57.0 (14.2)[a]	48.1 (15.9)[b]	46.4 (15.3)[b]	43.8 (15.8)[b]	47.2 (16.5)[b]	**<0.001**
PUFA, g/day	19.7 (6.8)	23.7 (6.4)[a]	19.4 (5.7)[b]	18.7 (6.2)[b]	18.2 (7.3)[b]	18.5 (6.9)[b]	**<0.001**
Calcium, mg/day	1,012.9 (391.0)	1,198.1 (403.5)[a]	1,001.6 (354.7)[b,c]	959.7 (362.2)[b,c]	896.9 (321.2)[b]	1,008.1 (440.3)[c]	**<0.001**
Vitamin A, μg/day	1,476.0 (1,465.2)	1,849.9 (1,719.5)[a]	1,542.8 (1,759.5)[a,b]	1,342.6 (1,107.7)[b]	1,177.2 (754.9)[b]	1,467.6 (1,637.2)[a,b]	**0.001**
Vitamin D, μg/day	5.1 (2.6)	6.1 (3.0)[a]	5.2 (2.4)[b]	4.8 (2.4)[b]	4.6 (2.5)[b]	4.7 (2.4)[b]	**<0.001**
(Polyp)phenols intake							
Flavonoids, mg/day	530.1 (331.3)	482.8 (314.0)[a]	440.1 (283.9)[a]	460.6 (263.3)[a]	490.4 (241.1)[a]	777.8 (406.1)[b]	**<0.001**
Phenolic acids, mg/day	97.8 (64.5)	117.2 (70.8)[a]	103.5 (74.4)[b]	89.6 (53.8)[b]	89.4 (60.4)[b]	89.3 (56.5)[b]	**<0.001**
Stilbenes, mg/day	0.2 (0.3)	0.2 (0.3)	0.2 (0.3)	0.2 (0.3)	0.2 (0.3)	0.2 (0.4)	0.677
Lignans, mg/day	3.8 (5.1)	5.4 (6.1)[a]	3.2 (3.7)[b]	3.5 (5.3)[b]	3.7 (5.7)[b]	3.4 (4.1)[b]	**0.002**
Other, mg/day	51.3 (34.4)	59.5 (44.0)[a]	55.1 (34.7)[a,b]	46.5 (29.7)[b]	48.9 (30.8)[b]	46.5 (28.6)[b]	**<0.001**

Data are expressed as mean (SD).
Q, quintiles of flavonoids from cocoa-based products; n, number; SD, standard deviation; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Statistical analyses were conducted using one-way ANOVA for continuous variables and the chi-square test for categorical variables.
[a,b,c]Data sharing the different letters are statistically different after Bonferroni post-hoc test. P-for trend were obtained using orthogonal contrasts test. P < 0.05 are considered statistically significant.
Significant differences are bolded.

TABLE 4 | Association between flavonoid intake from cocoa-based products (mg/day) and anthropometric measurements.

Anthropometric variables	Q1 (<12.1)	Q2 (12.1–32.0) (ß, 95% CI)	Q3 (32.1–53.2) (ß, 95% CI)	Q4 (53.3–83.8) (ß, 95% CI)	Q5 (>83.8) (ß, 95% CI)	*P-for trend*
BMI *z*-score	Reference	0.25 (0.10; 0.40)	0.29 (0.17; 0.41)	0.06 (−0.04; 0.15)	−0.07 (−0.25; 0.10)	**<0.001**
WC *z*-score	Reference	0.09 (−0.01; 0.19)	0.23 (0.17; 0.29)	0.06 (−0.13; 0.24)	−0.04 (−0.07; −0.01)	**0.045**
WHtR	Reference	−0.00 (−0.01; 0.01)	0.01 (0.01; 0.01)	−0.00 (−0.00; −0.00)	−0.01 (−0.02; −0.01)	**<0.001**
%FM	Reference	0.72 (0.51; 0.94)	2.17 (1.95; 2.38)	0.09 (−0.14; 0.32)	−1.15 (−3.36; 1.05)	0.160
FMI *z*-score	Reference	0.41 (0.18; 0.64)	0.52 (0.39; 0.66)	0.21 (0.02; 0.40)	0.02 (−0.53; 0.56)	0.242

Q, quintiles of flavonoids from cocoa-based products (mg/day); ß, (beta) regression coefficient; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; %FM, percentage of fat mass; FMI, fat mass index.
Statistical analyses were conducted using multilevel mixed-effect linear regression analysis. The fixed effects were gender, age, Tanner maturation stage, physical activity, parental education, intake of energy, breakfast cereals, pastries, sugar-sweetened beverages, meat, processed meat, fruits, vegetables, legumes, nuts, and extra olive oil. Municipalities and schools were included as random effects. P-for trend were obtained using orthogonal contrasts test across quintiles. P < 0.05 are considered statistically significant.
Significant differences are bolded.

higher intake of flavonoids from cocoa-based products tended to consume lower fish, meat, processed meat, refined grains, (poly)phenol-rich foods (legumes, vegetables, fruits, and nuts), and sugar-sweetened beverages (**Table 2**). In addition, compared to the lowest quintile, participants in the highest quintile of flavonoids from cocoa-based products had a lower energy intake and macro and micronutrients (**Table 3**), except for vitamin A. A higher intake of total dietary flavonoids was observed in the highest quintile, but lower values of phenolic acids, lignans, and other (poly)phenol intake were observed in the same group (**Table 3**).

Association of Dietary Flavonoids From Cocoa-Based Products With Adiposity Parameters

The results from the multivariate-adjusted linear regression analyses showed that a higher intake of flavonoids from cocoa-based products was associated with lower values of BMI z-score (*P-for trend* < 0.001); however, no significant difference was observed between the highest and lowest quintiles. Moreover, participants with highest intake of flavonoids from cocoa-based products had lower values of WC z-score [B = −0.04, 95% CI (−0.07; −0.01), *P-for trend* = 0.045] and WHtR [B = −0.01,

TABLE 5 | Association between flavonoids from cocoa powder, dark chocolate, and milk chocolate (mg/day) and anthropometric measurements.

Anthropometric measurements	Cocoa powder *N* = 700 (ß, 95% CI)	*P*	Dark chocolate *N* = 294 (ß, 95% CI)	*P*	Milk chocolate *N* = 644 (ß, 95% CI)	*P*
BMI *z*-score	−0.08 (−0.12; −0.05)	**<0.001**	−0.11 (−0.23; 0.12)	0.076	−0.02 (−0.11; 0.06)	0.593
WC *z*-score	−0.06 (−0.11; −0.02)	**0.003**	−0.06 (−0.08; −0.05)	**<0.001**	−0.01 (−0.02; 0.01)	0.403
WHtR	−0.01 (−0.01; −0.00)	**<0.001**	−0.003 (−0.01; 0.00)	0.110	−0.001 (−0.00; 0.00)	0.646
%FM	−1.11 (−1.48; −0.75)	**<0.001**	−0.42 (−1.63; 0.79)	0.494	0.112 (−0.49; 0.72)	0.718
FMI *z*-score	−0.18 (−0.20; −0.17)	**<0.001**	−0.16 (−0.36; 0.06)	0.147	−0.01 (−0.13; 0.11)	0.822

N, number of participants who reported cocoa powder, dark chocolate, or milk chocolate intake; ß, regression coefficient; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; %FM, body fat percentage; FMI, fat mass index.
Statistical analyses were conducted using multilevel mixed-effect linear regression analysis. The fixed effects were gender, age, Tanner maturation stage, physical activity, parental education, intake of energy, breakfast cereals, pastries, sugar-sweetened beverages, meat, processed meat, fruits, vegetables, legumes, nuts, and extra olive oil. Municipalities and schools were included as random effects. Data from flavonoids were normalized with the inverse normal distribution before this analysis. P < 0.05 are statistically significant.
Significant differences are bolded.

TABLE 6 | Association between flavonoids from cocoa-based products (mg/day) and adiposity parameters.

Adiposity parameters	Q1 (<12.1)	Q2 (12.1–32.0) (OR, 95% CI)	Q3 (32.1–53.2) (OR, 95% CI)	Q4 (53.3–83.8) (OR, 95% CI)	Q5 (>83.8) (OR, 95% CI)	*P-for trend*
BMI ≥ 85th to <95th percentile	1	1.17 (1.11; 1.22)	0.94 (0.86; 1.03)	0.93 (0.51; 1.71)	0.52 (0.20; 1.35)	**0.023**
BMI ≥ 95th percentile	1	1.11 (0.76; 1.61)	2.86 (2.40; 3.41)	1.41 (1.22; 1.62)	0.96 (0.63; 1.45)	0.908
WC ≥ 90th percentile	1	0.52 (0.34; 0.80)	1.48 (1.16; 1.87)	0.83 (0.61; 1.14)	0.79 (0.68; 0.93)	0.520
WHtR ≥ 0.5	1	0.97 (0.54; 1.74)	1.14 (0.91; 1.42)	0.96 (0.68; 1.36)	0.60 (0.33; 1.09)	**<0.001**
%FM ≥ 85th percentile	1	0.79 (0.69; 0.90)	1.95 (1.45; 2.62)	1.02 (0.53; 1.98)	1.02 (0.53; 1.98)	0.736
FMI ≥ 75th percentile	1	0.75 (0.49; 1.14)	1.59 (1.42; 1.77)	0.92 (0.74; 1.16)	0.56 (0.14; 2.22)	0.859

Q, quintiles of flavonoids from cocoa-based products; OR, odds ratio; CI, confidence interval; BMI, body mass index; WC, waist circumference; WHtR, waist-to-height ratio; %FM, body fat percentage; FMI, fat mass index.
Statistical analyses were conducted using multilevel mixed-effect logistic regression model. The fixed effects were gender, age, Tanner maturation stage, physical activity, parental education, intake of energy, breakfast cereals, pastries, sugar-sweetened beverages, meat, processed meat, fruits, vegetables, legumes, nuts, and extra olive oil. Municipalities and schools were included as random effects. P-for trend were obtained using orthogonal contrasts test. P < 0.05 are considered statistically significant.
Significant differences are bolded.

95% CI (−0.02; −0.01), *P-for trend* < 0.001] (**Table 4**). However, quartiles 2 and 3 had higher values of BMI *z*-score, %FM, and FMI *z*-score compared to quartile 1. No interaction with gender was found in the regression analysis.

Table 5 shows the association of flavonoids from each cocoa-based product and anthropometric parameters. Inverse associations were observed between flavonoids from cocoa powder and BMI *z*-score [B = −0.08, 95% CI (−0.12; −0.05), $P < 0.001$], WC *z*-score [B = −0.06, 95% CI [−0.11; −0.02], $P = 0.003$], WHtR [B = −0.01, 95% CI (−0.01; −0.00), $P < 0.001$], %FM [B = −1.11, 95% CI (−1.48; −0.75), $P < 0.001$], and FMI *z*-score [B = −0.18, 95% CI (−0.20; −0.17), $P < 0.001$]. Regarding dark chocolate, an inverse association only with WC *z*-score [B = −0.06, 95% CI (−0.08; −0.05), $P < 0.001$] was found. However, no association was observed between flavonoids from milk chocolate intake and anthropometric parameters.

Multivariate-adjusted logistic regression analyses revealed a tendency of having less probability of having overweight (BMI at or above the 85th percentile to less than the 95th percentile) and high WHtR (≥0.5 thresholds) in participants with higher flavonoid intake from cocoa-based products (**Table 6**). In addition, participants in the highest quintile had less probability of having abdominal obesity [OR 0.66, 95% CI (0.52; 0.85), *P-for trend* = 0.001] compared to the lowest quintile (**Figure 2**). However, participants in quintiles 4 and 3 had a higher probability of obesity (BMI at or above 95th percentile) compared to quintile 1.

Finally, in the correlation analysis between individual flavonoids from cocoa-based products and adiposity parameters, weak inverse correlations between WHtR and catechins (R = −0.08, FDR value = 0.027), epicatechins (R = −0.09, FDR value = 0.014), and proanthocyanidins (R = −0.08, FDR value = 0.021) were observed (Data not shown).

DISCUSSION

In the present study, a higher intake of flavonoids from cocoa-based products was inversely associated with individual adiposity parameters and abdominal obesity in adolescents. To our knowledge, this is one of the first studies to explore these associations in this target population.

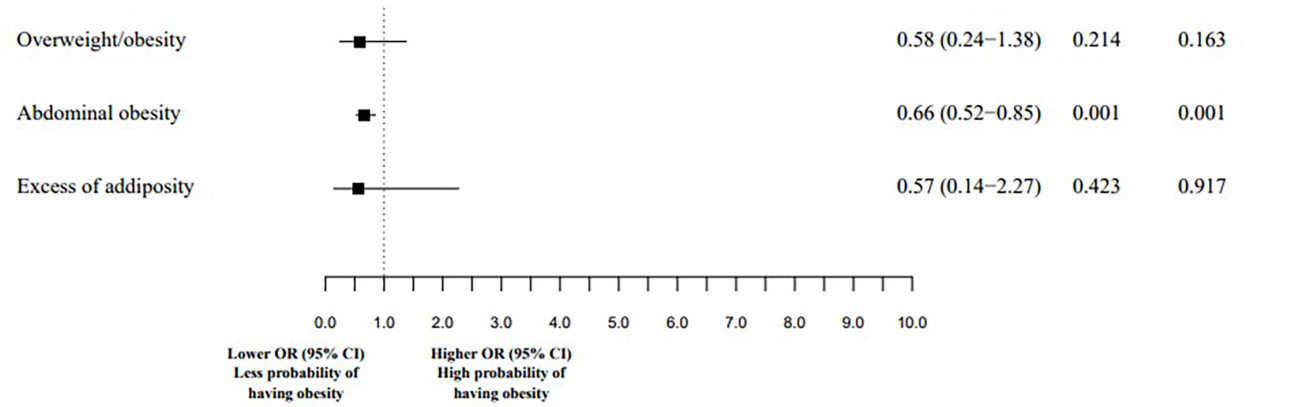

FIGURE 2 | Association between the highest and the lowest quintiles of flavonoids from cocoa-based products (mg/day) intake and obesity. Overweight/obesity was defined by body mass index percentile equal to or above the age- and gender-specific 85th percentile. Abdominal obesity was defined by WC at or above the 90th percentile and/or WHtR equal to or above the 0.5 threshold. Excess of adiposity was defined by %FM and or FMI greater than or equal to the age- and gender-specific 75th percentile. Q, quintiles of flavonoids from cocoa-based products; OR, odds ratio; CI, confidence interval. Statistical analyses were conducted using multilevel mixed-effect logistic regression model. The fixed effects were gender, age, Tanner maturation stage, physical activity, parental education, intake of energy, breakfast cereals, pastries, sugar-sweetened beverages, meat, processed meat, fruits, vegetables, legumes, nuts, and extra olive oil. Municipalities and schools were included as random effects. *P*-values between Q5 vs. Q1 and *P- for trend* were obtained using orthogonal contrasts test. $P < 0.05$ are considered statistically significant.

Cocoa Flavonoid Intake in Adolescents

Cocoa-based products are an important dietary source of flavonoids. In our study, adolescents consumed a mean of 57.4 mg/day of flavonoids from cocoa-based products, representing 11% of the total dietary flavonoid intake (mean 530.1 mg/day). A lower intake of flavonoids from cocoa-based products was reported by Bawaked et al. in Spanish children aged 6 to 11 years, who consumed 10.9 mg/day of flavonoids from cocoa powder and chocolate, which provided 23.5% of the total flavonoid intake (mean 70.7 mg/day) (36). In the Healthy Lifestyle in Europe by Nutrition in Adolescence study, chocolate products were once the major source of dietary (poly)phenols and flavonoids in European adolescents (37).

Flavonoids From Cocoa-Based Products and Adiposity Parameters

The inverse association of flavonoids from cocoa-based products with adiposity parameters is in accordance with previous studies, although most of them were conducted in adults. The fact that the results remained consistent when using different adiposity parameters (BMI *z*-score, WC *z*-score, WHtR, %FM, and FMI *z*-score) further strengthens the study findings. In addition, in our cross-sectional, multivariate-adjusted model, clinical relevance was observed between extremes of quintiles of flavonoids from cocoa-based products (Q5 vs. Q1) and less probability of having abdominal obesity. These results were independent of physical activity, puberty development, parental education, intake of energy, sweetened products, meat, and processed meat, as well as other (poly)phenol-rich foods intake such as fruits, vegetables, legumes, nuts, and extra olive oil. Fruits and vegetables represent the main food source of flavonoids in the diet of adolescents and their consumption could influence the association of flavonoids from cocoa-based products with adiposity parameters (36, 38–40). According to the results of a cross-sectional study of European adolescents, consumption of energy-dense foods is associated with a higher probability of obesity (41). In our study, a tendency to consume less energy-dense and sugary foods was observed in participants with a higher intake of flavonoids from cocoa-based products.

In agreement with our findings, Cuenca-García et al. recently reported that higher chocolate consumption was associated with lower BMI, body fat, and WC in European adolescents (40). Similar results were obtained in a large cross-sectional analysis in non-diabetic US adults, where the BMI and WC of individuals who reported chocolate intake were lower by 0.92 Kg/m^2 and 2.07 cm, respectively, compared to the non-reporters, a difference that could be attributed to the intake of cocoa flavonoids (42). However, the authors did not define the type of chocolate being consumed (for example, dark or milk), and its flavonoid content was not calculated. In our study, inverse associations between flavonoids from cocoa powder and BMI *z*-score, WC *z*-score, WHtR, %FM, and FMI *z*-score were observed, but WC *z*-score was inversely associated only with dark chocolate. No association between anthropometric parameters and milk chocolate was found. These results could be attributed to the highest concentration of flavonoids in cocoa powder compared to dark or milk chocolate (17, 18, 43). In addition to this, it could be also explained by the fact that cocoa powder was consumed by most participants (75%), with fewer consuming milk (68%) and dark chocolates (31%).

Evidence between cocoa flavonoid consumption and adiposity in adults is conflicting. The results of a meta-analysis based on randomized clinical trials suggested that the consumption of at least 30 g/day of cocoa/dark chocolate for 4–8 weeks decreases BMI in adults (12). Similarly, weight reduction in overweight/obese adults (BMI $\geq$ 25 kg/m^2) was related to the consumption of flavanol-containing products such as tea, cocoa, and apple in a subgroup meta-analysis (13). In contrast, a meta-analysis of short-term trials did not find significant associations between flavonoid intake from cocoa-based products and BMI in adults, although this could have been due to the short-term nature of the studies (44). Longer-term randomized controlled clinical trials are needed to examine the magnitude of the effect of flavonoids from cocoa-based products on adiposity parameters. Instead, the limitations of BMI as an adiposity indicator are well-known, because it does not provide information on adiposity distribution, and therefore additional anthropometric measurements are required, such as WC, WHtR, %FM, and FMI (24, 45).

Cocoa-based products also contain other bioactive compounds like theobromine, a methylxanthine highly associated with body weight, lipid, and glucose metabolism (46). In our study, theobromine was not quantified so their possible association with adiposity parameters has been not determined.

The effect of cocoa-based product intake on body fat and obesity could be explained by an associated reduction in plasma adipokine (leptin and adiponectin) concentrations, although the mechanisms involved still need to be clarified (8, 47). Leptin and adiponectin are hormones mainly secreted by adipose tissue and delivered into the systemic circulation to modify glucose and lipid metabolism, insulin sensitivity, and cardiovascular function (48). Leptin promotes fatty acid oxidation and reduces lipogenesis by regulating peripheral metabolic pathways in skeletal muscle, adipose tissue, the liver, and the pancreas (49). Meanwhile, plasma adiponectin improves insulin sensitivity, activates muscle utilization of glucose, induces muscle and hepatic fatty acid oxidation, and reduces hepatic glucose production (48). In the present study, adipokine levels were not analyzed and their possible relationship with the intake of flavonoids from cocoa-based products was not determined.

The relationship between the consumption of cocoa-based products and adiposity parameters has been attributed to their flavanol (flavan-3-ol) content. Flavanols from cocoa include mainly monomers and polymers of catechin and epicatechin (7, 9, 10, 43). In our exploratory analysis, we observed a negative correlation between catechins, epicatechins, proanthocyanidins (polymers of flavanols), and WHtR. Catechins and epicatechins, both flavanols monomers, are rapidly absorbed from the upper portion of the small intestine and could influence metabolic pathways related to body weight (50, 51). Gutiérrez-Salmeán et al. suggested that epicatechin decreases the expression of proteins associated with mitochondrial function and increases the expression of protein-induced thermogenesis (51). Instead, although proanthocyanidins are the most abundant (poly)phenols in cocoa-based products, they are poorly absorbed in the small intestine due to their large number of hydrophilic hydroxyl groups (9, 50). Most proanthocyanidins reach the colon and are transformed by the gut microbiota into phenylvalerolactones and phenolic acids, such as hydroxyphenylpropionic acid, hydroxyphenylacetic acid, and benzoic acid (43, 50, 52, 53). These microbial metabolites might be responsible in part for health beneficial effects of proanthocyanidins and could be implicated in adipogenesis and lipogenesis mechanisms (6). Results from a cross-sectional study, based on 2,734 women twins aged 18–83 years, revealed that women with a higher dietary intake of proanthocyanidins-rich foods, which included apples and cocoa drinks, had lower fat mass and central fat mass, both measured by dual-energy-X-ray-absorptiometry (54). In another way, according to the results shown by Lee et al., 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone, a microbial flavanols metabolite, reduces lipid accumulation in 3T3-L1 mature adipocytes regulating free fatty acids metabolism through the suppression of the expression of lipogenic proteins (6). However, evidence for the effect of flavanols from cocoa-based products and their microbial metabolites on adipogenesis and lipogenesis metabolic pathways is yet inconclusive and further studies are needed to better understand the mechanisms of action implicated in weight maintenance.

Although cocoa-based products are an important source of flavonoids that might contribute to the improvement of adiposity parameters, their consumption should be promoted with caution, considering that most commercial formulations are high in calories, sugars, and fats (7). Thus, from a public health perspective, cocoa-based products low in fats and sugars might be recommended.

Limitations and Strengths

A limitation of the present study is its cross-sectional design, which precludes causal assumptions about flavonoid intake from cocoa-based products and differences in adiposity parameters. In addition, data derived from food frequency questionnaires are prone to bias because misreporting is common in dietary self-assessment in adolescents (55). Misreporting in adolescents is associated with several factors, specifically weight status, weight loss or weight maintenance, body image dissatisfaction, and skipping breakfast (56, 57). Adolescents with high values of BMI tend to report a lower consumption of food rich in energy, fats, and sugars, like cocoa-based products. Misreporting may reflect socially desirable answers where adolescents with self-image dissatisfaction are more likely to under-report the consumption of high fat/high sugar foods. Another plausible reason could be that under-eating is the result of a dietary regimen to lose or maintain weight, so there could be a control in the intake of cocoa-based products. Instead, adolescents with normal weight status could real over-eating to reflect higher intakes due to a growth spurt. Regarding the dietary flavonoids assessment, although our validated food frequency questionnaire specifies portion size, measurement error will be present with any assessment of the flavonoid content of cocoa-based commercial products because they depend on the manufacturing process like alkalinization treatment (58). Furthermore, a limitation of using a food frequency questionnaire is that there is no possible

way to determine the exact content of flavonoids from specific cocoa-based products since the percentage of cacao varies for each commercial product. In addition to this, flavonoid intake was estimated through a database, which may not reflect the true concentration of compounds reaching the target organs after digestion, absorption, and metabolism. Therefore, the association between flavonoids from cocoa-based products and adiposity parameters might be distorted by the dietary data bias, so these results should be interpreted with caution. Further longitudinal analyses will be necessary to clarify the true direction of these associations.

