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Original Research Article

Effect of 1-year lifestyle intervention with energy-reduced Mediterranean diet and physical activity promotion on the gut metabolome and microbiota: a randomized clinical trial

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ABSTRACT

Background: The health benefits of the Mediterranean diet (MedDiet) have been linked to the presence of beneficial gut microbes and related metabolites. However, its impact on the fecal metabolome remains poorly understood.

Objectives: Our goal was to investigate the weight-loss effects of a 1-y lifestyle intervention based on an energy-reduced MedDiet coupled with physical activity (intervention group), compared with an ad libitum MedDiet (control group), on fecal metabolites, fecal microbiota, and their potential association with cardiovascular disease risk factors.

Methods: A total of 400 participants (200 from each study group), aged 55–75 y, and at high cardiovascular disease risk, were included. Dietary and lifestyle information, anthropometric measurements, blood biochemical parameters, and stool samples were collected at baseline and after 1 y of follow-up. Liquid chromatography-tandem mass spectrometry was used to profile endogenous fecal metabolites, and 16S amplicon sequencing was employed to profile the fecal microbiota.

Results: Compared with the control group, the intervention group exhibited greater weight loss and improvement in various cardiovascular disease risk factors. We identified intervention effects on 4 stool metabolites and subnetworks primarily composed of bile acids, ceramides, and sphingosines, fatty

Abbreviations: 3-MAA, 3-methyl-adipic acid; CG, control group; CI, confidence interval; CVD, cardiovascular disease; DPA, docosapentaenoic acid; FDR, false discovery rate; IG, intervention group; MedDiet, Mediterranean diet; MET, metabolic equivalent of tasks; PERMANOVA, permutational multivariate analysis of variance; PREDIMED, PREvención con DIeta MEDiterránea; WGCNA, Weighted gene coexpression network analysis.

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acids, carnitines, nucleotides, and metabolites of purine and the Krebs cycle. Some of these were associated with changes in several cardiovascular disease risk factors. In addition, we observed a reduction in the abundance of the genera *Eubacterium hallii* group and *Dorea*, and an increase in alpha diversity in the intervention group after 1 y of follow-up. Changes in the intervention-related microbiota profiles were also associated with alterations in different fecal metabolite subnetworks and some cardiovascular disease risk factors.

Conclusions: An intervention based on an energy-reduced MedDiet and physical activity promotion, compared with an ad libitum MedDiet, was associated with improvements in cardiometabolic risk factors, potentially through modulation of the fecal microbiota and metabolome.

This trial was registered at https://www.isrctn.com/ as ISRCTN89898870 (https://doi.org/10.1186/ISRCTN89898870).

Keywords: lifestyle intervention, Mediterranean diet, cardiovascular disease risk factor, metabolic syndrome, fecal microbiota, fecal metabolome

Introduction

The traditional Mediterranean diet (MedDiet) is characterized by a high intake of vegetables, fruits, legumes, whole cereals, and nuts; moderate consumption of fish and seafood; moderate-low consumption of dairy products; low consumption of meat and meat products; moderate alcohol intake (in the form of red wine during meals); and the use of olive oil as the main source of fat [1]. It has been widely demonstrated that the MedDiet pattern represents a nutritional strategy with significant beneficial effects for the prevention of cardiovascular diseases (CVD) [2], obesity [3], and related metabolic consequences [4], and reducing all-cause mortality [5].

Greater adherence to the MedDiet has also been positively associated with beneficial gut bacteria and derived microbiota-related metabolites [6]. These effects have been partially explained by the increase of fiber-degrading species and anti-inflammatory responses in the human body [7]. However, the effect of the MedDiet on gut microbiota and plasma metabolome is heterogeneous across the studies and the potential effects on cardiovascular disease risk factors remain unsettled [8,9].

Blood metabolome is commonly used in human studies to explore the associations of gut microbiota-derived metabolites with cardiometabolic diseases. Combining the results of plasma metabolomics and 16S sequencing, it is possible to identify specific networks that suggest an interplay between diet, circulating metabolites, and gut microbiota [10]. For instance, higher adherence to the MedDiet improved postprandial glucose metabolism and insulin sensitivity in subjects with obesity/overweight possibly mediated by gut microbiota metabolites, such as butyric acid derived from the fermentation of dietary fiber in the colon [11]. Although the effect of diet on gut microbiota and plasma or urine metabolites and its relationship with cardiovascular disease risk factors has been reported by different studies, few of them have been focused on the fecal metabolome.

In participants with obesity and overweight exposed to the MedDiet intervention, a decrease in plasma and urine concentrations of carnitine, and significant reductions in plasma cholesterol and fecal bile acid concentrations were reported [12]. In addition, the metagenomic analysis showed increased levels of the fiber-degrading bacteria and genes for microbial carbohydrate degradation linked to butyrate metabolism [12].

After 2 mo on a MedDiet intervention, participants with metabolic syndrome showed enrichment in gut bacterial genera related to bile acid metabolism and increased levels of fecal cadaverine and these changes were associated with an improvement in insulin sensitivity [13].

Even if the effects of the MedDiet in reducing the risk of numerous noncommunicable diseases have been described, more studies are needed to help understand the potential effects using a more detailed examination of microbes and metabolites [9], especially considering recent findings highlighting potential difficulties when inferring microbiome-cardiometabolic disease associations from either blood or fecal metabolome data [8].

Hence, within the framework of the PREvención con DIeta MEDiterránea (PREDIMED)-Plus randomized trial, we explored the effects of a 1-y intensive lifestyle intervention based on an energy-reduced MedDiet, physical activity, and behavioral support (intervention group [IG]) compared with an ad libitum MedDiet (control group [CG]), on fecal metabolites and fecal microbiota of 400 individuals with overweight/obesity and metabolic syndrome.

Methods

Participants and study design

This study was conducted within the frame of the PREDIMED-Plus trial, with further details provided in the Supplementary material.

This study encompasses the analysis of a subsample of 400 participants (CG, $n = 200$; IG, $n = 200$) from the PREDIMED-Plus recruiting centers of Alicante, Barcelona, Reus, and Valencia, with available fecal microbiota 16S data and fecal metabolomics data at both time points (baseline and 1 y of intervention) to evaluate the effect of 1-y lifestyle intervention on fecal metabolome and microbiota.

Intervention

The PREDIMED-Plus intervention was designed to last 6 y plus 2 y of follow-up. Participants randomly assigned in the IG were trained by a dietitian to modify their lifestyle through an er-MedDiet (energy reduction of 30% of individual estimated energy requirements) and increase their physical activity to reduce their body weight [14]. IG participants received lifestyle recommendations (related to diet and physical activity) and behavioral support through a face-to-face educational program. In addition, during the first year of the intervention, IG participants attended 2 monthly visits (1 group session and 1 individual session) and received 1 monthly telephone call.

A 17-item questionnaire was used to assess adherence to the er-MedDiet [15]. This 17-item questionnaire is a modified version of the previously validated 14-item Mediterranean Diet Adherence Screener questionnaire [16]. Specifically, this modified questionnaire limits the consumption of the less recommended food groups for weight loss (i.e., red and processed meats, butter and margarine, sugary drinks, and refined cereals).

All participants were also encouraged to increase their usual physical activity by recommending brisk walking for $\geq$45 min/d or equivalent activity and performing specific exercises to increase strength, balance, and flexibility (aim to increase moderate-to-vigorous physical activity $\geq$150 min/wk). These activities and sedentary behavior were evaluated with questionnaires validated for the Spanish population and administered periodically [17]. Total physical activity was calculated in metabolic equivalent of tasks (MET) min/wk and sedentary behavior in h/d, as previously described [18]. Participants in

the CG received recommendations to improve their adherence to the MedDiet in twice-a-year group sessions and did not receive recommendations to increase physical activity. A schematic representation of the PREDIMED-Plus intervention is reported in Figure 1.

Clinical variables, anthropometric measurements, and blood biochemistry

Detailed information regarding dietary assessments, collection of nondietary variables, anthropometric measurements, and blood biochemical parameters is provided in the Supplementary material.