Strengths of the present study include the large sample size ($n = 944$) of well-characterized participants, the standardization of measures performed in the SI! Program for Secondary Schools trial, and the inclusion of a range of anthropometric variables, not only BMI, to evaluate adiposity.

In conclusion, a higher intake of flavonoids from cocoa-based products was associated with lower adiposity parameters and less probability of abdominal obesity. These findings are relevant for hypothesis generation regarding mechanisms underlying potential therapeutic effects of cocoa flavonoids against obesity and should stimulate further prospective studies and clinical trials to determine the health beneficial effects of cocoa flavonoids on adolescents.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because there are restrictions on the availability of the data for the SI! Program study, due to signed consent agreements around data sharing, which only allow access to external researcher for studies following project purposes. Requestor wishing to access the database used in this study can make a request to the Steering Committee (SC) chair. For the present study, the database was requested from the SC on 24 February 2022. Requests to access the datasets should be directed to gsantos@fundacionshe.org, rodrigo.fernandez@cnic.es, juanmiguel.fernandez@cnic.es, restruch@clinic.cat, lamuela@ub.edu, bibanez@cnic.es, and vfuster@cnic.es.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Instituto de Salud Carlos III in Madrid (CEI PI 35_2016), the Fundació Unió Catalana d'Hospitals (CEI 16/41), and the University of Barcelona (IRB00003099). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RL-R: conceptualization. EL-S, AT-R, and RL-R: methodology. EL-S and CA-R: formal analysis. EL-S, CA-R, and RL-R: investigation. AC-G: data curation. EL-S, CA-R, AT-R, and RL-R: writing—original draft preparation. AT-R, RF-J, JF-A, GS-B, MM, PB, AC-G, CR, AR-L, SC-B, RC, RE, and RL-R: writing—review and editing. EL-S: visualization. AT-R and RL-R: supervision. RF-J, JF-A, GS-B, MM, PB, AC-G, JM-G, AT-R, RE, and RL-R: funding acquisition. All authors have read and agreed to the published version of the manuscript.

FUNDING

The SI! Program for Secondary Schools trial was supported by the SHE Foundation, the la Caixa Foundation (LCF/PR/CE16/10700001), the Fundació la Marató de TV3 (grant number 369/C/2016), and by the funding from Idilia Foods (FBG 311240). Support was also provided by the Ministerio de Ciencia, Innovación y Universidades (PID2020-114022RB-I00), CIBEROBN from the Instituto de Salud Carlos III, ISCIII from the Ministerio de Ciencia, Innovación y Universidades (AEI/FEDER, UE), and Generalitat de Catalunya. JM-G was a postgraduate fellow of the Ministerio de Ciencia e Innovación of Spain at the Residencia de Estudiantes (2020–ongoing). RF-J was a recipient of grant PI19/01704 funded by the Fondo de Investigación Sanitaria- Instituto de Salud Carlos III (ISCIII) and co-funded by the European Regional Development Fund/European Social Fund a way to make Europe/Investing in your future. The CNIC was supported by the ISCIII, the Ministerio de Ciencia e Innovación (MCIN), the Pro CNIC Foundation, and was a Severo Ochoa Center of Excellence (CEX2020-001041-S). GS-B was the recipient of grant LCF/PR/MS19/12220001 funded by la Caixa Foundation (ID 100010434). AT-R is a Serra Húnter fellow. EL-S was a FI-SDUR (EMC/3345/2020) fellowship from the Generalitat de Catalunya.

ACKNOWLEDGMENTS

The authors wish to thank all the volunteers and their families, teachers, and schools for their contribution to the SI! Program for Secondary Schools.

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Conflict of Interest: RL-R reports receiving lecture fees from Cerveceros de España and receiving lecture fees and travel support from Adventia and Idilia Foods SL. RE reports grants from Fundación Dieta Mediterránea, Spain, Cerveza y Salud, Spain, personal fees for given lectures from Brewers of Europe, Belgium, Fundación Cerveza y Salud, Spain, Pernaud-Ricard, Mexico, Instituto Cervantes, Alburquerque, USA, Instituto Cervantes, Milan, Italy, Instituto Cervantes, Tokyo, Japan, Lilly Laboratories, Spain, Wine and Culinary International Forum, Spain, non-financial support to organize a National Congress on Nutrition, and also feeding trials with products from Grand Fountain and Uriach Laboratories, Spain.

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.

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frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 22 July 2022
DOI 10.3389/fnut.2022.933557

OPEN ACCESS

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

RECEIVED 01 May 2022
ACCEPTED 28 June 2022
PUBLISHED 22 July 2022

CITATION
Elmugabil A, Alhabrdi NM, Rayis DA, Al-Wutayd O and Adam I (2022) Evaluation of the association between haemoglobin levels and preterm birth at Khartoum, Sudan: A hospital-based study.
Front. Nutr. 9:933557.
doi: 10.3389/fnut.2022.933557

Evaluation of the association between haemoglobin levels and preterm birth at Khartoum, Sudan: A hospital-based study

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Objective: The objective of this study was to determine the association between haemoglobin level and PB.

Methods: A cross-sectional study was conducted in Khartoum, Sudan. Questionnaires on demographics and medical and obstetric factors were completed. A logistic regression analysis was performed.

Results: Of the 1,716 pregnant women, approximately two-thirds (65.7%) had anaemia (haemoglobin < 11 g/dl) and six (0.3%) had severe anaemia (haemoglobin < 8 g/dl). Of the 1,716 women, 283 (16.5%) had a PB. In multivariable logistic regression, parity (AOR = 1.15, 95% CI = 1.09–1.21, $P < 0.001$) was positively associated with PB. Compared to those with haemoglobin levels of 10–10.9 g/dl, pregnant women with haemoglobin levels of 8–8.9 (AOR = 0.41, 95% CI = 0.22–0.77), 9–9.9 (AOR = 0.59, 95% CI = 0.38–0.91), and 11–11.9 g/dl (AOR = 0.53, 95% CI = 0.36–0.77) were at a lower risk of PB. Women with haemoglobin levels of 12–13 g/dl were at a higher risk of PB (AOR = 1.62, 95% CI = 1.06–2.45). There was no significant association between women with haemoglobin levels < 8 g/dl and > 13 g/dl and PB.

Conclusion: This study showed different levels of association between haemoglobin levels and PB.

KEYWORDS

haemoglobin, preterm, birth, Sudan, anaemia

Introduction

Preterm birth (PB) refers to the birth of a baby before 37 completed weeks of gestation or 259 days from the final day of the last menstrual period (1). PB is a worldwide health problem that affected 14.84 million births in 2014 (2). More than three-quarters (81.1%) of all PBs occur in Africa and South Asia (2). PB is a major

cause of perinatal death and a significant cause of long-term consequences among the survivors (3). PB is the main direct cause of neonatal death and is associated with 50–75% of all neonatal mortality and half of all neonatal morbidity worldwide (4). PB also presents an economic burden, due to the requirement of neonatal intensive care units, and is associated with socioeconomic disadvantages and disruptive life events during pregnancy (5). Recent studies showed a high prevalence of PB in sub-Saharan African countries (6, 7). Several factors such as age, short interpregnancy interval (6, 8), being a rural resident, inadequate antenatal care (9), previous PB, multiple pregnancies, and malaria (6) are significantly associated with PB. It is of paramount importance that the factors associated with PB (especially haemoglobin) in different settings are well documented, if the goal of the WHO and United Nations 2010 of reducing mortality due to PB by 50% before 2025 is to be achieved. Risk factors for PB should be correctly identified and properly managed to reduce the incidence of PB. Maternal nutrition may impact both haemoglobin synthesis and foetal growth, development, survival, and PB (10, 11). Previous studies on the association between the haemoglobin level and PB showed inconsistent results. While some found that anaemia was associated with PB (8, 12), others showed that a high haemoglobin level carries an increased risk of PB (13, 14). Although the prevalence of PB is high in many African countries (6, 7), pertinent information on the association between haemoglobin level and PB and spontaneous PB is not adequately documented in sub-Saharan Africa, including Sudan. This study was conducted to investigate the association between the haemoglobin level and spontaneous PB in Khartoum, Sudan.

Materials and methods

A cross-sectional study was conducted at Saad Abuelela Maternity Hospital in Khartoum, Sudan, from February to November 2020. The inclusion criteria were pregnant women with a single live baby. The exclusion criteria were post-term birth ($\geq$ 42 weeks of gestation), unknown gestational age, women with unknown body mass index (BMI) in early pregnancy, seriously ill women, multiple births, stillbirths, and congenital malformed deliveries. After signing an informed consent form, trained medical residents conducted face-to-face interviews with the pregnant women included in the study. Questionnaires on demographics and medical and obstetric factors were filled out in the local language (Arabic). The questionnaires recorded information concerning the age, parity, education, residence, occupation, antenatal care status, history of previous miscarriages/PBs, gestational age, interpregnancy interval, haemoglobin level, and infant's sex. Gestational age was calculated using a combination of the dates of the last menstrual period and early pregnancy ultrasound. PB refers to the birth of the baby before 37 completed weeks of gestation. Additional information was extracted from the clinical notes on pregnancy complications, such as data on hypertension, preeclampsia, or diabetes (defined as gestational or chronic). Early pregnancy ($<$ 14 weeks) weight and height were used to calculate BMI as weight in kilograms divided by the squared height in metres. The WHO classification was used to group the women, according to their BMIs, like normal weight (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), or obese (30–34.9 kg/m^2) (15). Following this, 2 mL of blood was withdrawn from every participant (before delivery) in an ethylenediaminetetraacetic acid and analysed for a complete blood count including haemoglobin, using an automated haematology analyser and following the manufacturer's instructions (Sysmex KX-21, Japan).

Sample size

The sample size was calculated considering the assumed prevalence of spontaneous PB of 13% (the ratio was 6.6:1) among all the deliveries, guided by the recent reports from Ethiopia (16). Assuming a type I error of 5% and adequate power of 80% (β = 0.2), based on the results of our previous meta-analysis (17), we assumed that 40% of the women who had a PB and 30% of women who had no PB would have anaemia, which resulted in a sample size of 1,716, considering that 10% of the women might not respond or might have incomplete data. The sample size was calculated using the OpenEpi Menu (18).

Statistics

Data were entered into a computer, and SPSS for Windows was used for data analysis. Continuous data were checked for normality using the Shapiro–Wilk test. Descriptive statistical [mean (standard deviation), median (interquartile range), frequency, and percentage] were used to present the characteristics of the participants. The Mann-Whitney test was used to compare the median (interquartile range) between the two groups. A logistic regression analysis was performed with spontaneous PB as the dependent factor. The covariates (independent factors) were the sociodemographic, medical, and obstetric factors, age of women, parity, education, residence, occupation, antenatal care status, history of previous miscarriages/PB, gestational age, interpregnancy interval, BMI, haemoglobin level (before delivery), and sex of the infant as independent factors. Variables with a p-value of <0.2 were entered into the multivariable logistic regression model using the backward stepwise method (likelihood ratio). The crude odds ratio (COR), adjusted odds ratio (AOR), and 95% CI were computed to show the strength of the association. A two-sided p-value of $<$ 0.05 was considered statistically significant.

Results

A total of 1,716 parturient women were enrolled in the study. The median (interquartile range) of their age, parity, IPI, BMI, and haemoglobin was 27 (23–32) years, 2 (1–4), 23 (15–23) months, 26.1 (23.3–27.5) kg/m^2, and 10.5 (9.8–11.4) g/dl, respectively. Of the 1,716 women, 939 (54.7%) were educated up to at least secondary level (8 years), 1,584 (92.3%) attended at least two antenatal visits, and 255 (14.9%) were obese. The details of the sociodemographic characteristics are shown in Table 1.

Of the 1,716 parturient women, 21 (1.2%) were underweight, 759 (44.2%) were of normal weight, 681 (39.7%) were overweight, and 255 (14.9%) were obese.

Approximately two-thirds (65.7%) of the women had anaemia and six (0.3%) had severe anaemia. Parturient women were classified into seven groups according to haemoglobin levels, as follows: < 8, 8–8.9, 9–9.9, 10–10.9, 11–11.9, 12–13, and > 13 g/dl. Their numbers and proportions are shown in Table 2.

Of the 1,716 parturient women, 283 (16.5%, 95% CI = 14.7–18.2) had a PB. Compared to women who had a term birth, women who had a PB had significantly higher age, higher parity, more history of miscarriage/PB, and were more likely to be obese (see Table 2). The median (IQR) of the haemoglobin level was significantly (Mann-Whitney test) higher in women with PTB

TABLE 1 Frequency and proportion of pregnant women in Khartoum, Sudan, 2020.

Variables	Frequency (n = 1,716)	Proportion (100)
Education level		
≥ Secondary	939	54.7
< Secondary	777	45.3
Antenatal care		
≥ Two visits	1,584	92.3
<Two visits	132	7.7
Occupation		
Housewife	1,509	87.9
Employed	207	12.1
History of miscarriage/preterm birth		
Yes	389	22.7
No	1,327	77.3
Body mass index		
Underweight	21	1.2
Normal weight	759	44.2
Overweight	681	39.7
Obese	255	14.9
Anaemia		
Yes	1,128	65.7
No	5,888	34.3
Gender		
Men	860	50.1
Women	856	49.9

TABLE 2 Comparing sociodemographic and clinical variables between mothers with preterm birth and mothers with term birth in Khartoum, Sudan, 2020.

Variables	Preterm birth (283)	Term birth (1,433)	OR (95%CI)	P
Age, years	29.0 (24.0–34.0)	27.0 (22.0–32.0)	1.04 (1.02–1.06)	< 0.001
Parity	3.0 (1.0–5.0)	2.0 (1.0–4.0)	1.13 (1.08–1.19)	< 0.001
Interpregnancy interval, months	21.0 (15.0–37.0)	23.0 (15.0–36.0)	1.0 (0.99–1.01)	0.459
Miscarriage/preterm birth				
Yes	82 (29.0)	307 (21.4)	1.49 (1.12–1.99)	0.007
No	201 (71.0)	1,126 (78.6)	Reference	
Occupation				
Housewife	249 (88.0)	1,260 (87.9)	Reference	
Employee	34 (12.0)	173 (12.1)	1.01 (0.67–1.48)	1.000
Antenatal care				
≥Two visits	295 (91.5)	1,325 (92.5)	Reference	
<Two visits	24 (8.5)	108 (7.5)	1.13 (0.71–1.80)	0.625
Body mass index				
Underweight	3 (1.3)	18 (1.3)	0.61 (0.43–0.88)	0.008
Normal weight	117 (41.3)	642 (44.8)	Reference	
Overweight	105 (37.1)	576 (40.2)	0.65 (0.16–1.98)	0.375
Obese	58 (20.5)	197 (13.7)	0.61 (0.43–0.88)	0.009
Maternal diseases				
Yes	36 (12.7)	111 (7.7)	1.73 (1.16–2.58)	0.010
No	247 (87.3)	1,322 (92.3)	Reference	
Gender				
Women	717 (50.0)	139 (49.1)	Reference	
Men	716 (50.0)	144 (50.9)	0.96 (0.74–1.24)	0.778
Hemoglobin level, g/dl				
< 8.0	1 (0.4)	24 (2.7)	0.16 (0.22–1.23)	0.079
8.0–8.99	14 (4.9)	123 (8.6)	0.44 (0.24–0.83)	0.011
9.0–9.99	44 (15.5)	278 (19.4)	0.62 (0.40–0.95)	0.029
10.0–10.99	81 (28.6)	563 (39.3)	Reference	
11.0–11.99	61 (21.6)	240 (16.7)	0.56 (0.39–0.82)	0.002
12.0–13.0	60 (21.2)	147 (10.3)	1.60 (1.1–2.42)	0.024
> 13.0	22 (7.8)	58 (4.0)	1.49 (0.84–2.62)	0.165

[11.3 (10–12) g/dl vs. 10.3 (9.7–11.2), $P < 0.001$]. Moreover, compared to women who had a term birth, women who had a PB are less likely to be anaemic [140/283 (49.5%) vs. 988/1,433 (68.9%), $P < 0.001$]. In multivariable logistic regression, parity (AOR = 1.15, 95% CI = 1.09–1.21, $P < 0.001$) was positively associated with PB. Anaemia was associated with reduced OR of PB (AOR = 0.4, 95% CI = 0.32–0.54, as seen in Table 3).

Compared to those with haemoglobin levels of 10–10.9 g/dl, parturient women with haemoglobin levels of 8–8.9 (AOR = 0.41, 95% CI = 0.22–0.77, $P = 0.006$), 9–9.9 (AOR = 0.59, 95% CI = 0.38–0.91, $P = 0.019$), and 11–11.9 g/dl (AOR = 0.53, 95% CI = 0.36–0.77, $P = 0.002$) were at lower risk of PB. Parturient women with haemoglobin levels of 12–13 g/dl were at a higher risk of PB (AOR = 1.62, 95% CI = 1.06–2.45, $P = 0.23$). There was no significant association between women with low haemoglobin levels < 8 g/dl and women with haemoglobin levels > 13.g/dl and PB (see Table 3 and Figure 1).

TABLE 3 Logistic regressions of sociodemographic and clinical variables associated with preterm birth in Khartoum, Sudan, 2020.

Variables	Adjusted	
	AOR (95% CI)	*P*
Age, years	0.99 (0.971.03)	0.958
Parity	1.15 (1.09–1.21)	<0.001
Hemoglobin level, g/dl*	1.39 (1.26–1.53)	<0.001
Miscarriage/preterm birth		
Yes	1.33 (0.99–1.80)	0.056
No	Reference	
Maternal disease		
Yes	0.66 (0.44–1.01)	0.056
No		
Body mass index		
Underweight	0.85 (0.239–3.073)	0.813
Normal weight	Reference	
Overweight	0.880 (0.649–1.193)	0.409
Obese	1.133 (0.753–1.706)	0.548
Hemoglobin level, g/dl*		
< 8.0	0.13 (0.01–1.03)	0.053
8.0–8.99	0.41 (0.22–0.77)	0.006
9.0–9.99	0.59 (0.38–0.91	0.019
10.0–10.99	Reference	
11.0–11.99	0.53 (0.36–0.77)	0.001
12.0–13.0	1.62 (1.06–2.45)	0.023
>13.0	1.48 (0.83–2.63)	0.175
Anaemia*		
Yes	0.41 (0.32–0.54)	<0.001
No	Reference	

*Were entered one by one in the model, AOR, adjusted odds ratio; CI, confidence interval.

Discussion

The prevalence (16.5%) of spontaneous PB in our study was found to be comparable to the prevalence of PB in other African countries, e.g., 16.9% in Ethiopia (16), 18.3% in Kenya (7), and 16.8% in Nigeria (19). However, a prevalence of 16.5% was outside the range (9.5–15.8%) reported by WHO for sub-Saharan Africa (20). The differences in the prevalence of PB could be explained by the differences in the methods (definition, inclusion criteria, and exclusion criteria) and risk factors as well as differences in social and other factors. Moreover, it was a hospital-based study that might not reflect the nature of the overall community. This might offer a plausible explanation for the high prevalence of PB in this study, especially since there is a high rate of home deliveries in Sudan (21).

In the current study, age, history of miscarriage/PB, education, ANC, BMI, duration, and IPI were not associated with PB. Similar findings were reported in other studies, which showed that IPI was not associated with PB (7). Our findings contrast with several studies showing that rural residence, short interpregnancy interval, presence of chronic illness (6), maternal age (8, 19), education, failure to attend antenatal care clinic, previous abortion (22), and previous PB were associated with PB (7).

Our results showed that, compared to those with haemoglobin levels (at the time of the delivery) of 10–10.99 g/dl, women with haemoglobin levels of 8–8.99 (AOR = 0.414), 9–9.99 (AOR = 0.59), and 11–11.99 (AOR = 0.53) g/dl were at lower risk of PB. Women with haemoglobin levels of 12–13 g/dl were at a higher risk of PB (AOR = 1.62). Previous studies refuted any association between high haemoglobin levels and PB (23, 24). In China, a high haemoglobin level in the second trimester has been shown to carry an increased risk of PB (13). Zhou et al. reported a slightly increased risk of PB associated with high haemoglobin levels (14). In Turkey, incidences of PB were significantly higher for both high and low haemoglobin levels (25). Moreover, it has been found that both low and high haemoglobin concentrations tend to be associated with an increased risk of PB, in a U-shaped pattern (26). Similarly, previous studies reported that women with high haemoglobin levels had higher risks of PB (27, 28). Interestingly, previous studies reported a U-shaped curve for the risk of PB against maternal haemoglobin concentrations (29, 30). During pregnancy, haemoglobin levels decrease due to an increase (expansion) in plasma volume. This results in a reduction in blood viscosity, which can interfere with the proper placental perfusion (31). Thus, a high haemoglobin level during pregnancy could lead to placental infarcts, poor functionality, and PB.

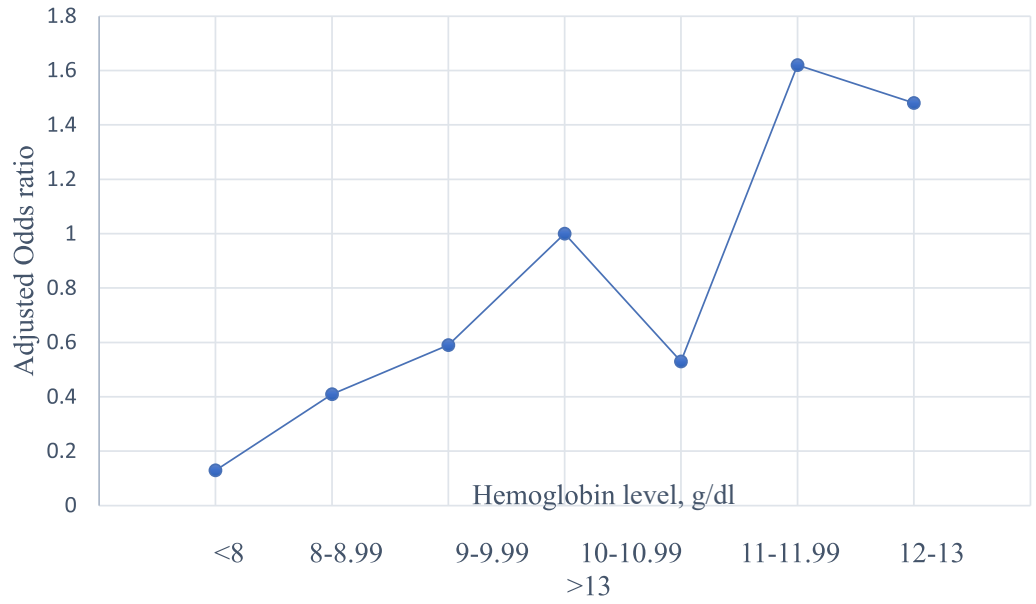

FIGURE 1
The adjusted odds ratio of haemoglobin level and preterm birth in Khartoum, Sudan 2020.