Stool samples collection

Stool samples at the baseline visit and 1 y were collected by the participants in a sterilized airtight flask. They were instructed to bring the sample to the study center within 12 h of excretion under refrigerated conditions (i.e., to be kept frozen at –20°C at home until delivery to the laboratory). Participants who were using antibiotics or pre/probiotics 15 d before sample collection ($n = 4$) were identified and excluded from the final sample size. The stool samples were then divided into 250 mg aliquots and stored at –80°C until analysis.

Fecal metabolomics analyses

Metabolomics analyses of stool samples collected at baseline and after 1 y of follow-up were conducted using a liquid chromatography-tandem mass spectrometry metabolomics platform. A detailed description is provided in the Supplementary material.

Fecal bacterial DNA extraction and 16S amplicon sequencing

A detailed description of the fecal microbial DNA extraction, amplicon libraries preparation, 16S sequencing procedure, and pipeline utilized to obtain the final data are provided in the Supplementary material.

Statistical analyses

The baseline clinical characteristics and changes during the follow-up were described according to the study groups. Numeric variables were summarized using means and SDs, whereas categorical variables were described as numbers and percentages. Group differences in anthropometric, biochemical, and lifestyle parameters were tested with Student's t test, and P value of <0.05 was deemed significant in the exploratory analysis.

A detailed description of the metabolomics statistical analyses is provided in the Supplementary material (**Supplementary Methods**). Briefly, linear regression models were used to assess differences in 1-y changes in stool metabolites (i.e., the change in the metabolite data over time) between study groups. Weighted gene coexpression network analysis (WGCNA) [19] was used to identify metabolomic subnetworks based on correlation patterns using baseline stool metabolomics data. The associations between the 1-y changes in the subnetworks and the study groups were computed using multivariate linear regression models. In the same way, we assessed the associations between the changes in the 4 subnetworks that were significantly modified by the intervention and 1-y changes in cardiovascular disease risk factors, using linear regression models.

A detailed description of the microbiota statistical analyses is provided in the Supplementary material (Supplementary Methods). Briefly, linear regression models were used to assess the effect of a 1-y PREDIMED-Plus intervention on calculated fecal microbiota alpha diversity indices. The effect of the intervention on fecal microbiota community dissimilarity was assessed with permutational multivariate analysis of variance (PERMANOVA) on the Bray–Curtis distance. Per-feature analysis was performed using the R package MaAsLin2 [20] (version 1.10.0), to detect the intervention effect on taxonomic feature changes over time. The associations between intervention-related fecal microbiota features and fecal metabolites subnetworks were assessed through linear mixed models. In addition, the association between changes in calculated alpha diversity indexes and changes in fecal metabolites subnetworks was assessed through linear regression models.

Results

General characteristics of the study population

A flowchart of selected participants is represented in Supplemental Figure 1. Participants were selected from 4 PREDIMED-Plus recruiting centers (Alicante, Barcelona, Reus, and Valencia) and available sequencing data from stool samples ($n = 782$). Participants without available 1-y sequencing data ($n = 125$), low-sequencing quality ($n =$

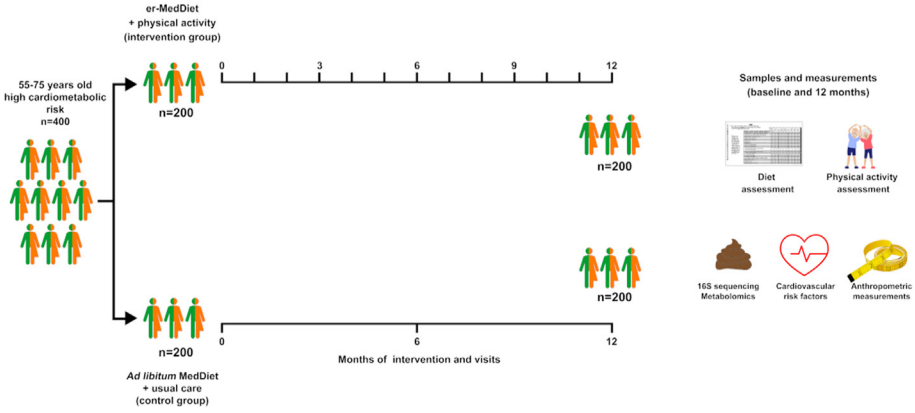

FIGURE 1. Schematic representation of the PREDIMED-Plus lifestyle intervention. Participants randomly assigned in the intervention group ($n = 200$) were exposed to a weight-loss energy-reduced Mediterranean diet (er-MedDiet) and increased physical activity, also attending 2 additional monthly visits. Participants in the control group ($n = 200$) received recommendations to improve their adherence to the Mediterranean diet in twice-a-year group sessions and did not receive recommendations to increase physical activity. Diet and physical activity assessments, stool samples for metabolomics and 16S sequencing, cardiovascular disease risk factors, and anthropometric measurements were collected at baseline and 1 y of follow-up.

26), and reported antibiotic use ($n = 4$) were excluded. From this subset ($n = 627$), 400 participants were randomly selected by age, sex, BMI [kg/m^2], and study group ($n = 200$ for each study group), and fecal metabolomics analysis was conducted.

The general baseline characteristics of the study population according to the PREDIMED-Plus study groups are shown in Table 1. The baseline and changes after a 1-y follow-up in anthropometric, biochemical, and lifestyle parameters according to different PREDIMED-Plus study groups are described in Table 2. Participants included in the IG showed greater weight loss (–4.2 ± 4.8 kg) and lower waist circumference (–4.4 ± 7.4 cm), BMI (–1.5 ± 1.8 kg/m^2), as well as a total energy intake (–113.9 ± 714.0 kcal) after 1 y of lifestyle intervention compared with the participants in the CG. In addition, the participants in the IG showed a reduction in glycated hemoglobin (–0.1% ± 0.8% over total) and increased adherence to MedDiet (3.4 ± 4.5) and physical activity (117.3 ± 501.9 METs/d) compared with those in the CG.

Fecal metabolomics and network analysis

Of the 532 fecal metabolites, only 4 showed significant differences in changes [false discovery rate (FDR) < 0.05] between study groups after 1 y of intervention (Figure 2). Compared with the participants in the CG, the 4,7,10,13,16-docosapentaenoic acid (DPA) (IG mean: –0.40 ± 1.44; CG mean: –0.08 ± 1.61) and adrenic acid decreased (IG mean: –0.33 ± 1.20; CG mean: –0.08 ± 1.33), and oleic acid (IG mean: 0.17 ± 0.94; CG mean: –0.10 ± 1.01) and 3-methyl-adipic acid (3-MAA) (IG mean: 0.25 ± 1.11; CG mean: 0.02 ± 1.10) increased in those in the IG. In addition, significant differences were observed in another 56 metabolites that disappeared after FDR correction (FDR > 0.05) (Supplemental Table 1).

WGCNA grouped 532 baseline metabolites into 16 subnetworks of different sizes (Supplemental Table 2). Grey60 network was the subnetwork with fewer connected metabolites ($n = 5$), whereas the brown network was the highest connected subnetwork ($n = 265$ metabolites). Four subnetworks (Black, Midnight blue, Pink, and Salmon) showed statistically significant between-group differences in changes after 1 y of intervention (Table 3). Metabolites selected in the Black subnetwork included mainly ceramides and sphingosines, the Midnight blue subnetwork included purines, the Pink included fatty acids and carnitines, and the Salmon subnetwork included bile acids. Compared with the CG, the participants in the IG showed a decrease in the Black, Midnight blue, and Pink subnetworks. The Salmon subnetwork increased in the IG compared with the CG.

The pair-wise partial correlations between metabolites accounting for the metabolites within the same networks are shown in Supplemental Figures 2–5. At baseline, the most connected hub for each subnetwork according to their intramodular connectivity was ceramide 18:1; 02/18:0 (kWithin = 10.72) for the Black subnetwork; fumaric acid or maleic acid (kWithin = 2.02) for the Midnight blue subnetwork; 4,7,10,13,16-DPA (kWithin = 6.68) for the Pink subnetwork; and glycochenodeoxycholic acid (kWithin = 4.27) for the Salmon subnetwork. After 1 y of intervention, the main hubs were similar: ceramide 18:1; 02/18:0 (kWithin = 9.60) for the Black subnetwork; 6,8-dihydroxy purine (kWithin = 1.67) for the Midnight blue subnetwork; 4,7,10,13,16-DPA (kWithin = 6.66) for the Pink subnetwork; and glycochenodeoxycholic acid (kWithin = 4.36) for the Salmon subnetwork.