In Kenya (7), anaemia was not found to be associated with PB. We previously showed that the risk of PB increases significantly with anaemia (especially the severe form) (32). In a recent (2020) meta-analysis of 58 studies including a total of 134,801 women, anaemic women were found to be at higher risk of PB (8). A previous (2019) meta-analysis (of 117 studies including a total of 4,127,430 pregnancies), revealed that maternal anaemia was significantly associated with PB (12). Several mechanisms may explain the increased risk of PB in anaemia. Hypoxia and an increase in the level of norepinephrine concentrations can lead to maternal and foetal stress. This may stimulate the secretion of the corticotrophin-releasing hormone. Moreover, anaemia may also increase the risk of maternal infection, which is a known predisposing factor for PB (33). Amino acids, which can be influenced by maternal protein and quality of protein intake, play a significant role in the placenta's function, foetus growth, and development. Low maternal protein intake can be important in reducing haemoglobin synthesis and in causing placental insufficiency. High protein intake may cause an excess of haemoglobin synthesis, leading to a relative excess of haemoglobin concentration. Moreover, plasma ammonia toxicity is potentially responsible for intrauterine growth restriction and excessive production of the metabolites of amino acids, hindering the development of the foetus (34). Also, there is accumulating evidence for changes in the maternal microbiota that might be associated with PB (35).

The study had some limitations: While there may be differential recall bias in women, haemoglobin in the first and second trimesters was not checked, serum ferritin and inflammatory biomarkers were not investigated, and several other factors and haemoglobinopathies/thalassaemia were not assessed in our cohort. These factors [e.g., malaria (6), HIV (19, 22)] and alcohol consumption (16) have been reported to be associated with PB. The prevalence of HIV and malaria is low in Khartoum. Smoking and alcohol consumption are not common in women in Sudan. Due to the sensitivity of such topics, cooperation from women may have been reduced or lost if they were asked about smoking or consuming alcohol.

Conclusion

Haemoglobin levels have different effects on the risk of PB; while some haemoglobin levels were associated with a lower risk of PB; other haemoglobin levels were associated with an increased risk or were not associated with PB. A large longitudinal study assessing other factors (especially inflammatory factors) is required.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee at the Department of Obstetrics and Gynaecology, Faculty of Medicine, University of Khartoum, Sudan (reference number: 2019/09). All procedures performed in the study were in accordance with the ethical standards of the institutional and/or National Research Committee and with the 1964 Helsinki declaration and its later amendments. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AE and NA: conceptualisation and writing—original draft, review, and editing. AE and DR: data curation. IA: formal analysis. AE: investigation. IA and OA-W: methodology. DR: project administration. All authors have read and agreed upon the published version of the manuscript.

Acknowledgments

We thank the Deanship of Scientific Research, Qassim University for funding the publication of this project.

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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frontiers | Frontiers in Nutrition

TYPE Perspective
PUBLISHED 30 September 2022
DOI 10.3389/fnut.2022.936929

OPEN ACCESS

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

RECEIVED 05 May 2022
ACCEPTED 16 September 2022
PUBLISHED 30 September 2022

CITATION
Garrabou G, García-García FJ, Presmanes RE, Feu M and Chiva-Blanch G (2022) Relevance of sex-differenced analyses in bioenergetics and nutritional studies. *Front. Nutr.* 9:936929. doi: 10.3389/fnut.2022.936929

Relevance of sex-differenced analyses in bioenergetics and nutritional studies

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Sex-biased analyses still remain as one of the biggest limitations to obtain universal conclusions. In biomedicine, the majority of experimental analyses and a significant amount of patient-derived cohort studies exclusively included males. In nutritional and molecular medicine, sex-influence is also frequently underrated, even considering maternal-inherited organelles such as mitochondria. We herein illustrate with in-house original data examples of how sex influences mitochondrial homeostasis, review these topics and highlight the consequences of biasing scientific analyses excluding females as differentiated entities from males.

KEYWORDS

mitochondria, sex bias, nutrition, metabolism, endosymbiosis

Introduction

Role of women in history and society

The organization of mutual aid, care, empathy, and cooperation is the order of a social network that became strong and resilient for millennia of prehistory, where women sought the wellbeing of group members (1). In fact, the existence of pre-patriarchal matrilineal societies in prehistory, from the Paleolithic period of 6,500 Before the Common Era (BCE) to 3,000 BCE, is well-established by many studies in archeology. These original matrilineal societies were organized around motherhood and the offspring. Additionally, bone remains with deformities have been found in these societies, confirming that the disabled and sick were not abandoned or eliminated. These cultures were peaceful, woman-centered based on reciprocity rather than asymmetry, and in communities of up to 15,000 members (2). In this context, the construction of relations among the community members, such as symbiosis, is crucial.

Despite this lack of historic trail, materno-linearity is known as the social architecture in ancient civilizations and, still today, ranks as the preferred structure in some societies. Usually, these societies rely on cooperative networks and equitable social roles, in all life

aspects including medicine. In the rest of societies, for the last 5 millennia, the paradigm of a predominantly male perspective invaded most aspects of life, including medicine and research.

The aim of this perspective is to highlight the relevance of understanding sex-specific conditions in health and disease, specifically in bioenergetics and nutritional matters, and the adverse consequences of biasing scientific and medical conclusions underscoring women's needs. In this sense, and according to the World Health Organization (WHO), sex refers to the biologically determined characteristics of females and males, whereas gender refers to the social construction as a learned behavior or identity (3). Given the biological nature of the concepts herein treated, the present work will be focused on the sex bias in relation to the study cohorts included traditionally in clinical and biomedical research, and thus human beings will be referred as males or females in this perspective.

The endosymbiotic theory

Cooperation and networking are the aspects that have led us to move forward successfully as a species. Both aspects were highlighted, at social level, by materno-linial cultural structures as mentioned in the introduction, and at biological level, by the endosymbiotic theory postulated by Lynn Margulis (4). This theory explains the relevance of symbiotic unions in understanding the origins and the evolution of living things. The endosymbiotic theory postulates that current eucaryotic cells and other forms of life evolve together by symbiogenesis, in mutual cooperative benefit terms. Endosymbiotic theory is in contraposition to some aspects of the classical competitive Darwinian evolution, based on the survival of the fittest, and the competition to gather resources and leave genes and traits into the next generation offspring, which are currently being questioned (5, 6).

At a cellular level, symbiogenesis would explain the incorporation of plasts or mitochondria, and even a symbiogenetic origin of flagella and cilia has been suggested (undulipodia), despite there is no evidence so far (7). In the case of mitochondria, endosymbiosis is the most accepted theory to explain how we acquire mitochondrial organelles, responsible for food metabolism and energy supply. For the mitochondria, this theory postulates that the ancestral eukaryotic cell that forms our bodies engulfed, millions of years ago, an ancient proteobacterium to obtain the ability to metabolize nutrients through aerobic metabolism (thus, consuming oxygen) while providing the bacteria with food and environmental protection. Both cooperated to live together from that moment to mutually benefit from obtaining energy through the consumption of nutrients and oxygen (4).

Interestingly, mitochondria are transmitted exclusively through the maternal lineage, because during fetal conception and egg fertilization, sperm only provides half of the genetic material of the nucleus of the former embryo, while the female oocyte provides the other half of nuclear genes and all the rest of embryo components, thus including embryo mitochondria. Remarkably, mitochondria are the unique organelle of our cells that have their own genetic material that is, therefore, maternally inherited. Thus, natural selection on mitochondria operates only in females. Consequently, most of our genetic material (half the nuclear and all the mitochondrial genome) is transmitted through the maternal lineage.

Notably, the crosstalk between the nuclear and the mitochondrial genome is crucial for the cellular regulation of mtDNA integrity, copy number and, overall, mitochondrial homeostasis. Among others, because the nucleus encodes for 1,500 proteins of mitochondrial location, including those responsible of mtDNA replication and maintenance. The intergenomic communication is an additional example of bilateral cooperation regulated by many actors, including nutrients and sex-hormones (8).

Mitochondria as an endosymbiotic evolution: A metabolic perspective

Despite these maternal-related mitochondrial genetic and organelle transmissions involved in nutrition and health, little interest is given in understanding sex's role in mitochondrial or nutritional pathophysiology or, in general, in females' specific biomedical needs. Accumulated knowledge stands for sex-dependent metabolism of nutrients and mitochondrial bioenergetic regulation (9–15). We herein present, as a proof of concept, novel data confirming differential sex-mitochondrial performance, in this case, related to mitochondrial DNA content (mtDNA; Figure 1). Mitochondrial DNA is present in multiple copies per mitochondria (10, 16) and, since there are thousands of mitochondria per cell, mitochondrial genome content per cell can vary from thousands to millions of copies per cell, and varies depending on the tissue considered (13). Higher mtDNA levels have been associated with more active mitochondrial function and, usually, are present in those tissues that mostly rely on oxidative metabolism, which also show a higher number of mitochondrial genomes. Conversely, low mtDNA content has been associated with disease and is usually measured for research and diagnosis of mitochondrial pathologies and associated disorders. Interestingly, their levels have rarely been associated with sex condition. We herein present original data suggesting that mtDNA levels may depend on sex assignment and that, at least in our cohort, mtDNA levels are significantly lower in the skeletal muscle of studied females (Figure 1A). When we stratify mtDNA content according to patients' age (Figure 1B), we observe that the significant differences observed in the mtDNA content between males and females, are apparently associated with the age of menopause onset (established in 52 years old) (17). This age-dependent mtDNA decline in females, although

not statistically significant in this small cohort ($p = 0.08$), may be part of the metabolic reprogramming associated with physiologic aging, frailty, and disease (18).

As previously mentioned, levels of mtDNA content have been related to mitochondrial activity and disease (16). Consequently, the relevance of finding fewer mtDNA genomes in skeletal muscle of studied females, if confirmed in larger cohorts, might contribute to the understanding of differential sexual behavior in cell bioenergetics, nutrient consumption, and, eventually, health and disease. Similarly, mtDNA decrease in females according to menopause onset might confirm hormonal regulation of mitochondria or, the same way around, a potential mitochondrial contribution in menopause regulation. In this line, estrogens have been demonstrated to regulate mitochondrial function through direct and indirect mechanisms (19). In fact, they are synthetized from cholesterol in the mitochondria, together with the other steroid hormones (progestins, glucocorticoids, mineralocorticoids and androgens) (20). Consequently, estrogens are not the unique hormones related to mitochondrial function and metabolism. In fact, most of these hormones and others (such as catecholamines) have been shown to participate in a complex feedback conditioning most of the mitochondrial functions (21), related to health and disease (22).

Globally, these findings suggest differential metabolic sex and aging reprogramming of mitochondria. However, in this analysis other clinical aspects such as BMI or body fat and lifestyle choices have not been considered, and might potentially influence our results. In consequence, further studies should deepen on this topic, considering all potential confounders, as one example of how sex influence may underlie physiologic and physiopathological responses. In this setting, sex differences are frequently ignored in pursuit of simplification and understanding. In accordance with the predominant male perspective, females are usually excluded to meet this aim, leading to biased interpretation of derived conclusions.

Despite past and current literature still exhibits a clear sex bias in clinical and experimental research, there is a growing body of evidence that sexual dimorphism and gender disparity regulate mitochondrial function, metabolism, and, consequently, the response to diet and nutrition. For instance, it has been established that muscle fiber type distribution (greater for type I fibers in females), substrate availability or consumption (higher for lipids and lower for carbohydrates and amino acids in females), as well as ROS production or ADP and oxygen-sensibility (lower in females), are different between sexes (23–28), thus conditioning mitochondrial function and metabolism in physiologic conditions or exercise (glucose turnover, glycogen use, lipid sources, AMPK signaling, lipid droplets metabolism and metabolic gene expression among others) (28, 29). Additionally, the regulation of such metabolic and mitochondrial functions also differs among sexes. For instance, higher levels of circulating adipokines (as adiponectin and leptin) in females, or the presence of 17-β estradiol receptors in muscle (the most important female sex hormone), provide evidence of differential sexual regulation (23), that could vary depending on the menstrual cycle phase (28). Interestingly, such differential sexual metabolic performance in physiologic conditions can constrain the response to disease, for instance in metabolic complications including type 2 diabetes mellitus (30), steatosis or even hepatic failure (31–33), but also in response to exercise (34) or aging (35).

Herein presented data of lower mtDNA content in skeletal muscle of human females, would reinforce the idea of sexual dimorphism in bioenergetic and metabolic interplay, specially studied during exercise (27–29, 33, 36–42), encompassing a wide spectrum of physiologic adaptations, such as those concerning gene expression (38). Moreover, apparent mtDNA decline in females associated with the age of menopause onset may also strengthen estrogen regulation of mitochondrial performance (27–29, 36, 39). Notwithstanding, mtDNA is the unique genome entirely dedicated to metabolic and bioenergetic performance and estrogens, the master regulator of female metabolism and the bioenergetic system (43), thus being the potential base of further adaptations.

Although we have still a long path to explore, these examples provide evidence that we won't understand the basis of health and disease unless sex influence is considered.

Sex bias in medicine research

In theory, biomedicine and medical research are aimed at understanding health and disease processes, developing new drug therapies, overall, with the objective to reduce the burden of diseases, improve health, and increase lifespan with a minimum quality of life for the overall population. However, in (bio)medicine, most experimental designs (from both cellular and animal models) and a significant amount of patient-derived cohort studies, exclusively included males [reviewed in (44–46)], which account for about only half of the worldwide population (47). This male bias will explain the poor knowledge in the biology and physiology of females. Consequently, several guidelines still do not distinguish differences in the manifestation and treatment of diseases between males and females.

The arguments used to exclude females in its experimental design are diverse and include both objective and subjective approaches. As objective approaches, for instance, the systematic exclusion of females at fertile age to "prevent" them from being submitted to a potentially harmful intervention, or the higher prevalence of a disease in males in a certain range of age. The main subjective approach is the assumption that what is observed in males can be extrapolated to females. The latter two approaches can be easily refuted with the example, for instance, of cardiovascular disease (CVD). CVD is more prevalent in middle-aged male subjects, although overall, CVD kills more females than males, being the first cause of death in

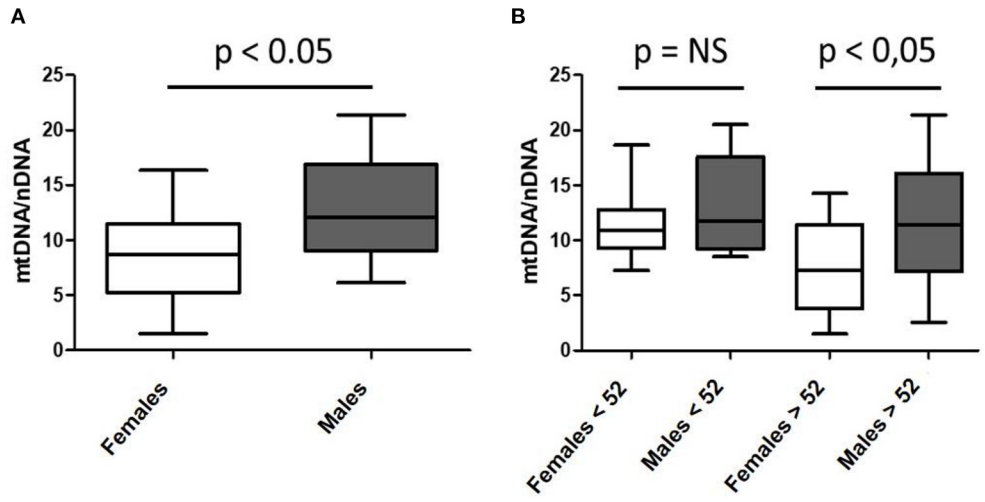

FIGURE 1
Sex-differential mitochondrial DNA (mtDNA) content in skeletal muscle of a cohort of healthy subjects according to mean age of menopause onset. **(A)** $n = 53$; 34 females and 19 males; mean age 55.94 ± 19.50 and 57.68 ± 15.06 years; **(B)** pre- and post-menopausal females ($n = 13$ and $n = 21$, respectively) and age-paired males ($n = 8$ and $n = 11$, respectively).

females, at least in Europe (48). Moreover, the physiopathology of CVD differs between males and females. Stage 1 diastolic hypertension has been associated with double the risk of an acute coronary syndrome (ACS) in females compared with males (49), and the symptoms of ACS largely differs between sexes, being more often than desirable not identified as ACS symptoms in females (50). Again, this responds to the assumption that the symptoms in females are the same than in males. In addition, the disparities in the prevention, diagnosis and treatment of ACS have been recently brought to light (51), always in detriment of females' health.

Moreover, gender issues such as lifestyle, including nutrition and stress, education and psychological aspects, might influence the outcome of several pathologies. For instance, the prevalence of coronary disease in females is higher and present worst prognosis due to the double exposure to stress from work and family (52). Besides CVD, females have increased risk of experiencing adverse and more severe drugs reactions compared to males (53). A potential explanation of such phenomena might be that female liver cells have increased cytochrome P450 (54), which is the complex responsible of the metabolism of about half of the drugs, thus reducing potential drug's therapeutic efficacy.

In addition, among several others, simplification is another argument used to exclude females from biomedical studies, as half the sample size is then eventually required. In this setting, the hormonal argument (the variability introduced by the menstrual cycle) has been repeatedly used to justify the exclusion of females in (bio)medicine studies. However, it has been largely shown that interindividual variability is usually higher in males (55). Interestingly, males are also subjected to the effects of hormones that vary according to daily and monthly rhythms and, longitudinally, along with their lives (56), and this has not hampered their inclusion in clinical studies. Fortunately, new policies and initiatives such as the Sex As a Biological Variable (SABV) (57), or the GenderMedDB (58), are increasingly being taken into consideration, and within the last years, there is a growing body of research considering both sexes and/or sex differences both in preclinical and clinical studies, which will be further discussed.

Sex bias in nutritional studies

In nutritional and molecular medicine, sex influence is also frequently underrated, even considering maternal-inherited organelles such as mitochondria. The mitochondrial respiratory chain is the final step for nutrient metabolism and energy production, but sex-differences in nutrient metabolism are largely unexplored, even considering that mitochondrial activity and body fat distribution and percentage largely differs between sexes (59), and that the nutritional requirements differ between males and females (60). Additionally, nutritional requirements within females also differ during pregnancy or after menopause compared to the rest of the adult life. Several current studies which consider the complexity of nutritional and mitochondrial metabolic pathways are unable to consider sex influence on the derived conclusions. Moreover, it has been recently outlined that the micronutrient requirements differ between sexes and across the lifespan (61), although current guidelines do not distinguish between sexes, only having special guidelines for childhood (of both sexes) and pregnancy. Interestingly, a very recent review (62) shows that human breast milk contains lower carbohydrates, lipids and energy for female-term compared to male-term infants, again, showing different sex-associated nutritional requirements even in newborns.

A recent study has analyzed the sex specific effects of a diet-induced obesity, and significant sex-differences in energy expenditure in response to a high-fat diet were found (63). This might have clinical and epidemiological implications, as (severe) obesity is more prevalent in females (64), although a large body of evidence relies on studies performed on males. Moreover, a recent study observed that in children and adolescents with type 1 diabetes, a higher dietary percentage of lipids was more associated with higher levels of LDL cholesterol in girls than in boys (15).

As previously stated, the hormonal argument is recurrent to exclude females in experimental models or cohort studies. This has been the case of the study of the effects of polyphenols, in which, unfortunately, some authors of this perspective have been contributors to this bias in the past (65, 66). Polyphenols are bioactive compounds found in plants with antioxidant and anti-inflammatory activities (67). Given its estrogenic activity (68), it has been argued that the effects of polyphenols may be dependent on the hormonal fluctuations in females, and this may hinder the execution of studies and the interpretation of the results. However, this argument should not be further used, because the effects of nutrients, bioactive compounds, or even dietary patterns might be sexually dimorphic independently of the reproductive hormonal fluctuations in females. A recent study has reported that a dietary pattern rich in energy-dense foods at the age of 4 is associated with a higher body mass index, a higher percentage of body fat, and insulin resistance at the age of 10 in girls, whereas this association was not found in boys (14).

Finally, it is worth mentioning that adherence to dietary patterns is different between males and females (69), which might be also taken into consideration in nutritional epidemiology.

Conclusion

Cooperation, synergy, and unbiased analysis should be the three pillars in which science should approach any social and health challenge, and sex-biased analyses remains as one of the biggest challenges and limitations to obtaining universal conclusions.

Sex bias in social and historic topics, as well as in medicine and research, can no longer be justified in any circumstance. Females are not a minority, they encompass, at least, half of the human beings. If any bias may be relevant leading to underrating and mistakes, ignoring sex differences in health can cost lives due to the postulation of wrong clinical and therapeutic interventions relying exclusively on man-based studies, thus threatening woman's health and life expectancy.

In this era of OMICs analysis, big data and supercomputational studies, where covariates and confounding factors are included in any calculation, modeling, and conclusion, we can no longer admit studies where sex-based differences are not considered.

In this brief perspective, we have discussed and exposed the sex bias historically inherent to (bio)medical research and nutrition. Considering the genotypic, phenotypic and metabolic biological differences between females and males, and of course, excluding sex-specific research (i.e., pregnancy), sex-biased science should not be acceptable anymore. Therefore, experimental design, at the cellular, animal, and human levels, should include a balanced sex sample, and sex differences might be analyzed and reported for the sake of the overall population.

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 Ethics Committee of the Hospital Clínic de Barcelona. The patients/participants provided their written informed consent to participate in this study.

Author contributions

GG, FG-G, and GC-B wrote the first draft of the manuscript. All authors contributed to the conception and design of the manuscript, edited the manuscript, suggested improvements at several stages during the preparation, and revised the manuscript.

Funding

We are grateful for the financial support from the Nutricia Research Foundation and the Spanish Society of Atherosclerosis (a2022-25, and OBN21PE02/2021, respectively, to GC-B). This work was also supported by the Instituto de Salud Carlos III (ISCIII) (PI1800451, PI1800498, and PI2100935 to GG), the Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER) (ER20P2AC722 to GG) all initiatives of ISCIII and FEDER ('Unamanera de hacer Europa'), as well as Fundació Privada Cellex.

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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frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 18 November 2022
DOI 10.3389/fnut.2022.950900

OPEN ACCESS

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

RECEIVED 23 May 2022
ACCEPTED 13 September 2022
PUBLISHED 18 November 2022

CITATION
Hernando-Redondo J, Toloba A, Benaiges D, Salas-Salvadó J, Martínez-Gonzalez MA, Corella D, Estruch R, Tinahones FJ, Ros E, Goday A, Castañer O and Fitó M (2022) Mid- and long-term changes in satiety-related hormones, lipid and glucose metabolism, and inflammation after a Mediterranean diet intervention with the goal of losing weight: A randomized, clinical trial. *Front. Nutr.* 9:950900. doi: 10.3389/fnut.2022.950900

Mid- and long-term changes in satiety-related hormones, lipid and glucose metabolism, and inflammation after a Mediterranean diet intervention with the goal of losing weight: A randomized, clinical trial

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Background: Obesity is produced by the enlargement of the adipose tissue. Functioning as an endocrine organ, it releases and receives information through a complex network of cytokines, hormones, and substrates contributing to a low-chronic inflammation environment. Diet and healthy habits play key roles in the prevention of obesity and its related pathologies. In this regard, there is a need to switch to healthier and more appetizing diets, such as the Mediterranean one.

Objective: To compare the mid-and long-term effects of two Mediterranean diet (MedDiet) interventions, one energy-reduced plus physical activity promotion versus a non-restrictive diet, on peripheral satiety-related hormones, weight loss, glucose/lipid metabolism, and pro-inflammatory markers in subjects with obesity/overweight and metabolic syndrome.

Materials and methods: A randomized, lifestyle intervention was conducted in 23 Spanish centers, with a large cohort of patients presenting metabolic syndrome. Our study is a subproject set in IMIM (Hospital del Mar Research

Institute). Participants were men and women, aged 55–75 and 60–75, respectively, who at baseline met at least three metabolic syndrome components. Subjects were assigned to two intervention groups: (1) an intensive lifestyle intervention with an energy-reduced MedDiet and physical activity promotion (intervention group) with the aim of weight loss; and (2) a normocaloric MedDiet (control). We quantified in a subsample of 300 volunteers from Hospital del Mar Research Institute (Barcelona), following analytes at baseline, 6 months, and 1 year: glucose, HbA1c, triglycerides, total cholesterol, high-density lipoprotein cholesterol, LDL cholesterol, C-peptide, ghrelin, GLP-1, glucagon, insulin, leptin, PAI-1, resistin, and visfatin. Anthropometric and classical cardiovascular risk factors were also determined. A multivariate statistical model was employed to compare the two groups. Linear mixed-effect models were performed to compare changes in risk factors and biomarkers between intervention groups and over time.

Results: Compared to participants in the control group, those in intervention one showed greater improvements in weight, waist circumference, insulin ($P < 0.001$), glucose metabolism-related compounds ($P < 0.05$), triglyceride-related lipid profile ($P < 0.05$), leptin, blood pressure, and pro-inflammatory markers such as PAI-1 ($P < 0.001$) at mid-and/or long-term. High-sensitivity C-reactive protein, resistin, and vifastin also decreased in both groups.

Conclusion: A weight loss intervention employing a hypocaloric MedDiet and physical activity promotion has beneficial effects on adiposity, glucose metabolism, lipid profile, leptin, and pro-inflammatory markers, such as PAI-1 in both mid-and long-term.

KEYWORDS

metabolic syndrome, Mediterranean diet (MedDiet), leptin, PAI-1, inflammation

Introduction

Over the past 40 years, obesity has come to be considered an emerging global pandemic. Described by the World Federation of Obesity "as a chronic relapsing disease process," it has proven influence on the development of hypertension, diabetes mellitus, and cardiovascular events (1). Current nutrition habits, which include the excessive consumption of sweetened beverages and high-density energy food, have notably increased the prevalence of overweight and obesity in both child and adult populations. Moreover, western society has embraced sedentary routines which further contribute to an augmented positive energy balance, thus worsening insulin resistance and perpetuating unhealthy behavioral patterns (2, 3).

Abbreviations: MedDiet, Mediterranean diet; HbA1c, glycated hemoglobin; HDL cholesterol, high-density lipoprotein cholesterol; LDL cholesterol, low-density lipoprotein cholesterol; hs-CRP, highly sensitivity C-reactive protein; K2-EDTA, dipotassium ethylenediaminetetraacetic acid; GLP-1, glucagon-like peptide-1; PAI-1, plasminogen activator inhibitor-1; CV, coefficient of variation.

Metabolic syndrome, characterized by high cardiovascular risk due to prediabetes/diabetes, hypertension, dyslipidemia, and overweight/obesity, is associated with several comorbidities, including cardiovascular conditions, diabetes, cancer, and liver disease. Specific pharmacological agents apart, there is a need to switch to healthier diets, such as the Mediterranean one (MedDiet), given that diet is a key factor in the prevention of such comorbidities (4).

The traditional MedDiet, largely based on plant-derived products, is characterized by seasonal and proximity products. It includes: (a) olive oil as the main source of fat; (b) high consumption of cereals, vegetables, legumes, fruit, and nuts; (c) moderate intake of poultry, fish, eggs, milk, and dairy products; (d) regular, but moderate, consumption of red wine at meals; and (e) low intake of red meat, processed meat, and industrial confectionery (5). The protective effect of the traditional MedDiet against cardiovascular disease in primary prevention has been demonstrated with the PREDIMED Study. This randomized, controlled, multicenter clinical trial had three intervention groups: two with a traditional MedDiet supplemented with extra virgin olive oil and nuts, respectively,

and low-fat diet control (6). In addition, a meta-analysis of 50 epidemiological and clinical trials (534,906 participants) determined that adherence to the MedDiet was associated with a reduced risk of metabolic syndrome (7).

Obesity is characterized by an increase of adipose tissue which, due to its involvement in metabolic regulation functions, has been acknowledged as an endocrine tissue organ. A maze of cytokines, hormones, substrates, and products, with both pro-and anti-inflammatory effects, regulate feelings of hunger and satiety through signals from the gastrointestinal tract and adipose tissue. Dietary interventions accompanied by weight loss have been shown in mid-and long-term programs to substantially influence satiety hormones. Feelings of hunger and satiety involve complex interactions between ghrelin and leptin in the hypothalamus which integrates both signals to regulate the body's energy homeostasis (8–10). Leptin, an adipose tissue-specific adipokine, is crucial in the control of appetite, energy expenditure, behavior, and glucose metabolism. It crosses the blood–brain barrier and acts on specific receptors to decrease appetite and increase energy expenditure. Reduction in leptin levels has been observed short and mid-term (11, 12), while fewer studies have demonstrated MedDiet effectivity beyond a 12-month intervention (13). Physical activity and a caloric-restricted diet have jointly been reported to augment leptin decrease (14). Ghrelin, an endogenous peptide mainly secreted by the gut, contributes to orexigenic stimulus thus increasing appetite (15, 16). Higher circulating levels have been observed in short-term, with lesser evidence after 1 year of initial weight loss (13).

By interacting with different cell lineages (17–20), leptin acts as a pro-inflammatory adipokine and increases C-reactive protein levels in primary hepatocytes and human coronary endothelial cells (21, 22). Low-grade chronic inflammation is associated with adiposity, advanced age, dyslipidemia, and hyperglycemia. Inflammatory status can be counteracted by modifying diet patterns, including moderate physical activity (23–25). Several biomarkers engage in the complex process of inflammation, such as C-reactive protein, considered to reflect inflammatory reactions in atherosclerotic vessels, as well as circulating cytokines and necrosis in acute myocardial infarction (26). Plasminogen activator inhibitor-1 (PAI-1), a physiological inhibitor of plasminogen, acts as a biomarker of a pro-thrombotic state. MedDiet interventions have been reported to ameliorate pro-thrombotic status decreasing PAI-1 serum levels (27, 28). Smoking, alcohol consumption, and age are positively correlated with PAI-1 levels (29).

White adipose tissue has been broadly accepted as a metabolic active organ. However, some of its peptides are unclear, for instance, resistin, an antagonist polypeptide of insulin action that may play a role in obesity (30). Controversial results have been obtained regarding the identification of changes in its levels in both obesity and diabetes mellitus (31, 32). Regarding visfatin, an adipokine with arguably insulin-mimetic effects (33) and which is highly expressed in visceral fat (34, 35) appears to be upregulated in patients with obesity (36) and type 2 diabetes mellitus (37). Results, however, are inconsistent with respect to insulin sensitivity, waist circumference, body mass index (BMI), and HbA1c (38–40).

Our objective is to assess whether an intervention with a restrictive MedDiet plus physical activity promotion, versus a non-restrictive MedDiet, is associated with an improvement in satiety-related hormones, weight loss, pro-inflammatory biomarkers, and glucose/lipid metabolism at mid-and long-term (6-and 12-month follow-ups). In addition, we will establish the association of these markers with weight loss irrespective of the intervention group.

Materials and methods

Study design and population recruitment

The PREDIMED-PLUS is a multicenter lifestyle intervention with 6,874 eligible participants. It is a 6-year randomized trial conducted in 23 Spanish centers with a large cohort presenting metabolic syndrome recruited from primary healthcare centers. Inclusion criteria were: men aged 55–75 years and women 60–75 years, with overweight/obesity (BMI: 27–40), and meeting at least three metabolic syndrome components at baseline (41, 42).

Patients were randomly allocated either to the intervention group or control (41). Those in the former followed an energy-reduced MedDiet with physical activity promotion and behavioral support so as to meet specific weight loss objectives. The participants received recommendations based on a 17-item energy-restricted score. In addition, physical activity counseling to gradually increase exercise intensity to 150 min/week, and attitudinal lifestyle advice through frequent sessions with dietitians (both individual and collective), were provided. Participants in the control group received educational sessions on an *ad libitum* MedDiet based on a 14-item non-energy-restricted score. No specific advice for increasing physical activity or losing weight was provided.

Regarding the individual sessions, participants in both groups received periodical group sessions and telephone calls (once a month in the intensive intervention group and two times a year in the control one).

Adherence to diet was assessed with a previously validated 14-item questionnaire employed in the PREDIMED Study for the control group (43, 44), which was adapted to the 17-item energy-restricted diet questionnaire for the intervention

group. According to the score obtained, the scale was estimated as approximate tertiles: low ($\leq$ 7), medium (8–10), and high (11–17) (45). Physical activity practice was evaluated at the beginning of the study and during follow-up. Participants reported activities through the Regicor Short Physical Activity Questionnaire, a validated version adapted from the Minnesota leisure time physical activity questionnaire (46, 47). Physical activity was measured in MET·min/week.

Hormone and inflammation-related determinations were performed in a subsample of 300 patients at baseline, with measurements at 6-and 12-month follow-ups of 298 and 266 subjects, respectively. The sample size of glycosylated A1c hemoglobin (HbA1c) was made up of 300, 353, and 369 individuals at the three visits, respectively. Due to sample availability, high sensitivity C-reactive protein (hs-CRP) was analyzed in 228 individuals.

Laboratory, anthropometric, and clinical data

The following information was gathered before and after the intervention: (i) the participants' general clinical status (sex, age, BMI, waist circumference, systolic/diastolic blood pressure); (ii) adherence to the energy-reduced MedDiet (with a 17-point questionnaire); and (iii) levels of physical activity. Sample collection was performed after an overnight fasting period at baseline, 6-months, and 12-months of follow-up. Venous blood samples were collected in vacuum tubes with a silica clot activator and K_2-EDTA anticoagulant (Becton Dickinson, Plymouth, United Kingdom) to yield serum and plasma, respectively. Serum tubes were centrifuged after the completion of the coagulation process, and plasma tubes immediately after collection, both for 15 min at 1.700 g room temperature. With the exception of HbA1c which was analyzed with K_2-EDTA anticoagulated whole blood, the following analytes were quantified in serum with an ABX Pentra-400 auto-analyzer (Horiba-ABX, Montpellier, France): glucose, HbA1c, triglycerides, high-density lipoprotein (HDL) cholesterol, and total cholesterol. Low-density lipoprotein (LDL) cholesterol was calculated according to the Friedewald formula whenever triglycerides were $<$ 300 mg/dL. Remnant-C was estimated as total cholesterol minus LDL cholesterol minus HDL cholesterol. Finally, leptin, ghrelin, glucagon-like peptide-1 (GLP-1), C-peptide, glucagon, insulin, PAI-1, resistin, and visfatin were simultaneously analyzed in plasma by Bio-Plex Pro methodology, a bead-based multiplexing technology with specific capture antibodies coupled with magnetic beads to discriminate analytes using an XMAG-Luminex assay (Bio-Rad, Hercules, CA, USA). The fluorescence signal was read on a Bio-Plex 200 equipment (Bio-Rad) (14). After several washes to remove unbound protein, a biotinylated detection antibody conjugated with fluorescent dye reporter. Homeostatic model assessment for insulin resistance (HOMA) was calculated as fasting plasma glucose (mg/dL) x fasting serum insulin (μ units/mL)/405. The inter-assay coefficients of variation (CVs) of these determinations were between 4.92 and 12.43%, except for GLP-1 (24.11%) and vifastin (32.42%). Values under the methodological limit of detection were reported with the limit of detection itself. Leptin measurements from six individuals were removed from the database due to analytical sampling error, and two hs-CRP values were considered outliers.

Statistical analysis

The assessment of the normality distribution of the variables was performed based on normality probability plots and boxplots. Continuous variables were normally shaped, except for triglycerides which were normalized by Napierian logarithm, and median and interquartile ranges were displayed. Lifestyle categorical variables were compared between groups with the Chi-square test.

A descriptive statistic table stratified by intervention and control group was summarized including mean values (or median if non-normally shaped), and mean differences between 6-and 12-month intervals. In addition, multivariate linear regression models adjusted for sex, age, energy intake baseline value, and baseline value of the variable under study were fitted. Mean differences between groups were estimated and 95% confidence intervals were reported. To identify possible statistical differences across time, we performed the paired *t*-test among baseline, 6 months, and 12 months in each group (Mann–Whitney *U* test was carried out for non-normal variables).

Weight loss and waist circumference changes were stratified according to the tertiles of the population at the different time points (baseline, 6 months, and 12 months). To estimate the extent of variation among the first, second, and third tertiles, the analysis of variance was calculated by adjusting for baseline value and baseline weight. Major weight and waist circumference losses corresponded to the first tertile. The linear mixed-effect models were constructed considering potential significant covariates with age, sex, time, weight, and adherence to MedDiet as fixed effects. Given that time affects individuals differently, it was contemplated as a varying covariate and a random slope constructed. The model contains both linear and quadratic time components so as to determine which trend better fits the model. We also included possible interaction between sex and weight, using the latter to correct the model in all variables (except for weight itself). Linear mixed-effect estimation was carried out with the use of restricted maximum likelihood. Graphical representation of variables that showed significant results for the and/or group: time interaction (linear and/or quadratic component) was performed. In addition,

analysis of 1-year weight loss correlation with these variables was calculated with Pearson's correlation formula. A p-value of < 0.05 was considered significant.

Sample size

Accepting an alpha risk of 0.05 and a beta risk of < 0.2 in a bilateral contrast, 116 subjects in both groups allow the detection of a difference $\geq$ 1,2 pg/mL for leptin circulating levels, when the standard deviation is assumed to be 3.26 pg/mL.

Results

Our study population was a sample of 407 (215 women) participants from the IMIM (Hospital del Mar Research Institute) site within the framework of the PREDIMED PLUS Study. The mean age was 65.44 years ($\pm$ 4.62 years). With respect to participants' lifestyles at baseline, the diet and physical activity questionnaire scores did not show significant differences between groups, and they met the minimal physical activity requirements suggested by the American Heart Association (450–750 MET·min·week^{-1}) (48). Diabetes, dyslipidemia, hypertension, and smoking conditions were equally distributed between the two groups without significant differences.

Baseline, 6-and 12-month follow-ups, characteristics of continuous variables regarding clinical features, lifestyle, lipid/glucose metabolism, satiety-related hormones, and studied pro-inflammatory markers are shown in **Table 1**. The main food items and nutritional parameters are shown in **Table 2**. In comparison to the control group, the adjusted multivariate of MedDiet adherence, physical activity, weight, waist circumference, remnant cholesterol, triglyceride levels, and HDL cholesterol showed an improvement at 6-month follow-up which was maintained at 12 months. Systolic and diastolic blood pressure presented significant improvements at 6-month follow-up but did not reach significance at 12 months. Regarding carbohydrate metabolism, we found differences between the two groups at 6-and 12-month follow-ups in HOMA, insulin, and C-peptide. Borderline inter-group P-value these explanations were aimed to clarify the meaning of borderline to reviewer 2. Borderline inter-group was observed for glucose at 6 and 12 months [a tendency to ameliorate results over time: $\beta_{6m} = -3.58$ (-7.39, 0.23) and $\beta_{12m} = -4.22$ (-9.16, 0.72)] and a significant decrease for HbA1c only at 12 months. Changes in leptin and PAI-1 levels were reported at 12 months, with a 6-month P-value close to significance in the case of PAI-1. Mean multivariate-adjusted differences (95% CI) for 6-and 12-month follow-ups were estimated and are depicted in common units of baseline standard deviations in **Supplementary Figure 1**.

As expected, the weight loss tertiles showed improvements at mid-and long-term follow-up for MedDiet adherence and physical activity practice regardless of the group. In particular, we observed changes in the triglyceride-related measurements (total cholesterol, HDL cholesterol, triglycerides, and remnant cholesterol), systolic/diastolic blood pressure, and carbohydrate metabolism (HOMA, HbA1c, insulin, glucagon, C-peptide, GLP-1). In addition, changes in leptin, PAI-1, and visfatin levels were observed at 6-and 12-month follow-ups (**Table 3**). Waist circumference change tertiles showed similar results to body weight tertiles (**Table 4**).

Changes were graphically examined through linear mixed-effect models of cardiovascular risk factors at 6-and 12-month follow-ups to observe the behavior of the repeated measures in both groups. The time:group (linear and quadratic) interaction as a potential predictor of the outcome variable was significant in weight, waist circumference, HDL, and remnant cholesterol, systolic/diastolic blood pressure, triglycerides, and PAI-1 levels (**Supplementary Figure 2**). Pearson's correlation at 1 year yielded a moderately positive correlation ($r > 0.20$) between weight loss and reduction of leptin, glucagon, PAI-1, HbA1c, and insulin levels. Comparably, moderately positive correlations ($r > 0.20$) between waist circumference changes and reduction of leptin, PAI-1, and insulin levels were observed. Weight change with moderate positive correlation was reported (**Supplementary Figure 3**).

Discussion

The intervention with an energy-reduced MedDiet and physical activity, versus a non-reduced one, was associated with an improvement in weight, waist circumference, glucose metabolism, triglyceride-related lipid profile, satiety-related hormones (leptin), and pro-inflammatory markers (PAI-1) at mid-and long-term in subjects with metabolic syndrome.

Such changes being maintained over time have been previously reported. Moreover, it has been hypothesized that MedDiet pattern interventions lead to greater compliance and adherence rates, in fact, the number of dropouts registered in trials has been reported to be larger in the control groups (7, 49–52). The MedDiet fat component is of vegetable origin (olive oil and nuts) and includes an abundance of plant foods (vegetables, fruit, whole grains, and legumes), limited fish consumption, and red wine in moderation (usually during meals). The intake of red and processed meats, refined grains, potatoes, dairy products, and ultra-processed foods (ice cream, sweets, creamy desserts, industrial confectionery, and sugar-sweetened beverages) (41, 53).

The hypothesis that the MedDiet is an eating pattern that can be maintained in mid-and long-term with a high degree of acceptance has been reflected in several studies introducing behavioral and nutritional patterns into small population groups (52, 54, 55). During other interventions,

TABLE 1 Baseline and 6-and 12-month changes (mean and standard deviation) stratified in the control and intervention groups of the participants on the 17-item questionnaire, physical activity, biomarkers, and anthropometric measurements, lipid profile, carbohydrate metabolism, and hormones. Adjusted for sex and age.

	Control group			Intervention group			Control group vs. Intervention group		
	Baseline	6 month-change	12 month-change	Baseline	6 month-change	12 month-change	Baseline	6 month-adjusted model	12 month-adjusted model
Diet adherence and physical activity									
Mediterranean diet adherence (17-point item score)	7.18 (2.36)	3.03* (3.03)	2.55 + ^ (2.94)	7.52 (2.60)	4.13* (3.27)	3.89^ (3.38)	0.30 (−0.17, 0.77)	1.37 (0.88, 1.86)	1.62 (1.13, 2.12)
Physical activity (MET·min/week)	2477 (2132.37)	275.77 (2311.75)	367.91^ (2272.46)	2648 (2216.67)	838.78* (2430.42)	872.23 ^ (2207.52)	150.64 (−269.65, 570.93)	649.28 (233.83, 1064.72)	591.67 (206.63, 976.71)
Lipid profile									
Total cholesterol (mg/dL)	218.30 (41.22)	−3.69 (31.71)	−0.16 (33.93)	221.88 (41.65)	−4.50* (32.54)	−1.70 (32.66)	3.62 (−4.06, 11.30)	0.69 (−4.95, 6.34)	−0.34 (−6.53, 5.85)
HDL cholesterol (mg/dL)	54.29 (11.06)	0.91 (6.89)	−0.13 + (7.06)	52.73 (11.21)	2.53* (7.79)	2.62 ^ (7.83)	−1.74 (−3.69, 0.22)	1.35 (−0.10, 2.79)	2.29 (0.84, 3.74)
LDL cholesterol (mg/dL)	133.67 (35.19)	−4.17* (25.22)	2.03 + (28.77)	139.22 (38.03)	−3.03 (27.71)	−0.36 (27.70)	5.67 (−1.45, 12.80)	3.44 (−1.38, 8.26)	−0.41 (−5.74, 4.92)
Triglycerides (mg/dL)	144 [107: 186]	−5.93 (55.03)	−13.48^ (55.65)	134 [104:182]	−13.48* [−38:6]	−16^ [−42:10]	0.00 (−0.09, 0.08)	−0.12 (−0.18, −0.07)	−0.08 (−0.14, −0.02)
Remnant cholesterol (mg/dL)	29.13 (10.60)	−1.08 (8.66)	−1.52^ (8.05)	28.52 (10.88)	−3.72* (7.82)	−3.03^ (9.49)	−0.48 (−2.62, 1.67)	−2.81 (−4.30, −1.33)	−1.78 (−3.43, −0.13)
Blood pressure and anthropometric measurements									
Systolic pressure (mmHg)	139.25 (13.46)	−2.04* (14.30)	−3.64^ (14.61)	140.01 (12.90)	−6.38* (13.90)	−5.18^ (15.43)	0.63 (−1.92, 3.18)	−3.90 (−6.46, −1.35)	−1.09 (−3.79, 1.61)
Diastolic pressure (mmHg)	74.59 (10.74)	−0.81 (11.22)	−2.10^ (11.11)	75.59 (9.77)	−4.99* (10.20)	−4.06^ (10.87)	1.13 (−0.80, 3.05)	−3.39 (−5.24, −1.53)	−1.21 (−3.13, 0.71)
Weight (kg)	88.98 (13.71)	−2.66* (3.47)	−2.67^ (3.99)	87.54 (13.87)	−6.31* (4.09)	−7.41 + ^ (4.07)	−1.12 (−3.46, 1.21)	−3.74 (−4.46, −3.03)	−4.84 (−5.60, −4.08)
Waist circumference (cm)	111.47 (9.56)	−2.81* (3.70)	−2.83 ^ (4.48)	110.25 (9.74)	−6.21* (4.73)	−7.28 + ^ (4.37)	−1.10 (−2.84, 0.64)	−3.52 (−4.33, −2.70)	−4.57 (−5.44, −3.70)
Carbohydrate metabolism									
HOMA	3.14 (3.18)	−0.30* (1.82)	−0.34 ^ (1.52)	2.97 (2.17)	−0.75* (1.24)	−0.70^ (1.38)	−0.17 (−0.79, 0.45)	−0.49 (−0.83, −0.14)	−0.38 (−0.70, −0.06)
Glucose (mg/dL)	119.90 (33.19)	−2.92 (22.11)	−0.53 (27.27)	120.86 (31.32)	−6.67* (17.69)	−4.95^ (21.75)	1.04 (−5.25, 7.33)	−3.58 (−7.39, 0.23)	−4.22 (−9.16, 0.72)