One-y changes in the Pink subnetwork were positively associated with 1-y changes in body weight [β: 0.11, 95% confidence interval (CI): 0.01, 0.21], HOMA-IR index (β: 0.12, 95% CI: 0.01, 0.24), insulin (β: 0.11, 95% CI: 0.01, 0.22), and fasting plasma glucose (β: 0.11, 95% CI: 0.01, 0.23), and negatively with changes in LDL cholesterol (β: –0.11, 95% CI: –0.21, –0.01). One-y changes in the Black subnetwork were negatively associated with changes in LDL cholesterol (β: –0.11, 95% CI: –0.20, –0.01) (Figure 3).

TABLE 1
General baseline characteristics of the study population according to the PREDIMED-Plus study groups

	CG ($n = 200$)	IG ($n = 200$)
Sex, female, *n* (%)	88 (44.0)	88 (44.0)
Age (y) mean ± SD	64.7 ± 5.0	64.5 ± 4.9
Recruiting center, *n* (%)		
Alicante	49 (24.5)	42 (21.0)
Barcelona	20 (10.0)	26 (13.0)
Reus	114 (57.0)	110 (55.0)
Valencia	17 (8.5)	22 (11.0)
Education, *n* (%)		
Primary school or less	107 (53.5)	111 (55.5)
High School	51 (25.5)	58 (29.0)
College	42 (21.0)	31 (15.5)
Civil status, *n* (%)		
Married	163 (81.5)	146 (73.0)
Single/divorced/separated	22 (11.0)	35 (17.5)
Widower	15 (7.5)	19 (9.5)
Smoke status, *n* (%)		
Never smoker	93 (46.5)	99 (49.5)
Former smoker	81 (40.5)	76 (38.0)
Smoker	26 (13.0)	25 (12.5)
Disease prevalence, *n* (%)		
Overweight (BMI = 25–29.9 kg/m^2)	144 (72.0)	157 (78.5)
Obesity (BMI ≥ 30 kg/m^2)	56 (28.0)	43 (21.5)
Hypertension prevalence	162 (81.0)	163 (81.5)
Type 2 diabetes prevalence	44 (22.0)	44 (22.0)

Abbreviations: CG, control group; IG, intervention group.

Microbiota profiles associated with 1-y PREDIMED-Plus intervention

We observed an increase in alpha diversity indexes Chao1 (mean and SD: 5.5 ± 17.5) and Shannon (0.1 ± 0.5) after 1 y of follow-up in participants in the IG compared with those in the CG ($\beta = 6.376$, $P = 0.0005$; $\beta = 0.131$, $P = 0.013$, respectively) (Figure 4). The top 2 axes from principal coordinate analysis calculated over the Bray–Curtis distance explained 36% of the total variance between samples. The cluster based on interventions was not obvious (Supplemental Figure 6). PERMANOVA test based on the Bray–Curtis distance did not show statistically significant differences between study groups after 1 y of lifestyle intervention (Supplemental Table 3).

We observed a decrease in the abundance of the *Eubacterium hallii* group (–0.02 ± 1.1) in the IG compared with the CG after 1 y of follow-up ($\beta = –0.365$, FDR = 0.046). We also reported a marginal decrease in the abundance of genus *Dorea* (–0.2 ± 1.2) in the IG compared with the CG ($\beta = –0.346$, FDR = 0.169) after 1 y of follow-up (Figure 5). The effect of the intervention on total fecal microbiota genera abundances is shown in Supplemental Table 4.