(Continued)

10.3389/fnut.2022.950900

TABLE 1 (Continued)

	Control group			Intervention group			Control group vs. Intervention group		
	Baseline	6 month-change	12 month-change	Baseline	6 month-change	12 month-change	Baseline	6 month-adjusted model	12 month-adjusted model
HbA1c (%)	6.40 (1.11)	−0.27* (0.72)	−0.06 + (0.55)	6.33 (0.84)	−0.33* (0.45)	−0.21 + ^ (0.50)	−0.07 (−0.30, 0.15)	−0.06 (−0.19, 0.07)	−0.14 (−0.27, −0.02)
Insulin (pg/mL)	351.81 (230.53)	−24.19 (160.44)	−33.22^ (142.44)	336.79 (189.70)	−77.56* (129.63)	−79.59^ (131.15)	−14.65 (−62.49, 33.19)	−57.09 (−90.06, −24.12)	−49.39 (−79.44, −19.33)
Glucagon (pg/mL)	455.03 (163.32)	−36.43* (114.51)	−39.24^ (115.95)	446.44 (154.60)	−47.00* (128.95)	−48.99^ (125.23)	−6.35 (−41.12, 28.43)	−13.46 (−39.90, 12.98)	−11.33 (−38.03, 15.37)
C-peptide (pg/mL)	1054.99 (522.52)	−68.82* (303.26)	−89.74^ (313.09)	1066.02 (432.07)	−155.66* (327.24)	−170.98^ (316.98)	11.15 (−97.34, 119.65)	−83.93 (−149.45, −18.41)	−82.90 (−149.68, −16.12)
GLP_1 (pg/mL)	172.39 (134.29)	−8.33 (81.05)	−12.46 (98.24)	166.78 (120.69)	−9.71 (91.22)	−23.20^ (82.12)	−5.75 (−34.43, 22.93)	−4.45 (−22.92, 14.01)	−13.74 −33.43, 5.95)
Hormones and inflammation biomarkers									
Ghrelin (pg/mL)	807.38 (415.02)	−30.86 (245.98)	−31.21 (236.83)	831.31 (412.41)	−11.05 (207.24)	5.69 (227.47)	26.33 (−67.05, 119.72)	26.96 (−24.03, 77.95)	40.68 (−12.90, 94.25)
Leptin (pg/mL)	7746.99 (4152.80)	−730.11 (2486.83)	−776.03 (2727.56)	7246.06 (3850.42)	−1020.43 (3141.41)	−1310.85 (2524.41)	−385.77 (−1145.57, 374.04)	−425.74 (−1036.51, 185.04)	−698.56 (−1295.48, −101.64)
PAI_1 (pg/mL)	2631.62 (838.39)	−250.14* (715.26)	−129.02 + ^ (729.33)	2652.39 (828.08)	−425.63* (837.60)	−371.54^ (692.88)	29.66 (−158.92, 218.24)	−153.42 (−312.00, 5.16)	−252.41 (−403.90, −100.92)
Resistin (pg/mL)	4625.87 (2138.00)	−286.75* (1670.16)	−362.79^ (1815.51)	4254.35 (1635.32)	−74.65* (1343.08)	−12.26 + ^ (1176.19)	−378.91 (−812.86, 55.05)	55.85 (−247.20, 358.89)	151.36 (−160.26, 462.98)
Visfatin (pg/mL)	1309.09 (1620.44)	−302.97* (1314.76)	−300.98^ (1375.44)	1194.63 (1093.55)	−270.33* (668.66)	−258.90^ (613.18)	−93.64 (−387.15, 199.87)	−52.22 (−205.86, 101.41)	−47.97 (−214.70, 118.76)
hs-PCR (mg/dL)	0.45 (0.61)	−0.07 (0.81)	−0.13^ (0.47)	0.45 (0.61)	−0.13* (0.63)	−0.11 (0.81)	0.00 (−0.15, 0.16)	−0.01 (−0.09, 0.08)	0.02 (−0.11, 0.15)

#Median and interquartile range were displayed in non-normal distributed variables *: significant *P-value* between baseline and 6-month follow-up; + : significant *P-value* between 6-month follow-up and 12-month follow-up; ^: significant *P-value* between baseline and 12-month follow-up.

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TABLE 2 Baseline and differences at 6-and 12-month follow-ups (mean and standard deviation) stratified in the control and intervention groups in the consumption of key food items and dietary parameters between the control and intensive group adjusted for the baseline value.

	Baseline	6 month-change	12 month-change	Baseline	6 month-change	12 month-change	Baseline	6 month-adjusted model	12 month-adjusted model
Energy intake (kcal/day)	2464.42 (548.70)	−135.32 (559.65)	−160.20 (576.76)	2357.21 (528.53)	−111.46 (585.36)	−110.20 (581.21)	< 0.05	0.054	0.368
Carbohydrates (g/day)	227.24 (66.12)	−20.31 (73.14)	−20.84 (64.67)	218.22 (69.56)	−22.88 (72.54)	−18.93 (75.03)	0.181	< 0.05	0.241
Protein (g/day)	105.80 (19.94)	2.07 (20.69)	−1.74 (22.29)	102.25 (19.77)	6.84 (23.13)	6.23 (22.15)	0.072	0.288	< 0.05
Total fat (g/day)	118.22 (28.89)	−4.14 (30.72)	−5.15 (33.56)	113.22 (28.08)	−2.12 (33.58)	−3.45 (33.79)	0.077	0.272	0.255
Saturated fatty acids (g/day)	30.80 (9.50)	−5.47 (9.11)	−5.84 (9.62)	29.25 (9.03)	−6.18 (9.58)	−6.19 (9.89)	0.093	< 0.001	< 0.05
Monounsaturated fatty acids (g/day)	61.05 (15.04)	1.83 (19.54)	2.11 (20.07)	58.08 (14.90)	5.59 (21.29)	4.85 (19.85)	< 0.05	0.451	0.968
Polyunsaturated fatty acids (g/day)	19.01 (5.83)	3.12 (6.90)	2.29 (7.32)	18.82 (6.84)	3.64 (7.78)	2.62 (8.19)	0.761	0.528	0.800
Cholesterol (mg/day)	426.16 (105.26)	−40.19 (106.38)	−48.23 (121.61)	418.58 (118.16)	−35.06 (124.82)	−41.95 (132.46)	0.495	0.934	0.733
Trans-fatty acids (g/day)	0.72 (0.41)	−0.27 (0.39)	−0.28 (0.43)	0.70 (0.44)	−0.37 (0.45)	−0.37 (0.46)	0.579	< 0.001	< 0.001
Linolenic acid	1.74 (0.67)	0.52 (0.84)	0.37 (0.95)	1.72 (0.78)	0.63 (0.98)	0.44 (0.92)	0.775	0.248	0.538
Carbohydrate percentage (%)	36.72 (5.44)	−1.34 (6.41)	−1.01 (5.90)	36.72 (5.89)	−2.06 (6.67)	−1.35 (6.71)	1	0.076	0.462
Protein percentage (%)	17.44 (2.46)	1.28 (2.98)	0.84 (2.79)	17.62 (2.68)	1.88 (3.02)	1.83 (3.27)	0.481	< 0.001	< 0.001
Total fat percentage (%)	43.22 (5.36)	0.90 (6.52)	0.97 (6.14)	43.33 (5.70)	1.27 (6.70)	0.64 (6.93)	0.837	0.203	0.526
Saturated fatty acid percentage (%)	11.18 (1.97)	−1.44 (2.16)	−1.48 (1.96)	11.09 (1.82)	−1.86 (1.95)	−1.91 (1.99)	0.635	< 0.05	< 0.001
Monounsaturated fatty acid percentage (%)	22.41 (3.58)	1.91 (5.33)	2.27 (4.86)	22.34 (4.10)	3.16 (5.55)	2.89 (5.49)	0.856	0.004	0.219

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TABLE 2 (Continued)

	Baseline	6 month-change	12 month-change	Baseline	6 month-change	12 month-change	Baseline	6 month-adjusted model	12 month-adjusted model
Polyunsaturated fatty acid percentage (%)	6.95 (1.65)	1.64 (2.25)	1.37 (2.15)	7.21 (2.17)	1.82 (2.48)	1.38 (2.51)	0.185	0.014	0.198
Meat and meat products (g/day)	174.48 (59.31)	−17.35 (60.32)	−24.14 (61.51)	166.54 (52.82)	−7.08 (60.80)	−9.02 (63.08)	0.155	0.279	< 0.05
Fish (g/day)	120.91 (42.98)	17.11 (49.01)	6.91 (47.91)	126.49 (46.18)	15.53 (60.00)	14.27 (57.86)	0.207	0.523	0.005
Vegetables (g/day)	343.34 (149.73)	42.53 (186.18)	33.25 (171.62)	354.26 (122.21)	49.97 (176.83)	57.45 (143.06)	0.422	0.226	< 0.05
Total cereals (g/day)	129.44 (58.75)	−5.29 (69.71)	−7.00 (61.00)	119.38 (63.50)	−0.15 (63.61)	6.69 (78.60)	0.098	0.248	0.450
Dairy products (g/day)	370.65 (181.52)	14.88 (209.43)	−10.69 (208.67)	339.42 (168.83)	27.83 (214.99)	35.79 (189.12)	0.073	0.629	0.105
Nuts (g/day)	15.69 (15.92)	21.24 (26.01)	19.97 (25.91)	15.83 (16.73)	28.66 (25.76)	25.12 (25.57)	0.933	< 0.05	< 0.05
Fruit (g/day)	351.48 (174.25)	0.63 (224.44)	35.68 (223.37)	351.14 (174.26)	19.97 (221.11)	22.88 (209.81)	0.984	0.255	0.479
Legumes (g/day)	20.53 (10.15)	3.99 (12.26)	3.35 (13.21)	19.73 (9.02)	7.26 (12.01)	5.32 (11.78)	0.399	0.007	0.145
Olive oil (g/day)	47.77 (13.78)	−0.11 (17.34)	1.35 (16.49)	45.47 (14.44)	1.89 (17.33)	2.17 (16.41)	0.100	0.906	0.244
Virgin olive oil (g/day)	30.39 (20.44)	13.40 (22.33)	13.94 (21.70)	31.61 (20.07)	12.29 (21.66)	13.61 (21.43)	0.544	0.963	0.580
Sunflower oil (g/day)	0.74 (2.81)	−0.65 (2.77)	−0.40 (2.56)	1.35 (6.44)	−1.31 (6.65)	−1.19 (6.24)	0.214	0.755	0.327
Dietary fiber (g/day)	24.93 (7.18)	5.80 (8.72)	5.08 (8.75)	25.25 (6.93)	7.29 (8.83)	6.93 (8.16)	0.645	< 0.05	< 0.001
Alcohol (g/day)	9.76 (12.52)	−3.59 (10.38)	−3.36 (8.86)	8.05 (9.84)	−4.02 (9.45)	−4.04 (7.81)	0.128	< 0.05	< 0.05

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TABLE 3 Tertiles of weight loss change (mean, standard deviation, and their comparison) adjusted for weight and baseline value of the participants on the 17-item questionnaire, physical activity, biomarkers and anthropometric measurements, lipid profile, carbohydrate metabolism, and hormones.

	First tertile of 6-month weight-loss change	Second tertile of 6-month weight-loss change	Third tertile of 6-month weight-loss change	Global *P-value*	First tertile of 12-month weight-loss change	Second tertile of 12-month weight-loss change	Third tertile of 12-month weight-loss change	Global *P-value*
Diet adherence and physical activity								
Mediterranean diet adherence (17-point item score)	4.93 (3.12)	3.31* (2.91)	2.42 + ˆ (3.04)	< 0.001	4.56 (3.10)	2.70* (3.18)	2.34 + ˆ (2.98)	< 0.001
Physical activity (MET·min/week)	1084.68 (2503.52)	390.80* (2197.49)	167.02ˆ (2356.66)	< 0.001	933.72 (2398.89)	596.32* (1969.18)	311.94ˆ (2346.88)	< 0.05
Lipid profile								
Total cholesterol (mg/dL)	−8.99 (30.57)	−4.73 (36.49)	1.64ˆ (28.05)	< 0.05	−2.52 (32.39)	−3.51 (36.13)	3.76 (30.54)	0.414
HDL cholesterol (mg/dL)	3.34 (8.04)	0.71* (6.96)	0.96ˆ (6.80)	< 0.05	3.54* (7.87)	0.71* (7.12)	−0.73ˆ (7.11)	< 0.001
LDL cholesterol (mg/dL)	−8.00 (25.82)	−1.21 (30.34)	−1.29ˆ (22.28)	0.113	−1.17 (27.43)	0.03 (30.99)	4.05 (25.70)	0.370
Triglycerides (mg/dL)	−33.70 (61.93)	−14.25* (53.21)	1.22ˆ (53.76)	< 0.001	−27.33 (53.10)	−21.30* (57.28)	−2.96 + ˆ (60.50)	< 0.001
Remnant cholesterol (mg/dL)	−5.35 (8.44)	−1.90* (7.54)	0.33ˆ (8.05)	< 0.001	−4.72 (9.12)	−2.59* (8.30)	0.90 + ˆ (8.12)	< 0.001
Blood pressure and anthropometric measurements								
Systolic pressure (mmHg)	−7.71 (13.62)	−4.48* (14.46)	−0.14 + ˆ (13.74)	< 0.05	−7.86 (13.49)	−3.96* (15.40)	−1.18ˆ (15.50)	< 0.05
Diastolic pressure (mmHg)	−5.41 (10.11)	−2.84 (11.21)	−0.21 + ˆ (10.89)	< 0.001	−4.71 (10.98)	−3.39 (11.29)	−0.95 + ˆ (10.51)	< 0.05
Waist circumference (cm)	−8.20 (4.24)	−3.88* (3.28)	−1.19 + ˆ (2.87)	< 0.001	−9.32 (4.64)	−4.45* (2.83)	−1.05 + ˆ (3.21)	< 0.001
Carbohydrate metabolism								
HOMA	−0.94 (1.25)	−0.38* (2.08)	−0.02ˆ (1.01)	< 0.001	−1.02 (1.18)	−0.49* (1.61)	0.13 + ˆ (1.36)	< 0.001
Glucose (mg/dL)	−10.73 (19.68)	−4.47* (21.84)	1.20 + ˆ (16.96)	< 0.001	−8.16 (18.06)	−2.87 (31.30)	3.46ˆ (21.29)	< 0.001
HbA1c (%)	−0.44 (0.54)	−0.30 (0.77)	−0.07 + ˆ (0.33)	< 0.001	−0.35 (0.47)	−0.05* (0.48)	0.06ˆ (0.57)	< 0.05
Insulin (pg/mL)	−93.90 (134.85)	−30.79* (177.62)	−5.45ˆ (102.70)	< 0.001	−108.89 (124.15)	−43.90* (143.14)	1.47ˆ (126.86)	< 0.001
Glucagon (pg/mL)	−72.75 (131.53)	−29.64* (106.92)	−7.82ˆ (112.95)	< 0.001	−83.56 (126.80)	−30.87* (102.37)	−5.49ˆ (116.94)	< 0.001

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TABLE 3 (Continued)

	First tertile of 6-month weight-loss change	Second tertile of 6-month weight-loss change	Third tertile of 6-month weight-loss change	Global *P-value*	First tertile of 12-month weight-loss change	Second tertile of 12-month weight-loss change	Third tertile of 12-month weight-loss change	Global *P-value*
C-peptide (pg/mL)	−207.60 (322.66)	−71.29* (299.50)	−9.15ˆ (292.38)	< 0.001	−227.06 (305.38)	−104.96* (303.46)	−26.49ˆ (313.63)	< 0.001
GLP_1 (pg/mL)	−14.42 (84.28)	−3.39* (99.23)	−7.89 (68.07)	0.105	−21.13 (75.58)	−18.21 (88.02)	−12.87ˆ (111.02)	0.109
Hormones and inflammation biomarkers								
Ghrelin pg/mL)	−32.28 (205.27)	−21.59 (195.07)	−3.61 (295.65)	0.653	−8.91 (225.22)	−20.47 (224.18)	−9.35 (253.85)	0.966
Leptin (pg/mL)	−1549.05 (3235.36)	−724.65* (2355.01)	22.19ˆ (2400.70)	< 0.001	−1789.81 (2299.54)	−1194.54* (2562.08)	156.79 + ˆ (2762.06)	< 0.001
PAI_1 pg/mL)	−484.66 (811.69)	−261.77* (786.25)	−193.61ˆ (684.39)	< 0.05	−464.06 (640.85)	−239.00* (718.15)	31.85 + ˆ (735.85)	< 0.001
Resistin (pg/mL)	−142.90 (1542.54)	−183.42 (1322.29)	-−253.10 (1745.68)	0.588	−106.24 (1215.95)	−129.09 (1457.42)	−369.27 (1971.13)	0.710
Visfatin (pg/mL)	−352.27 (702.77)	−227.70 (568.43)	−263.99ˆ (1780.08)	< 0.001	−388.93 (627.74)	−226.48* (553.56)	−192.99ˆ (1765.19)	< 0.001
hs-PCR (mg/dL)	−0.21 (0.72)	−0.42 (2.94)	−0.04 (0.33)	0.297	−0.23 (0.65)	−0.01 (0.86)	−0.54 (3.35)	0.141

*: significant *P-value* between first and second tertile; + : significant *P-value* between second and third tertile; ˆ: significant *P-value* between first and third tertile.

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TABLE 4 Tertiles of waist circumference change (mean, standard deviation, and their comparison) adjusted for weight and baseline value of the participants on the 17-item questionnaire, physical activity, biomarkers and anthropometric measurements, lipid profile, carbohydrate metabolism, and hormones.

	First tertile of 6-month waist circumference change	Second tertile of 6-month waist circumference change	Third tertile of 6-month waist circumference change	Global *P-value*	First tertile of 1-year waist circumference change	Second tertile of 1-year waist circumference change	Third tertile of 1-year waist circumference change	Global *P-value*
Diet adherence and physical activity								
Mediterranean diet adherence (17-point item score)	4.76 (3.12)	3.38* (3.04)	2.55 + ^ (3.04)	< 0.001	4.73 (3.34)	2.92* (2.58)	2.48 + ^ (2.87)	< 0.001
Physical activity (MET·min/week)	932.61 (2453.74)	617.23 (2262.68)	122.75 + ^ (2462.50)	< 0.05	1202.29 (2242.48)	531.83* (2238.96)	263.94 + ^ (2051.53)	< 0.001
Lipid profile								
Total cholesterol (mg/dL)	−5.33 (31.52)	−4.50 (33.51)	−2.09 (31.08)	0.796	−3.66 (30.10)	2.11 (34.04)	−1.36 (35.69)	0.168
HDL cholesterol (mg/dL)	2.89 (8.22)	1.63 (6.61)	0.36^ (7.05)	< 0.05	2.01 (7.33)	2.60 (7.65)	−1.19 + ^ (7.24)	< 0.001
LDL cholesterol (mg/dL)	−3.70 (27.74)	−2.60 (26.89)	−4.74 (24.42)	0.567	0.74 (25.35)	1.77 (29.73)	−0.13 (29.73)	0.231
Triglycerides (mg/dL)	−31.64 (64.98)	−17.41* (53.28)	5.09 + ^ (48.90)	< 0.001	−33.86 (54.90)	−12.75* (50.83)	−5.30^ (64.12)	< 0.001
Remnant cholesterol (mg/dL)	−4.41 (8.71)	−2.81* (7.26)	0.60 + ^ (8.39)	< 0.001	−5.65 (8.85)	−1.48* (7.86)	0.61 + ^ (8.65)	< 0.001
Blood pressure and anthropometric measurements								
Systolic pressure (mmHg)	−7.86 (14.34)	−4.32* (13.46)	0.51 + ^ (13.90)	< 0.001	−7.00 (13.94)	−4.02 (15.69)	−1.99^ (15.07)	< 0.05
Diastolic pressure (mmHg)	−5.02 (10.19)	−3.57 (10.68)	0.68 + ^ (11.31)	< 0.001	−3.72 (11.06)	−4.64 (10.58)	−0.56 + (11.14)	< 0.05
Weight (kg)	−8.00 (4.49)	−4.07* (1.97)	−0.81 + ^ (2.24)	< 0.001	−9.16 (4.60)	−4.83* (2.59)	−1.15 + ^ (2.58)	< 0.001

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TABLE 4 (Continued)

	First tertile of 6-month waist circumference change	Second tertile of 6-month waist circumference change	Third tertile of 6-month waist circumference change	Global *P-value*	First tertile of 1-year waist circumference change	Second tertile of 1-year waist circumference change	Third tertile of 1-year waist circumference change	Global *P-value*
Carbohydrate metabolism								
HOMA	−0.83 (1.30)	−0.68 (1.54)	0.12 + ˆ (1.78)	< 0.001	−0.95 (1.22)	−0.57* (1.29)	−0.05 + ˆ (1.71)	< 0.001
Glucose (mg/dL)	−9.30 (19.90)	−5.65 (20.32)	1.84 + ˆ (18.58)	< 0.001	−5.92 (28.74)	−2.98 (20.17)	1.12ˆ (24.32)	0.051
HbA1c (%)	−0.45 (0.51)	−0.30* (0.71)	−0.09 + ˆ (0.49)	< 0.001	−0.36 (0.49)	−0.14* (0.43)	0.10 + ˆ (0.58)	< 0.001
Insulin (pg/mL)	−82.04 (132.49)	−59.17* (130.24)	5.55 + ˆ (176.87)	< 0.001	−95.44 (123.25)	−66.84* (128.61)	−5.78 + ˆ (149.81)	< 0.001
Glucagon (pg/mL)	−78.51 (122.47)	−31.70* (118.85)	−6.87ˆ (112.42)	< 0.001	−69.39 (139.30)	−47.78* (96.20)	−14.95 + ˆ (120.76)	< 0.001
C-peptide (pg/mL)	−184.51 (354.04)	−105.02* (277.13)	−21.46ˆ (299.70)	< 0.001	−216.09 (296.89)	−139.44* (295.42)	−34.91 + ˆ (336.88)	< 0.001
GLP_1 (pg/mL)	−7.86 (86.89)	−11.69 (93.79)	−6.64 (73.08)	0.620	−12.84 (73.14)	−25.92 (88.71)	−13.53 (107.03)	0.239
Hormones and inflammation biomarkers								
Ghrelin (pg/mL)	−36.13 (212.62)	−4.74 (183.25)	−25.43 (296.16)	0.657	−23.74 (259.87)	8.61 (182.88)	−26.26 (254.03)	0.541
Leptin (pg/mL)	−1654.22 (3115.84)	−545.42* (2525.77)	−281.55ˆ (2583.37)	< 0.001	−1741.04 (2490.24)	−924.76* (2237.87)	−468.22ˆ (3030.65)	< 0.001
PAI_1 (pg/mL)	−561.69 (620.62)	−295.04* (949.65)	−92.44ˆ (609.46)	< 0.001	−456.89 (603.42)	−243.57* (710.70)	−52.24 + ˆ (785.81)	< 0.001
Resistin (pg/mL)	−166.84 (1586.23)	−137.04 (1494.89)	−275.84 (1492.57)	0.668	−225.48 (1522.45)	−40.72 (1367.82)	−317.87 (1727.57)	0.935
Visfatin (pg/mL)	−379.89 (695.73)	−308.23* (1494.60)	−136.14 ˆ (571.59)	< 0.001	−355.01 (579.81)	−375.96* (1571.81)	−96.18ˆ (599.17)	< 0.001
hs-PCR (mg/dL)	−0.22 (0.77)	−0.39* (2.77)	−0.06ˆ (0.42)	< 0.05	−0.13 (0.87)	−0.17 (0.62)	−0.43 (3.01)	0.413

*: significant *P-value* between first and second tertile; + : significant *P-value* between second and third tertile; ˆ: significant *P-value* between first and third tertile.

10.3389/fnut.2022.950900

several participants reported freshness and palatability of food, with variance across the studies regarding taste (56–58). Meal plans resulted in hedonic appreciation and satisfaction by most participants (58), although this differed according to age and dishes (57). There were, however, a number of barriers, such as dislike of some foods (including olive oil) and/or reduction of red meat. In addition to diet acceptability, various limitations have been reported such as the perception of expense, expectation of time commitment, perceived impact on body weight, and cultural differences (56, 58–60). Among a group of schoolchildren, a study found that food neophobia correlated negatively with certain healthy dietary habits, such as fruit and vegetable consumption.

The intervention group was based on a hypocaloric diet with moderate fat consumption of vegetable origin: olive oil, tree nuts, and peanuts. Furthermore, it was designed to augment complex carbohydrates and fiber-rich products. Moderate intake of monounsaturated fat in the form of olive oil is one of the cornerstones of MedDiet due to its culinary versatility. Its beneficial effects on the reduction of cardiovascular disease include cardioprotective characteristics, improvement in lipid profile (decrease in total and LDL cholesterol and an increase of HDL cholesterol) and blood pressure decrease, amelioration of LDL cholesterol oxidation and low-chronic inflammation, and anti-atherogenic properties (61–67).

Weight and waist circumference

While short-term changes are relatively easy to accomplish, successfully maintaining them over time is considerably more difficult. The combination of diet-induced weight loss with exercise training has demonstrated greater improvement in cardiovascular risk factors than diet alone (68, 69). Our findings from the intervention group showed a decrease in waist circumference and weight at both 6-and 12-month follow-ups, and the comparison with the control was significant for both periods. The weight loss experienced by the control group, despite following a non-reduced diet, can be explained by their motivation to participate in a clinical trial for subjects with overweight/obesity. In the intervention group, the maximum weight loss was at 1 year. Such a finding is particularly relevant since in most studies on the effects of restrictive diets this occurs at 6 months followed by a reward effect. Interventions with hypocaloric diets which can be sustainable over time could, therefore, provide a better approach to weight loss. In this regard, a MedDiet is appropriate as its better palatability, due to its mainly vegetal content and use of olive oil leads to greater adherence.

Leptin–Ghrelin binomial

Hyperleptinemia is a characteristic manifestation of obesity in humans. Resistance to leptin action in obesity has been suggested, and elevated circulating concentrations may be necessary to maintain sensitivity to hormone and energy homeostasis (70, 71). Leptin, as a polypeptide secreted by adipocytes, might be decreased as a result of fat mass reduction (72, 73). We observed a significant reduction in its levels after both the intervention and control groups. The former displayed an overall stronger decrease probably caused by the further reduction of anthropometric measurements. In fact, a significant reduction was reported comparing the intervention arm to the control at 12-month follow-up.

Individuals with overweight/obesity have typically lower circulating ghrelin levels. This adipogenic hormone seems to indicate downregulation in human obesity, supposedly as an adaptive mechanism in response to positive energy balance (74, 75). Diet-induced effects usually show an increase in circulating levels, although reversion to baseline levels at 12 months after a 6-month peak has been reported (76). Our cohort reflected an initial reduction followed by a minor increase in circulating levels in the intensive group, with no statistical significance.

Carbohydrate metabolism-related hormones

Weight loss interventions lead to changes in carbohydrate homeostasis, and increased insulin sensitivity has been observed following dietary interventions, physical activity, and bariatric surgery (77, 78). Nevertheless, in contrast to isolated interventions, the combined effects of a restricted diet and physical exercise have been reported to improve to a greater extent such sensitivity and variables related to the cardiometabolic syndrome. In our intervention group, insulin levels decreased during the first 6 months and were maintained up to the 12-month follow-up. The control group also experienced a steady reduction although it presented higher levels at 6-and 12-month follow-ups. HOMA, C-peptide, HbA1c, and glucose levels followed a similar pattern.

Glucagon improvement caused by diet and exercise training has been reported in the literature. A meta-analysis made up of 29 interventions assessed body weight change, glucagon, insulin, and glucose fasting concentrations after two different weight reduction methods (bariatric surgery versus low-caloric diet intervention). More than half the diet interventions resulted in a decrease from 17 to 27%. The mean decrease in fasting glucagon, however, was not significantly different between both weight reduction approaches (77). Although no inter-group differences in the present study were obtained, a linear time component proved to be a predictor of weight loss regardless of the intervention.

Lipid profile

Triglyceride reduction is crucial in the management of dyslipidemia, particularly atherogenic dyslipidemia which is highly prevalent in metabolic syndrome subjects. Atherogenic dyslipidemia is characterized by high circulating triglyceride levels and low levels of HDL cholesterol, and even optimal concentrations of LDL cholesterol. We have recently reported in subjects with overweight/obesity at high cardiovascular risk, that triglycerides and remnant cholesterol levels, but not LDL cholesterol, were associated with cardiovascular outcomes irrespective of other risk factors (79, 80). Triglyceride concentration is an independent risk factor for cardiovascular disease and is strongly associated with subcutaneous abdominal adipose tissue. In fact, it has been suggested that triglycerides could be a predictor of cardiovascular disease (79). The MedDiet has been previously studied as a dietary tool to improve metabolic syndrome and subsequent events (6, 79, 81). In this respect, our results show an overall triglyceride reduction in both groups, with a greater reduction in the intervention group than in the control. In concordance, we have recently reported that an energy-reduced MedDiet plus physical activity improves HDL-related triglyceride metabolism versus a non-reduced MedDiet without physical activity (82). Regarding remnant cholesterol, its levels follow a similar pattern to that of triglycerides. Although we did not observe changes after the intervention in total cholesterol, remnant cholesterol decreased in mid-and long-term versus the control group. Such a finding could be a good indicator that the intensive intervention shifted toward protection against cardiovascular risk.

High-density lipoprotein (HDL) cholesterol lipoproteins are known for their atheroprotective effects through a number of anti-inflammatory, anti-oxidative, anti-thrombotic, and anti-apoptotic properties (83, 84). An inverse association between triglycerides and HDL cholesterol concentrations usually occurs. In fact, HDL lipoproteins are catabolized faster in the presence of hypertriglyceridemia in non-pathological states. In our study, while the intervention group experienced an increase in the first 6 months and kept a steady concentration at 12 months, the control group had increased HDL cholesterol in the first 6 months which was slightly decreased at 12 months.

Pro-inflammatory markers

High sensitivity C reactive protein (hs-CRP) is broadly used to monitor inflammatory processes, including autoimmune, infectious, tumoral, and metabolic diseases. Prospective epidemiological studies have reported elevated hs-CRP as an independent factor associated with cardiovascular events (26, 85). Dietary interventions usually lead to inflammatory profile improvement (86), we observed a reduction in hs-CRP levels across time in both groups, with no significant inter-group results.

Plasminogen activator inhibitor-1 plasma levels are positively associated with cardiovascular disease, thrombosis, fibrosis, and the progression of coronary syndromes (87). They are also positively correlated with individual risk factors (BMI, triglycerides, glucose, and mean arterial pressure) which may be indicative of their relevance in metabolic syndrome events (88). Diet composition has been demonstrated to affect circulating levels of PAI-1 and the fibrinolytic system as much as alcohol intake and smoking. High-fat diet consumption increases PAI-1 levels impairing clot lysis (29, 89). In our study, both groups produced a marked change in PAI-1 levels, although decreases were higher in the intensive group, mainly at the 12-month follow-up.

Cross-sectional studies have demonstrated that, compared to lean individuals, those with obesity have higher resistin levels (90–92). Some weight loss programs, however, have not always resulted in a decrease in circulating levels (31, 93, 94), while others reflect parallel reduction (95, 96). Regarding visfatin, weight loss programs have achieved a decrease in their levels, with no significant difference between them (94, 97). Nevertheless, there is evidence that a MedDiet has not always demonstrated an improvement in visfatin concentrations (98). In our study, resistin and visfatin levels displayed parallel behavior in both groups with an initial reduction at 6 months followed by steady maintenance at 12 months.

Strengths and limitations

Our large sample size and randomized design provide high-quality evidence that minimizes confounding and bias influences. We have comprehensively assessed diverse cardiovascular risk biomarkers and satiety-related hormones. There are, however, some limitations. First, results were obtained in adult/elderly participants with metabolic syndrome and excess body weight; therefore, our findings cannot be extrapolated to other populations. Second, we observed only moderate differences between the two intervention arms. Such a finding was to be expected as the control group was an active comparator following a healthy traditional MedDiet. Moreover, due to the physiological regulation of ghrelin, among other hormones, the measurement of post-prandial levels would have been inestimable contribution, further research is warranted. Nevertheless, this randomized trial provides high-level evidence of the benefits of an intervention with a restrictive MedDiet and physical activity, especially on weight, waist circumference, leptin levels, lipid/glucose metabolism, blood pressure, and the pro-inflammatory marker PAI-1 at mid-and long-term intervention in subjects with metabolic syndrome. Given that

such changes were maintained over time, and the marked palatability and acceptability of the MedDiet on the part of the consumers, MedDiet pattern interventions with hypocaloric diets could be a pertinent approach to weight loss.

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 Research Ethics Committees of all centers approved the study protocol during 2013 and 2014. The trial was registered in 2014 at (www.isrctn.com/ISRCTN89898870). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MF, JS-S, MM-G, DC, ER, FT, and RE designed the clinical trial. OC and MF designed the conceptualization sub-study. JH-R performed the formal and laboratory analysis. AT and JH-R carried out the statistical analysis. OC, MF, and JH-R drafted the manuscript. AT, DB, JS-S, MM-G, DC, RE, AG, OC, and MF revised and approved the final version. All authors contributed to the article and approved the submitted version.

Funding

This work was funded by the Instituto de Salud Carlos III (grant numbers: PI19/00017, PI15/00047, PI18/00020, PI16/00533, PI13/00233, PI21/00024, PI20/00012, and CP21/00097) and co-funded by the European Union. The funders played no role in the study design, collection, analysis, or interpretation of data, and neither in the process of writing the manuscript and its publication. JH-R had received the Contrato Rio Hortega CM20/00085 grant and is enrolled in the Ph.D. Program in Food Science and Nutrition, Universitat de Barcelona, Barcelona, Spain with file number: 5166968. OC is the recipient of the Miguel Servet PI20/00012, CP21/00097 grant, and previous JR17/00022. JS-S partially supported by the ICREA under the ICREA Academia programme.

Acknowledgments

We thank Daniel Muñoz-Aguayo, Gemma Blanchart, and Sònia Gaixas for their laboratory support, and Stephanie Lonsdale for her help in editing the English text. We also thank all PREDIMED and PREDIMED-Plus study collaborators, PREDIMED-Plus Biobank Network as a part of the National Biobank Platform of the ISCIII for storing and managing the PREDIMED-Plus biological samples. CIBER de Fisiopatología de la Obesidad y Nutrición (CIBERobn) is an initiative of the Instituto de Salud Carlos III (Madrid, Spain), and is financed by the European Regional Development Fund.

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/fnut.2022.950900/full#supplementary-material

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frontiers | Frontiers in Nutrition

TYPE Original Research
PUBLISHED 29 November 2022
DOI 10.3389/fnut.2022.970923

OPEN ACCESS

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

RECEIVED 16 June 2022
ACCEPTED 10 November 2022
PUBLISHED 29 November 2022

CITATION
Gaspar MCMP, Celorio-Sardà R, Comas-Basté O, Latorre-Moratalla ML, Aguilera M, Llorente-Cabrera GA, Puig-Llobet M and Vidal-Carou MC (2022) Knowledge and perceptions of food sustainability in a Spanish university population.
Front. Nutr. 9:970923.
doi: 10.3389/fnut.2022.970923

Knowledge and perceptions of food sustainability in a Spanish university population

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In 2015, the United Nations adopted the 2030 Agenda for Sustainable Development, with 17 Sustainable Development Goals (SDGs) at its core. Besides tackling climate change and the fight to reduce inequality, the SDG number 12 is specifically focused to develop strategies toward food sustainability. The aim of this study, aligned with SDG number 12, was to analyze the level of knowledge and perceptions of food sustainability in a university community from Spain. A descriptive cross-sectional study, based on an online questionnaire, was carried out between July and November 2021 with convenience sampling. The survey included 28 items and was distributed among students, teachers, researchers and administrative staff from a Spanish university. A total of 1,220 participants completed the survey. 70.4% of the respondents heard about the environmental impact of food and more than 50% were aware of the existence of the SDGs. The different aspects related to diet that concerned them the most were food waste, plastic usage, and environmental impact. They reported that a sustainable diet should be mainly based on local and seasonal products and with a low environmental impact as well as no or the minimum food waste. When asked if they were following a sustainable diet, 77% answered affirmatively. Moreover, the food groups more involved in a sustainable diet should be vegetables and fruits, olive oil, legumes, and whole grains. Regarding food waste, 60% of the surveyed population claimed to generate it at home, with the use of leftovers and planning shopping and meals being some of the most important domestic

actions to avoid it. Further initiatives must be implemented to increase the level of knowledge as well as to raise the awareness on the importance to translate it into individual and collective actions that allow a shift toward more sustainable practices.

KEYWORDS

sustainability, food, perception, knowledge, environmental impact, university population

Introduction

The modern food system faces an unprecedented challenge: on the one hand, to manage the environmental and socioeconomic consequences of the industrial production model, and on the other, to produce affordable and nutritious food in adequate quantities in a context of population growth in a sustainable and resilient manner, reducing environmental impacts and the overexploitation of natural resources (1–3). In this scenario, sustainability has become a key concept of new strategies promoting a global transformation of the current food system (4). Sustainability is a complex multidimensional notion that encompasses the simultaneous fulfillment of different objectives with productive, ecological, temporal, economic and socio-cultural dimensions (5, 6).

In 2015, the General Assembly of the United Nations adopted the 2030 Agenda for Sustainable Development, with 17 Sustainable Development Goals (SDGs) at its core. These SDGs are an urgent call for action to all countries in a global partnership to improve the future of people and the planet. Besides tackling climate change and inequality, the SDGs are also focused on developing strategies to foster a healthy and sustainable diet. According to Lang (7), defining what constitutes a sustainable diet is a major challenge because it is not only a matter of reconciling discourses of public health with those of ecology, but also includes the economic and cultural dimension of food (7). The Food and Agriculture Organization (FAO) defines a sustainable diet as: "those diets with low environmental impacts which contribute to food and nutrition security and to healthy life for present and future generations. Sustainable diets are protective and respectful of biodiversity and ecosystems, culturally acceptable, accessible, economically fair and affordable; nutritionally adequate, safe and healthy, while optimizing natural and human resources" (8). Although this definition is widely used, the notion of sustainability is mobilized in different ways by the multiple actors involved in the agri-food system (5). This polysemy is also reflected among consumers, who often have confused or superficial perceptions of the concept and express doubts about its meaning (9–12).

Universities have a great and undeniable potential as catalyzers for sustainability, being both formal learning institutions and places where informal, mutual influences and lay/expert knowledge meet (13–15). These organizations are also fundamental to achieving the SDGs proposed by the United Nations (16–18). In fact, one of the major challenges currently facing universities is to promote and improve training to create key professionals capable of acting in accordance with the principles of sustainability (19). Understanding how university communities perceive and understand the concept of a sustainable diet is fundamental to improve training and develop policies, educational activities and individual practices aimed at sustainability awareness and application (14, 20). However, studies addressing the perceptions of sustainability in large university communities are still lacking.

Sonetti et al. (14) analyzed the representations of sustainability and the SDGs among members of a polytechnic university in Italy and reported heterogeneous and sometimes contradictory representations, as well as a less than holistic conception of sustainability, even in those who define themselves as experts on the subject (14). A study in Spain found that university students considered sustainability to be important and that sustainability training should be included in all areas and at all levels of education. However, the study participants did not know how to define the concept of sustainability, mainly associating it with recycling and the balance between production-consumption and they were unable to express a holistic view (19). Another study, conducted with teaching staff at the University of Valencia (Spain) showed that teachers had a lack of environmental knowledge and inadequate training in sustainability-related issues (13). Busquets et al. (15) also verified among professors at several Spanish universities that their perceptions of sustainability often did not cover all its dimensions and were mainly focused on environmental aspects (15).

Taking this context into account, the aim of this study was to analyze the level of knowledge and perceptions of food sustainability in a university community from Spain.

Materials and methods

An exploratory and descriptive cross-sectional study, based on a quantitative methodology, was carried out between July and November 2021 by an interdisciplinary team composed of

researchers from the Food and Nutrition Torribera Campus of the University of Barcelona (UB).

Data production

A questionnaire was specifically designed for this study based on previous research on food, sustainability and risk perceptions (9, 12, 14). The instrument encompassed different main themes: food perception, food decisions, food concerns, food trust, level of knowledge concerning sustainability issues (environmental impact, SDGs, Green Deal, carbon footprint, biodiversity, local products, etc.), perceptions of one's own diet pattern, perceptions of food sustainability, barriers to a sustainable diet and food waste. It consisted of 28 items, 7 of which were for the socio-economic characterization of the sample and 21 concerned the level of knowledge and perceptions related to food and sustainability. Most of the questions were Likert-type and multiple-choice with predefined response options. In addition, there were two open-ended subjective free-association questions ("Which word do you associate with the concept 'food'?" and "Which word do you associate with the concept 'sustainable diet'?") (questionnaire available in **Supplementary material**).

The content of the questionnaire was validated using the Content Validity Index for Items (I-CVI) and Content Validity Index for Scale (S-CVI) (21–24). In accordance with these methods, nine experts from different fields related to the topic of food and sustainability (sociology, nutrition, food sciences, economics, anthropology, and public health) were invited to evaluate the questionnaire. These experts were selected from different UB research groups according to the relevance of their research in the field of food and sustainability. They evaluated each question of the questionnaire on a numerical scale from 1 to 4, considering the following aspects: relevance, simplicity, ambiguity and clarity. In addition, the experts were invited to make comments and suggestions for each question. The I-CVI for each question was obtained by adding the number of experts who gave the question a score of 3 or 4, divided by the total number of experts. The final score for each question ranged from 0 to 1, and the closer to 1, the greater the expert consensus. According to Lynn (21), for item acceptability the I-CVI should be no lower than 0.78, that is to say, every question with a lower value had to be compulsorily modified by the research team, based on the comments and suggestions of the experts. The S-CVI indicates the degree of consensus among experts regarding the relevance of the general content of the questionnaire. This index was calculated through the average of the I-CVIs for "relevance" by summing them and dividing by the number of items. The S-CVI also varies from 0 to 1, with 1 being the maximum consensus among experts regarding the relevance of the content. According to Polit and Beck (23), the criterion used for acceptability of the S-CVI was a score no lower than 0.90. Following this content validation, a pilot test was carried out online, *via* Google Forms, with 30 people from the university community. Once the questionnaire was completed, they were also able to comment and suggest changes to improve the instrument. The questionnaire was adjusted again after this pilot test to obtain its final version.

Context and sampling

The study was conducted within the community of the UB, one of the largest universities in Spain. The UB is composed of more than 25 centers, offering 73 bachelor's degrees and 173 university master's degrees that cover all knowledge areas: humanities, health sciences, social sciences, experimental sciences and engineering. The UB community comprises 72,161 students, 5,963 researchers and teaching staff, and 2,387 administrative and service employees.

For the study, all individuals working or studying in this university were invited to answer the questionnaire (convenience sample). No exclusion criteria were established with respect to the participant gender, age, faculty/center/scientific background, place of residence, and nationality. For the characterization of the sample, data were collected on gender, age, educational level, occupation (student, professor, or administrative staff), faculty or center affiliation, and average monthly household income.

The questionnaire was administered online between October and November 2021 *via* Google Forms and sent by email to all members of the UB community with the support of the university's administrative services. A total of 1,225 responses were obtained, 5 of which were excluded after a sensing cleaning data procedure checking for duplicates and missing data, resulting in a final data set of 1,220 responses.

Data analysis

Textual data collected with the free association questions were first pre-processed to reduce data dispersion; synonyms and multi-words were identified, and verbs were reduced to the infinitive. Four experts from the research team classified the 178 words mentioned by participants into nine analytical categories according to their semantic field. Chi-squared tests were performed for categorical variables to examine whether the proportion of participants was different across gender or affiliations (administrative staff, teaching staff, and students). When mean scores were analyzed, two-tailed independent sample *t*-tests were carried out to explore the differences between male and female and analyses of variance (ANOVA) when exploring differences among affiliations. *Post-hoc* tests were performed when differences among affiliations were detected. Spearman correlations were carried out to test the

putative relationship between perceptions of sustainability and healthiness. All these analyses were performed using SPSS for Windows 24.0. For the statistical analyses according to gender, considering the low rate of participants who indicated "others" in the questionnaire, only the participants who defined themselves as men and women were considered. In this sense, we refer to "sex" in the results.

Ethical aspects

The study was conducted according to the recommendations of the Code of Good Practice in Research of the University of Barcelona (25) and in accordance with the ethical standards laid out in the 1964 Declaration of Helsinki and subsequent updates (26). The questionnaire was anonymous, and participants accessed information about the study on the first page of the online survey, expressing their consent to participate by ticking "accepted."

Results

Participant profile

Out of the 1,220 participants (accounting for around a 2% of this university population) who completed the questionnaire, 68.3% were female, and most of them were between 51 and 65 years old (46.8%), teaching staff (48.3%), and from the health sciences academic field (33.2%). On the contrary, although students represent the largest group at the UB, they were underrepresented among the participants of this study (17.8%), which may reflect a low level of interest of this subgroup in the research topic and/or low engagement with this kind of initiatives promoted by the university. Moreover, most participants were from the field of health sciences, which may be related to their greatest awareness to health-related topics and to the affiliation of most researchers of the study. **Table 1** presents the effective response rates (i.e., percentage of potentially eligible affiliates who participated) distribution of the sample and its characteristics according to their affiliation (administrative staff, teaching staff or students).

Level of knowledge of sustainability

In general, most participants (70.4%) indicated that they had often heard about the environmental impact of food (only 4.8% had not). No statistical significant differences were found by sex (70.0% male and 70.2% female). In contrast, the analysis by affiliation showed statistical significant differences: the teaching staff were more likely to state that they had often heard about the environmental impact (76.6%) in comparison with administrative staff (71.3%) and especially in comparison with students (52.1%) (χ^2 = 73.71 and $p < 0.001$). When participants were asked if they knew about the SDGs of the UN, a statistical difference was observed related to sex more male (70.8%) than female participants (64.1%) indicated awareness of this concept (χ^2 = 5.18 and p = 0.023). Important differences were also identified among affiliations: teaching staff (78.1%) showed a higher level of knowledge than administrative staff (65.2%) and students (34.6%) (χ^2 = 134.08 and $p < 0.001$). Regarding the Green Deal, it is worth noting that 56.8% of the sample did not know about this European strategy; moreover, following the same pattern as in the previous questions, teaching staff (49.6%) presented a significantly higher level of knowledge than the other two groups (37.0% administrative staff and 37.8% students) (χ^2 = 18.93 and $p < 0.001$).

When informants were asked to evaluate from 1 to 5 their level of knowledge regarding specific concepts ("carbon footprint," "biodiversity," "greenhouse gases," etc.), the results indicated that participants tended to be more familiar with more general and less technical concepts, such as "local products/Km0" (common expression in Spanish-speaking populations that referred to local foods that have not traveled far after production) ($\bar{X}$ = 4.34, SD = 0.86) and "food waste/food lost" ($\bar{X}$ = 4.13, SD = 0.96). No significant differences were found by sex. Regarding the affiliations, once again teaching staff declared a higher level of knowledge than the other two groups, especially in comparison with students (**Figure 1**).

Food decisions and social perceptions of food sustainability

Almost all participants declared that their diet is often healthy (99.7%) and sustainable (96.3%). No statistical significant differences were observed according to sex or affiliation. Furthermore, 96.1% of the participants stated that a healthy diet corresponds to a sustainable diet.

Taking all the participants together, the factors that most influenced their eating decisions were the quality, ingredients, and nutritional composition of food (38.0%), followed by pleasure and taste (35.9%), and preventing chronic illness and its effect on health (33.0%). Factors related to food sustainability were not prioritized when food decisions were made: the origin of food and support for the agro-ecological territory (13.4%), ecology, environment, or animal welfare (8.5%) and seasonality (seasonal products) (5.2%). No statistical difference was found according to sex. The analysis by affiliation indicated several statistical significant differences, revealing that the rationalities mobilized in food choice vary according to the professional/occupational status: price was more important for the administrative staff (12.1%) than for students (10.1%) and teaching staff (7.3%) (χ^2 = 6.65 and

TABLE 1 Socio-demographic characteristics of the analyzed university community.

	Total		Affiliation (%)		
	n	%	Adm. Staff (*n* = 414)	Teach. Staff (*n* = 589)	Students (*n* = 217)
Sex					
Male	380	31.7	29.2	59.7	11.1
Female	819	68.3	36.6	42.6	20.8
Age range (years)					
18–30	233	19.1	7.3	5.2	87.6
31–50	382	31.3	38.5	58.4	3.1
51–65	571	46.8	48.3	56.0	0.2
>66	34	2.8	0.0	100.0	0.0
Academic field *					
Arts and humanities	148	12.1	34.5	62.2	3.4
Sciences	224	18.4	28.1	69.6	2.2
Health sciences	405	33.2	16.5	34.1	49.4
Social sciences	254	20.8	21.7	77.6	0.8
Technical services and associated centers	189	15.5	33.9	48.3	17.8

*Classification according to The National Agency for Quality Assessment and Accreditation of Spain, ANECA.

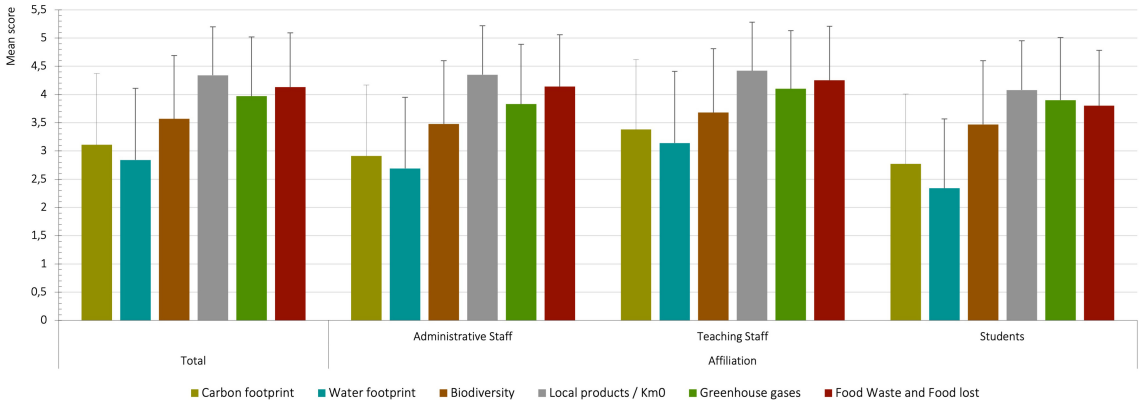

FIGURE 1
Level of knowledge of concepts related to sustainability.

$p = 0.036$); concerns about body weight or physical shape had more influence on students (10.1%) than administrative staff (5.3%) and teachers (3.2%) ($\chi^2 = 15.49$ and $p < 0.001$); the origin of food and support for the agro-ecological territory was taken into consideration far more by administrative staff (16.2%) and teaching staff (14.6%) than by students (5.1%) ($\chi^2 = 16.42$ and $p < 0.001$); the seasonality (seasonal products) was more relevant for teaching staff (7.1%) and administrative staff (4.3%) than for students (1.8%) ($\chi^2 = 9.93$ and $p = 0.007$); pleasure and taste was more important for students (48.8%) than teaching staff (37.2%) and administrative staff (27.3%) ($\chi^2 = 29.55$ and $p < 0.001$); preventing chronic illness and the effect on health had more impact on teaching staff (37.7%) and administrative staff (33.3%) than students (19.4%) ($\chi^2 = 24.18$ and $p < 0.001$); state of mind played a bigger role for students (9.2%) than teaching staff (2.7%) and administrative staff (4.1%) ($\chi^2 = 16.20$ and $p < 0.001$).

In general, when participants were specifically asked to indicate on a scale from 1 to 5 the importance given to food sustainability at the time of purchasing foodstuffs, the answers also showed that it was not a key motivation: the total average among all participants was 2.42 ($SD = 0.82$) and the average for either gender or affiliation, considered separately, reached 3 points. Statistical significant differences were found between the sexes and among affiliations. Male participants gave higher values ($\bar{X} = 2.48$, $SD = 0.86$) than females ($\bar{X} = 2.38$, $SD = 0.79$) ($F = 6.06$ and $p = 0.014$) and, contrary to the question in the previous paragraph, students gave higher values ($\bar{X} = 2.75$, $SD = 0.90$) than administrative staff ($\bar{X} = 2.43$, $SD = 0.77$)

TABLE 2 Level of concern for different food-related factors.

	Total	Sex		*P*-value	Affiliation			*P*-value
	Mean (SD)	Male	Female		Adm. Staff	Teach Staff	Students	
Pesticides	3.77 (1.12)	3.63 (1.27)	3.83 (1.18)	< 0.001	4.01 (1.10)	3.83 (1.19)	3.12 (1.27)	< 0.001*
Hygiene in the home and outside the home	4.06 (0.99)	3.91 (0.98)	4.14 (0.99)	< 0.001	4.16 (0.94)	3.96 (1.03)	4.15 (0.95)	< 0.001[ab]
Contamination by viruses and bacteria	3.78 (1.20)	3.55 (1.22)	3.90 (1.17)	< 0.001	3.96 (1.14)	3.58 (1.23)	3.99 (1.11)	< 0.001[ab]
Allergens	2.77 (1.42)	2.53 (1.34)	2.89 (1.44)	< 0.001	3.08 (1.40)	2.66 (1.38)	2.46 (1.44)	< 0.001[ac]
Presence of gluten and/or lactose	2.07 (1.34)	1.74 (1.09)	2.23 (1.41)	< 0.001	2.40 (1.39)	1.92 (1.26)	1.85 (1.31)	< 0.001[ac]
Residues of antibiotics and hormones in animal products	3.58 (1.28)	3.25 (1.31)	3.74 (1.23)	< 0.001	3.85 (1.20)	3.57 (1.26)	3.12 (1.33)	< 0.001*
Presence of chemical contaminants	4.00 (1.17)	3.87 (1.21)	4.07 (1.14)	< 0.001	4.17 (1.11)	3.99 (1.14)	3.73 (1.26)	< 0.001*
Animal welfare	3.73 (1.12)	3.50 (1.18)	3.83 (1.06)	< 0.001	3.95 (1.05)	3.61 (1.10)	3.64 (1.22)	< 0.001[ac]
Genetically modified organisms	3.12 (1.37)	2.87 (1.40)	3.24 (1.34)	< 0.001	3.59 (1.28)	3.03 (1.36)	2.45 (1.23)	< 0.001*
Chronic non-communicable diseases	3.77 (1.19)	3.66 (1.20)	3.83 (1.18)	0.020	3.97 (1.12)	3.68 (1.18)	3.67 (1.28)	< 0.001[ac]
Weight gain	3.70 (1.12)	3.70 (1.03)	3.70 (1.12)	0.980	3.89 (1.02)	3.65 (1.08)	3.45 (1.33)	< 0.001[ac]
Sugar and salt content	3.83 (1.03)	3.77 (1.06)	3.87 (1.00)	0.120	3.95 (0.96)	3.84 (1.01)	3.59 (1.14)	< 0.001[bc]
Fat and saturated fat content	3.94 (0.97)	3.82 (1.02)	4.00 (0.94)	< 0.001	4.06 (0.91)	3.99 (0.94)	3.60 (1.10)	< 0.001[bc]
Food additives	3.42 (1.19)	3.22 (1.20)	3.52 (1.17)	< 0.001	3.69 (1.06)	3.47 (1.17)	2.79 (1.26)	< 0.001*
Food waste	4.15 (0.95)	4.00 (1.02)	4.22 (0.90)	< 0.001	4.29 (0.85)	3.80 (1.14)	4.15 (0.95)	< 0.001[bc]
Use of plastic and plastic packaging	4.01 (1.03)	3.84 (1.11)	4.09 (0.98)	< 0.001	4.12 (0.97)	4.05 (0.96)	3.68 (1.24)	< 0.001[bc]
Environmental impact	3.73 (1.14)	3.58 (1.17)	3.79 (1.11)	< 0.001	3.87 (1.09)	3.72 (1.09)	3.47 (1.29)	< 0.001[bc]
Socioeconomic situation of local agriculture	3.73 (1.12)	3.67 (1.12)	3.77 (1.11)	0.130	3.93 (1.04)	3.80 (1.06)	3.15 (1.27)	< 0.001[bc]

*Indicates statistically significant differences among the three different groups of affiliation.
[a]Indicates statistically significant differences between administrative staff and teaching staff.
[b]Indicates statistically significant differences between teaching staff and students.
[c]Indicates statistically significant differences between administrative staff and students.

10.3389/fnut.2022.970923

TABLE 3 Frequency (%) of word categories associated with the concept of "sustainable diet" through the free association task.

Category name	N*	%
Values (e.g., adequate, responsible, kindly, conscious, intelligent)	280	23.0
Environment (e.g., nature, environment, planet, earth)	253	20.7
Proximity (e.g., local, Km0, seasonal)	203	16.6
Sustainable production (e.g., ecologic, organic, free from contaminants, artisanal, agroecology, circular)	122	10.0
Future (e.g., future, durable, conservation, preservation)	94	7.7
Health (e.g., healthy, nutrition, plant-based, vegan, wellbeing)	89	7.3
SDGs (e.g., food waste, climate change, reuse, sustainability)	70	5.7
Socioeconomics (e.g., justice, economy, equity)	55	4.5
Difficulties/criticism (e.g., price, expensive, impossible, fashion, utopia, laziness, inaccessible)	45	3.7

*Nine participants (0.8%) did not answer properly or indicated that they did not know.

and teaching staff ($\bar{X}$ = 2.29, SD = 0.79) (F = 26.0 and $p < 0.001$).

The food-related factors that concern the university community were also analyzed (Table 2). The three aspects that concerned the sample the most were: food waste/food lost ($\bar{X}$ = 4.15, SD = 0.95), hygienic conditions at home and outside the home ($\bar{X}$ = 4.06, SD = 0.99) and use of plastic and plastic packaging ($\bar{X}$ = 4.01, SD = 0.95). The three aspects that generated the least concern in the university community were: genetically modified organisms (GMOs) ($\bar{X}$ = 3.12, SD = 1.37), allergens ($\bar{X}$ = 2.77, SD = 1.42) and the presence of gluten and/or lactose ($\bar{X}$ = 2.07, SD = 1.34). Although the three aspects that most or least concerned female and male participants were almost the same, female participants indicated higher levels of concern for 17 of the 18 items proposed (the exception was weight gain) and statistical significant differences were observed in 15 of the 18 items. Among the affiliations, administrative staff showed greater concern for every factor except the presence of contamination by viruses (avian flu, norovirus, SARS-CoV-2, etc.) and bacteria (salmonella, listeria, etc.). Statistically significant differences were observed between affiliations for all the factors included in the survey ($p < 0.05$).

In this article the results of two main questions exploring the social perceptions of the concept "sustainable diet" were analyzed (an open-ended question "Which word do you associate with the concept 'sustainable diet'?" and a multiple choice question "Which are the three most important aspects for a sustainable diet?"). One hundred seventy-eight different words were associated with "sustainable diet," among which the five most frequently cited were: proximity (mentioned 145 times), ecological (136), environment (115), balance (63) and future (58). The categorization of these words revealed that participants associated a sustainable diet with words most related to values (responsible, kindly, etc.) and to the environmental dimension of sustainability such as: planet, environment, ecology, nature, etc. (Table 3). A third large category of words involved the idea of proximity (e.g., locality, Km0, etc.). It is worth mentioning that for this item, participants did not focus on social and economic dimensions in their answers.

When participants were asked to choose the most important aspects involved in following a sustainable diet (Table 4), the three most chosen options were the presence of locally produced, seasonal products (71.8%), that the diet was respectful of ecosystem biodiversity and had a low environmental impact (68.6%), and no or minimum food waste (37.7%). No significant differences were found between males and females. Regarding the affiliations, statistically significant differences were found for 6 out of the 10 aspects ($p < 0.05$). In general, administrative and teaching staff gave similar responses and were differentiated from the students, who gave more importance to locally produced food (81.6%), biodegradable and compostable packaging (46.1%), and the monetary cost (28.1%).

Participants were also asked to rate from 1 to 5 different foodstuffs regarding their healthiness and sustainability value (Figure 2). It should be mentioned that no correlation was found for these two aspects with sex or affiliation. In general, the three products perceived as most sustainable were vegetables ($\bar{X}$ = 4.71, SD = 0.75), fruit ($\bar{X}$ = 4.70, SD = 0.76), and olive oil ($\bar{X}$ = 4.59, SD = 0.84). The products perceived as the least sustainable were distilled alcoholic beverages ($\bar{X}$ = 1.40, SD = 1.22), snacks, sweets, and pastries ($\bar{X}$ = 1.16, SD = 1.06) and sweetened beverages ($\bar{X}$ = 1.05, SD = 1.03). Between the genders, statistically significant differences in the perceived sustainability value were found in the case of olive oil (male $\bar{X}$ = 4.53, female $\bar{X}$ = 4.64, t = 2.16 and p = 0.031), potatoes (male $\bar{X}$ = 4.30, female $\bar{X}$ = 4.45, t = 2.55 and p = 0.011), nuts (male $\bar{X}$ = 4.28, female $\bar{X}$ = 4.42, t = 2.22 and p = 0.027), whole grains (male $\bar{X}$ = 3.76, female $\bar{X}$ = 3.93, t = 2.10 and p = 0.036), fermented alcoholic beverages (male $\bar{X}$ = 2.45, female $\bar{X}$ = 2.25, t = 2.42 and p = 0.016), and refined grains (male $\bar{X}$ = 2.27, female $\bar{X}$ = 2.04, t = 2.67 and p = 0.008). Among affiliations, statistically significant differences were found in red meat (F = 4.30 and p = 0.014), fish (F = 5.31 and p = 0.005), dairy products (F = 3.78 and p = 0.023), eggs (F = 11.17 and $p < 0.001$), nuts (F = 8.09

TABLE 4 Distribution (%) of answers about the most important aspects of a sustainable diet.

	Total		Sex (%)		*P*-value	Affiliation (%)			*P*-value
	n	%	Male	Female		Adm. Staff	Teach. Staff	Students	
Rich in plant-based foods	106	8.8	10.8	7.9	0.105	8.2	12.4	1.8	<0.001
With no or the minimum amount of food waste	450	37.7	39.2	36.8	0.413	38.4	37.9	33.6	0.463
With biodegradable, compostable packaging	372	31.0	28.2	32.4	0.144	31.9	25.6	46.1	<0.001
With locally produced, seasonal products	863	71.8	74.5	70.8	0.190	67.1	71.5	81.6	0.001
Respectful of ecosystem biodiversity and with a low environmental impact	822	68.6	69.2	68.3	0.740	69.8	69.1	65.0	0.434
With products from companies that respect workers' social rights	165	13.8	25.5	15.0	0.064	14.7	13.8	12.9	0.807
Affordable	250	20.9	21.8	20.4	0.565	20.0	18.8	28.1	0.014
Organic/ecological	186	15.5	12.6	16.8	0.060	16.7	16.6	9.2	0.022
Simple, without additives, based on foods with few ingredients and little processed	339	28.3	30.0	27.5	0.366	28.0	31.7	18.0	0.001
Culturally acceptable	43	3.6	2.6	4.0	0.226	4.8	2.5	3.7	0.153

and $p < 0.001$), refined grains ($F = 5.11$ and $p = 0.006$), legumes ($F = 5.19$ and $p = 0.006$), olive oil ($F = 8.75$ and $p < 0.001$), snacks ($F = 3.46$ and $p = 0.032$), coffee and tea ($F = 3.09$ and $p = 0.046$), and fermented alcoholic beverages ($F = 16.69$ and $p < 0.001$).

Participants were also asked to rate from 1 to 5 the extent to which different factors could hinder them in following a sustainable diet (Table 5). The total average scores for all the factors were higher than 3, which may indicate that all of them can impede the implementation of sustainable practices. For the whole sample, in order of importance, the three factors considered to be the main barriers were: cost ($\bar{X} = 4.26$, $SD = 0.87$), lack of information ($\bar{X} = 4.14$, $SD = 0.95$), and ease of purchase (accessibility) ($\bar{X} = 3.99$, $SD = 0.98$). No significant differences were observed between male and female informants and among the three affiliations.

Food waste

Two questions specifically addressed the issue of food waste. The first one focused on the frequency with which participants waste food, in which they were asked to evaluate their food waste from 1 to 4. Among all the participants, the mean frequency score was 2.56 ($SD = 0.70$) out of 4, without significant differences according to sex or affiliation. The second question concerned the actions carried out by participants to avoid food waste (Table 6). Planning shopping and meals ($\bar{X} = 4.72$, $SD = 0.58$), reusing leftovers ($\bar{X} = 4.60$, $SD = 0.70$) and making a shopping list ($\bar{X} = 4.37$, $SD = 0.94$) are the three actions most employed by the informants. Four statistical significant differences were found between male and female individuals: reusing leftovers ($t = 5.82$ and $p < 0.001$), planning shopping and meals ($t = 3.63$ and $p < 0.001$), making a shopping list ($t = 5.17$ and $p < 0.001$) and consuming foods that last longer (frozen foods, preserves) ($t = 0.90$ and $p = 0.008$). Significant differences were observed among the three affiliations in six of the nine options proposed in the questionnaire ($p < 0.05$): planning shopping and meals, making a shopping list, learning cooking techniques to preserve foods, making organic compost, consuming foods that last longer (frozen foods and preserves) and taking part in initiatives to recover food.

Discussion

To our knowledge, this is the first study to analyze the level of knowledge and social perceptions of food sustainability of a whole university community in Spain. The study was carried out with the teaching staff, administrative staff and students of the UB, one of the largest universities in the country. Overall, the results indicated that a greater effort is needed to enhance knowledge of food sustainability and to increase the importance given to this dimension when food choices are made.

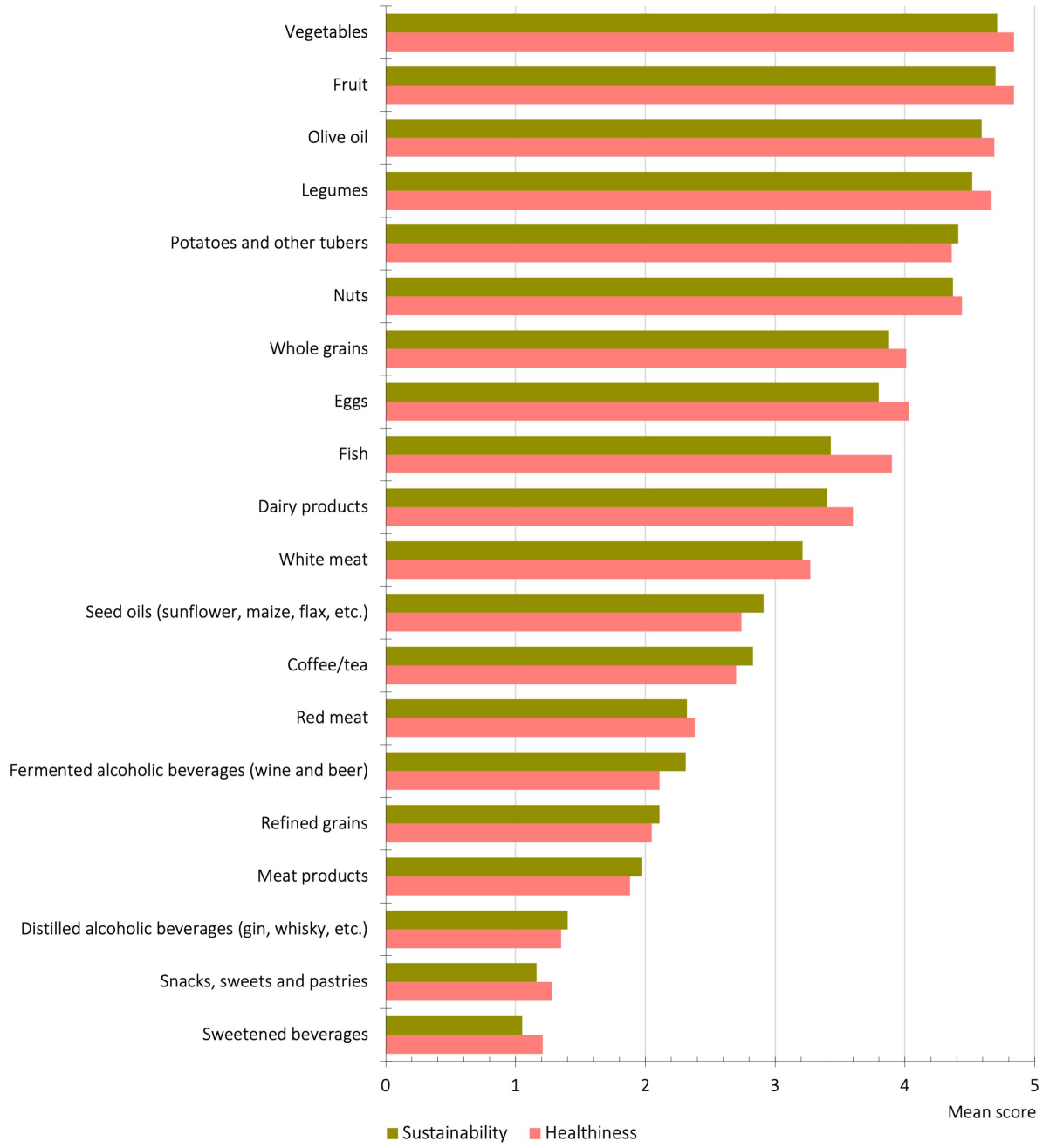

FIGURE 2
Perceptions regarding the level of sustainability and healthiness of different foods.

Level of knowledge of sustainability

Although most participants declared that they had often heard about the environmental impact of food or the SDGs, the level of knowledge was lower for specific or more technical aspects of sustainability, such as: "carbon footprint," "biodiversity," or "greenhouse gases." Respondents had higher levels of knowledge of concepts that appear more frequently in the media and that can be directly applied in personal practices, such as local food/Km0 and food waste. These concepts feature in many of the recommendations made by different institutions for the implementation of sustainable individual practices in Catalonia, such as the report Sustainable food: a handbook for cities by the Barcelona City Council (27). Burkhart et al. (28) also observed that Australian dietetic students were familiar with and concerned about sustainability, but in a superficial way, since the

TABLE 5 Factors perceived as barriers to following a sustainable diet.

	Total	Sex		*P*-value	Affiliation			*P*-value
	Mean (SD)	Male	Female		Adm. Staff	Teach. Staff	Students	
Cost	4.26 (0.87)	4.14 (0.94)	4.31 (0.839	0.319	4.33 (0.85)	4.21 (0.86)	4.25 (0.92)	0.112
Lack of information	4.14 (0.95)	3.97 (1.00)	4.22 (0.91)	0.160	4.16 (0.94)	4.11 (0.95)	4.15 (0.97)	0.738
Lack of culinary knowledge	3.35 (1.19)	3.31 (1.15)	3.38 (1.21)	0.059	3.46 (1.19)	3.29 (1.19)	3.31 (1.18)	0.680
Lack of time	3.62 (1.21)	3.57 (1.23)	3.64 (1.21)	0.436	3.68 (1.23)	3.61 (1.20)	3.52 (1.21)	0.291
Food preferences and taste	3.23 (1.21)	3.34 (1.18)	3.18 (1.22)	0.545	3.24 (1.18)	3.21 (1.23)	3.29 (1.23)	0.670
Food traditions	3.45 (1.15)	3.45 (1.14)	3.45 (1.15)	0.928	3.47 (1.14)	3.44 (1.14)	3.42 (1.19)	0.859
Accessibility to food	3.98 (0.98)	3.86 (0.95)	4.04 (0.98)	0.640	3.97 (1.00)	3.95 (0.96)	4.07 (0.99)	0.316

TABLE 6 Actions to avoid food waste at home.

	Total	Sex (%)		*P*-value	Affiliation (%)			*P*-value
	Mean (SD)	Male	Female		Adm. Staff	Teach. Staff	Students	
Use leftovers	4.60 (0.67)	4.44 (0.75)	4.68 (0.61)	< 0.001	4.60 (0.69)	4.63 (0.64)	4.53 (0.71)	0.163
Plan shopping and meals	4.72 (0.58)	4.63 (0.65)	4.76 (0.54)	< 0.001	4.78 (0.46)	4.72 (0.57)	4.59 (0.75)	0.001[bc]
Write shopping lists	4.37 (0.94)	4.17 (1.02)	4.46 (0.88)	< 0.001	4.51 (0.80)	4.30 (0.99)	4.29 (0.98)	0.001[ac]
Buy smaller quantities of food	3.98 (1.04)	3.85 (1.05)	4.05 (1.02)	0.480	4.08 (1.02)	3.98 (1.02)	3.80 (1.12)	0.007[c]
Learn cooking techniques to preserve foods	3.92 (1.04)	3.78 (1.04)	3.99 (1.02)	0.266	4.10 (0.97)	3.77 (1.07)	3.98 (1.00)	< 0.001[ab]
Make organic compost	2.96 (1.24)	2.76 (1.20)	3.05 (1.24)	0.950	3.04 (1.26)	2.79 (1.21)	3.24 (1.21)	< 0.001[ab]
Consume foods that last longer	2.90 (1.16)	2.95 (1.10)	2.89 (1.19)	0.008	2.94 (1.24)	2.74 (1.09)	3.27 (1.14)	< 0.001*
Take leftovers home from a restaurant	3.54 (1.21)	3.36 (1.18)	3.63 (1.21)	0.266	3.58 (1.19)	3.46 (1.20)	3.65 (1.25)	0.087
Take part in initiatives to recover food	3.31 (1.25)	3.04 (1.26)	3.44 (1.23)	0.657	3.36 (1.23)	3.11 (1.25)	3.73 (1.20)	< 0.001*

*Indicates statistically significant differences among the three affiliations.
[a]Indicates statistically significant differences between administrative staff and teaching staff.
[b]Indicates statistically significant differences between teaching staff and students.
[c]Indicates statistically significant differences between administrative staff and students.

level of familiarity was low when specific factors encompassed by the concept were assessed. García-González et al. (9) identified a similar situation in a Spanish sample: the most recognized concepts were "environmental impact" and "local food," whereas the least familiar were "carbon footprint" and "green water/blue water." Moreover, in the present study, the level of knowledge of topics related to sustainability depended mainly on the affiliation: while teaching staff presented a higher level of knowledge for almost all the aspects included in the questionnaire, students showed the lowest levels. Sonetti et al. (14) also found that researchers and professors from an Italian polytechnic university had the highest levels of knowledge of the SDGs, followed by postdoc and PhD students, the technical and administrative staff and students. This difference may be related to the level of formal education, as previous studies have revealed that the higher the level of education, the greater the understanding of sustainability (29, 30). Furthermore, this difference may also be related with the age of the participants, given that in a previous study with a general Spanish population, younger individuals were less motivated to adapt their diet to achieve a more sustainable pattern (29). Indeed, it is notable that students represent the largest group at the UB, but they had the lowest response rate to the survey. These data suggest that it would be worthwhile to develop strategies focused on improving the knowledge and motivation of students.

Food decisions and social perceptions of food sustainability

Almost all the sample perceived their food-associated practices to be sustainable. This self-perception of diet can be a barrier to motivation to make changes toward a more sustainable diet. Indeed, the results regarding food decisions indicated that factors related to sustainability were not prioritized and that overall this issue was not taken into account when purchasing food. The three most important factors that influenced participant food choices were: the quality, ingredients, and nutritional composition, followed by pleasure and taste and preventing chronic illness and the effect on health. According to the Eurobarometer on perceptions of food sustainability, when making their food purchases, Europeans prioritize taste, food safety and cost over sustainability concerns (31). According to Díaz-Méndez (32), the priorities of people

who cook in Spanish households are: having a varied, balanced and tasty diet, and eating in company (32). Her study found that health and taste predominate in Spanish food decisions. The present study did not reveal statistical significant differences according to sex in factors that influence food choice, but important differences were observed among the affiliations. Students, who were the youngest group, expressed concerns about the body and the hedonic and emotional dimensions of food choice to a greater extent than the other two affiliations. The latter were more influenced by health-associated issues and took sustainability-related factors more into account. These differences may be associated with age and level of education. The Eurobarometer indicated that the younger the respondent, the less likely they are to cite food safety as important compared to those who are aged 55 and over. Furthermore, among Europeans, the longer the respondent remained in education, the more likely they were to state that nutrient content is important in food choice (31).

The analysis of food-related issues of most concern for the university community revealed that in this case, sustainability-related factors, mainly related to food production, generated more concern than nutritional aspects. According to Contreras (33), the main food-associated problem facing Western industrialized society a century ago was scarcity, but the increased productivity following the Green Revolution and food industrialization has led to the emergence of a new set of issues related to food excesses, globalized food crises or food waste, engendering new concerns about production methods and their impact on the environment. Beck (34) states that with the industrialization of society, risks have become a constant in the daily lives of individuals in Western countries (34), especially concerning food consumption (35, 36). In lay social perceptions, foods derived from the industrial agri-food system are often associated with toxicity and are viewed as harmful to human and planetary health (37–39). Adamiec (40) further notes that beyond contemporary morals that encourage individuals to feel responsible for their health and their bodies, another morality is being forged that makes them feel responsible for their social and natural environment. Consequently, new food concerns and discourses have emerged (40).

The Eurobarometer on the perceptions of food risks confirms that Europeans associate them above all with chemicals and pesticides applied in production, antibiotics used in breeding, pollutants such as mercury and dioxins, animal welfare, etc., to the detriment of nutritional concerns (41, 42). In general, the concerns and perceptions related to food risks in Catalonia seem to follow the European trend (43). According to the Barometer of Food Safety in Catalonia (44), in 2015, compared to previous years, there was a higher perceived frequency of food risks, especially in relation to fruit or vegetables carrying pesticide residues (45). Another report indicated that food safety is no longer associated so much with problems related to access to food or its nutritional composition (such as fat content), but rather with the health and hygiene dimension and with the contamination and toxicity of products from production practices (46). This study revealed that a significant proportion of informants were suspicious and critical of the intensive agricultural production model, the use of pesticides and the techniques used in modern farming, such as the use of hormones. The insecurity derived from environmental pollution and production methods thus represented major concerns for the Catalan population.

However, it should be noted that food perceptions or food concerns are not always reflected in the day-to-day behaviors of individuals (47, 48), which are conditioned by a multitude of social, cultural, economic, symbolic and material aspects (33, 49). In fact, it is notable that the issue that generated most concern among the participants of the present study was wasting food, a widespread practice in the general population. According to the Ministry of Agriculture, Fisheries and Food Panel for the quantification of food waste in households, in 2020, three out of four Spanish households wasted some food and wastage reached 1,363 million kg or liters of food and beverages (50).

Female participants expressed higher levels of concern for 17 out of the 18 options proposed (the exception was weight gain). This stronger concern among women may be associated with differential historically and socially constructed gender roles (51). Women are more likely to establish a relationship between food and health, be involved in reproductive care and feeding activities at home (especially in relation to child nutrition education), internalize more food and nutrition recommendations, as well as control their diet (42, 51–54). The fact that almost 70% of the sample is made up of women may itself be an indication that this is a subject (food, health, or sustainability) that arouses greater interest and concern in the female than the male collective. However, it is surprising that no gender difference was found for concerns related to weight gain, considering that most scientific literature shows that the female body is more subjected to normativization and aesthetic pressure (55, 56).

Analyzing the perceptions of what constitutes a sustainable diet revealed that the social representations of the participants are not very holistic and are mainly associated with the ecological dimension at the cost of social and economic dimensions. The perceptions of the university population analyzed in this study are in this sense similar to those of the Spanish population in general. García-González et al. (9) point out that although the FAO definition of a sustainable diet emphasizes that sustainable food should not only be environmentally friendly, but also culturally acceptable, accessible, and economically fair, these aspects are underestimated by the Spanish population (9). Research with university samples in Spain and other countries has also found that this population lacks a holistic view of the concept of a sustainable diet (13–15, 19). These results suggest that it is crucial to develop training activities to foster a broader and

more complex conception of food sustainability among the university community. Moreover, the promotion of knowledge and sustainable practices may operate not only through teaching activities and research but also through actions that seek social impact and transformation, as well as the co-management of the university environment itself (57, 58).

Participants associate a sustainable diet above all with the consumption of local and seasonal products, respect for ecosystem biodiversity, a low environmental impact, and no or a minimum amount of food waste. It is worth noting that local products and food waste were also the topics that the participants knew most about, and that receive widespread attention in the media. The theme of local products in particular seemed to be of great importance to the participants. In studies with participants from different cultural backgrounds, including in Spain, local or proximity products have been increasingly valued and associated with trust, good quality and health (36, 39). The interest in local products is a reaction to the transformations provoked by the industrial food system that cause a weakening of the links between food and territory and among the eater and food and the natural/cultural environment (37, 59). "Eating local" would allow a return to tradition and the know-how that individuals are afraid of losing in a globalized society (60) and it would be a way to rediscover a sense of security with respect to modern food (59). Moreover, studies conducted in Spain have observed an increase in the purchase of food locally produced and/or sold through short-circuit retail during the COVID-19 pandemic (61–63). The Barometer of the Government of Catalonia and the Promoter of Catalan Exports (Prodeca) has reflected this trend by revealing that 37% of Catalan individuals bought more local products during the pandemic (only 8% bought less) (64). This phenomenon may be related to changes in perceptions of the agri-food chain that occurred during this period, and which has led to the questioning of the global agri-food production and distribution system and greater solidarity with and appreciation of local producers (63, 65).

With regard to the perception of different foods in terms of their healthiness and sustainability, in both cases, there is a more positive assessment of foods of plant origin, especially fruit, vegetables and olive oil, in concordance with their lower environmental impact (66), at the cost of foods of animal origin, mainly red meat and processed products. These results corroborate the analyses of other studies carried out in different socio-cultural contexts (9, 12, 36, 39). Fruit and vegetables are perceived as healthier and more sustainable because of their nutritional composition (rich in vitamins, minerals and fiber), because they are considered fresh and natural, and they are products that generate a link with regional or national agriculture (40, 67, 68). In general, studies also reveal a positive perception of olive oil (59, 69). Likewise, the Spanish population seems to associate itself directly with the Mediterranean Diet, which in their social representations evokes healthiness, tradition, proximity, and sustainability (70).

The consumption of meat and dairy products has been questioned due to the impact of their production on the environment (1, 71) and this problematization seems to be reflected in the perceptions of the studied university community. In all historical periods and socio-cultural groups, animal products, mainly red meat, are shrouded in ambiguous and ambivalent discourses (59, 64). Fischler (37) notes that meat is at the same time the most sought-after food for humans, and the most abhorred food. Moreover, meat is attached to contradictory meanings within Western societies (52). This ambivalence seems to be linked to objective aspects, associated with the nutritional composition and the effect on health, but also to subjective, symbolic and ethical aspects concerning animal welfare, the environment and man's relationship with animals and death. Moreover, this perception of meat has intensified in recent years following the World Health Organisation [WHO] (72) on the consumption of meat products and the prevalence of diseases (72), but also as a consequence of the discourses that warn about the impacts on the environment (1, 3).

As a whole, the data on food perceptions are indicative that the studied population has internalized institutional discourses regarding both nutrition and sustainability. Recommending a lower consumption of animal and processed foods while promoting plant-based diets is widespread, including in public health recommendations in Spain (73) as well as in the Eat-Lancet Report (3) or even the IPCC (74). However, data on individual practices confirm that internalized knowledge and norms are not always reflected in day-to-day practices, which are complex and conditioned by a multitude of factors, as observed in this study. It is noteworthy that the percentage of the population following a vegetarian or vegan diet has increased in the last decade (75) as social awareness of the environmental impact of food has also grown. However, meat consumption must be reduced still further to align human health with planetary health (66). A study carried out in Barcelona, for example, has shown that CO2 equivalent emissions generated by food and drink consumption amount to 2.5 million tons per year. Domestic food consumption by residents is responsible for 3/4 of the emissions. Some foods, such as meat, dairy, eggs and seafood, are targeted as the most problematic, being responsible for about 60% of the carbon footprint of household consumption. According to the authors of the study, if 25% of the city's residents cut back their consumption of animal protein, emissions would be reduced by 285,000 tons of CO_2 equivalents, corresponding to an 11% decrease in the city's carbon footprint (76).

All the factors proposed as possible barriers to sustainable diets received scores of more than three out of five, revealing that following a sustainable diet is perceived as difficult. Corroborating previous studies, these aspects include price, lack

of information and accessibility. A lack of money is seen as central to not being able to eat well in general, but especially healthy and sustainable food (33). Likewise, the price of food considered sustainable (organic, local, etc.) represents a barrier to sustainable eating, even for those who are not in a situation of poverty. Studies indicate that price is a crucial factor in the decision to buy sustainable products (11, 77, 78). According to a recent report on perceptions of organic products issued by the Catalan Government, among the factors that would encourage the purchase of organic products, a more affordable price clearly stands out (57.7%). The survey also reveals that among citizens who are familiar with organic products but do not consume them, price is the most cited reason (53.4%). Concerning the level of consumer information, research conducted in different Spanish cities shows that several factors limit the transition process from internalized values and knowledge to their application in sustainable purchasing decisions and practices, among which the level of education and information stand out. According to Eldesouky et al. (79), higher levels of consumer education tend to go hand in hand with a better understanding of environmental issues, and these consumers even show a higher degree of sensitivity or willingness to consider them as relevant attributes in their purchases (79). The accessibility of sustainable products is another important factor. The Eurobarometer on perceptions of food sustainability reveals that almost half of the respondents (especially individuals from disadvantaged social classes) state that affordability of healthy and sustainable food (49%) would help them to adopt a healthy and sustainable diet and for 45% having healthy and sustainable food options available where they usually buy food would help them adopt a healthy and sustainable diet (31). Accessibility can be directly related to the social environment and where people live. A study conducted in Barcelona's metropolitan area on the characteristics of the city's food environments and access to shops with organic products found that access to these shops is unevenly distributed across the city and is conditioned by the socio-economic status of neighborhoods (80).

Food waste

The final part of the questionnaire dealt with the issue of food waste. The mean score for the frequency with which food is wasted among all the participants was 2.56 out of 4 (without differences according to sex or affiliation), indicating that wasting food is a widespread practice, although most participants expressed concern about it and considered its reduction to be very important for food sustainability. Data linking diet quality and sustainability are typically focused on a limited set of markers, and normally do not include food waste as an indicator (81, 82), despite a growing focus on understanding where and how food is wasted in the food system (83). Globally, enough food is wasted every year to feed nearly 2 billion people a 2,100 kcal/day diet (81), which magnifies the negative environmental impact associated with agriculture and resource scarcity. Food waste is a useful indicator of sustainability as it embodies all the resources used to produce uneaten food (83).

Recently it has been observed that some strategies to prevent food waste have been implemented in households inspired by growing social awareness of the matter (84). In the present study, it was found that women seemed more likely than men to use strategies related to household food management when trying to avoid food waste at home. This may be directly related to socially constructed gender roles, which give women greater responsibility for reproductive activities in the domestic environment, mainly related to food management (51, 85). Among the affiliations, teaching staff and administrative staff were more likely to employ actions related to food purchasing, such as planning shopping and meals and buying smaller quantities of food, compared to the students.

Limitations

Despite the originality and relevance of this research, especially in Spain, some methodological limitations should be emphasized. First, the study was carried out within a single academic institution. Although the UB is one of the largest universities in Spain, the sample should be expanded to include other institutions and geographical contexts. Likewise, the convenience sample of this research may involve some risk of bias because the participants may already present a certain profile or interests related to the topic. Moreover, due to the underrepresentation of students among participants of this study, in future research it could be necessary to use other recruitment strategies to improve the student's participation, as they may be key agents for the transformation of the food system in the next few years. Furthermore, the study is based on a quantitative approach. Although this methodology is widely used for the study of food perceptions (14, 59, 86), qualitative data could provide more in-depth results and new insights (87).

Conclusion

This study has shown that in general the level of knowledge held by the analyzed university community about the more technical aspects of food sustainability is low, especially among students. Likewise, although different aspects of food sustainability generate a high level of concern in the student population, especially in women, sustainability is not among the main factors that influence food decisions. Finally, regarding perceptions, a less than holistic conception of sustainability has been revealed that does not include the social and economic dimensions.

The direction of the UB is committed to the SDGs and is carrying out various actions to implement them in the different areas of interest and among all the university groups with the aim of promoting sustainability in the academic sphere. Overall, the results indicate that a greater effort is needed to enhance knowledge of food sustainability and to improve the importance given to this dimension in food choice in the university community. Moreover, the findings of the present study highlight that these strategies should be designed taking into account the differences between the different affiliations.

Data availability statement

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

Author contributions

MG, RC-S, OC-B, ML-M, GL-C, MP-L, and MCV-C: conceptualization. MG, RC-S, OC-B, and ML-M: investigation and writing—original draft preparation. MG, RC-S, OC-B, ML-M, and MA: data analysis. MG, RC-S, OC-B, ML-M, MA, GL-C, MP-L, and MCV-C: writing—review and editing. GL-C, MP-L, and MCV-C: supervision. All authors have read and agreed to the published version of the manuscript.

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/fnut.2022.970923/full#supplementary-material

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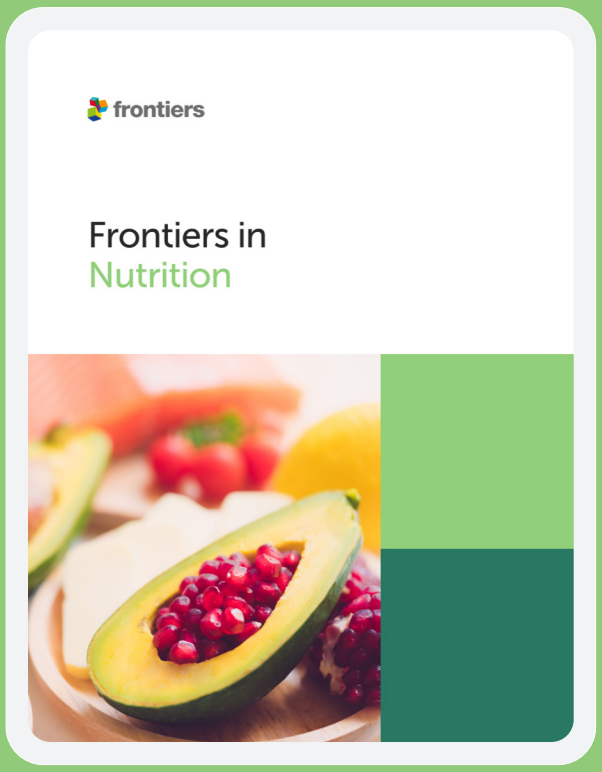

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