Associations between intervention-related fecal microbiota profiles, fecal metabolites subnetworks, and cardiovascular disease risk factors

We observed a negative association between 1-y change in metabolite Pink subnetwork and 1-y change in alpha diversity indexes Chao1 ($\beta = –0.0005$, $P = 0.0005$) and Shannon ($\beta = –0.012$, $P =$

TABLE 2
Baseline and 1-y changes in anthropometric, biochemical, and lifestyle parameters according to the PREDIMED-Plus study groups

	CG ($n = 200$)	IG ($n = 200$)	Between group difference (CG–IG)	P value
Body weight (kg)				
Baseline	86.7 ± 12.7	88.0 ± 13.4		
1-y change	–0.8 ± 2.8	–4.9 ± 4.1	–4.2 ± 4.8	<0.001
Waist circumference (cm)				
Baseline	107.4 ± 10.3	108.1 ± 10.0		
1-y change	–1.1 ± 4.1	–5.5 ± 6.2	–4.4 ± 7.4	<0.001
BMI (kg/m^2)				
Baseline	32.7 ± 3.6	33.0 ± 3.5		
1-y change	–0.3 ± 1.1	–1.8 ± 1.5	–1.5 ± 1.8	<0.001
Total cholesterol (mg/dL)				
Baseline	201.8 ± 38.8	201.6 ± 36.4		
1-y change	–0.4 ± 33.9	0.6 ± 30.6	1.0 ± 44.9	0.758
HDL cholesterol (mg/dL)				
Baseline	49.0 ± 10.5	49.9 ± 12.3		
1-y change	2.2 ± 7.7	2.8 ± 7.3	0.6 ± 11.2	0.491
LDL cholesterol (mg/dL)				
Baseline	122.2 ± 32.2	121.5 ± 31.6		
1-y change	–1.0 ± 29.6	–0.3 ± 25.1	0.7 ± 39.9	0.825
Triglycerides (mg/dL)				
Baseline	169.1 ± 107.6	160.3 ± 93.4		
1-y change	–3.5 ± 92.9	–11.8 ± 87.0	–8.4 ± 123.8	0.345
FPG (mg/dL)				
Baseline	116.0 ± 25.1	114.2 ± 22.5		
1-y change	–4.3 ± 21.0	–3.7 ± 17.7	0.6 ± 27.2	0.770
Insulin (mU/mL)				
Baseline	19.7 ± 9.7	19.2 ± 11.6		
1-y change	–1.9 ± 7.6	–2.8 ± 7.5	–0.9 ± 10.8	0.273
HOMA-IR				
Baseline	5.8 ± 3.5	5.6 ± 4.1		
1-y change	–0.7 ± 2.8	–1.0 ± 2.8	–0.2 ± 3.9	0.349
HbA1c (% over total)				
Baseline	6.0 ± 0.8	6.0 ± 0.8		
1-y change	0.1 ± 0.6	–0.1 ± 0.4	–0.1 ± 0.8	0.042
Total energy intake (kcal)				
Baseline	2543.1 ± 550.4	2516.6 ± 592.7		
1-y change	–141.2 ± 537.4	–255.2 ± 566.4	–113.9 ± 714.0	0.025
MedDiet adherence score				
Baseline	8.3 ± 2.5	8.0 ± 2.5		
1-y change	2.2 ± 2.9	5.6 ± 3.1	3.4 ± 4.5	<0.001
Alcohol intake (g/d)				
Baseline	11.0 ± 14.5	10.3 ± 11.9		
1-y change	–0.6 ± 10.1	–1.7 ± 8.4	–1.1 ± 13.4	0.246
Physical activity (METs/d)				
Baseline	367.1 ± 314.3	366.9 ± 329.8		
1-y change	51.1 ± 299.5	168.4 ± 407.4	117.3 ± 501.9	0.001

Data expressed as mean ± SD. Differences between-group differences according to the study groups tested with Students' t test and $P < 0.05$ were deemed significant.
Abbreviations: CG, control group; FPG, fasting, plasma glucose; HbA1c, glycated hemoglobin; IG, intervention group; MedDiet, Mediterranean diet.

0.010) (Figure 6). Furthermore, we observed a positive association between *E. hallii* group and *Dorea* genera abundance and metabolites subnetworks Black ($\beta = 0.007$, $P = 0.00007$; $\beta = 0.004$, $P = 0.012$, respectively), Midnight blue ($\beta = 0.007$, $P = 0.00005$; $\beta = 0.060$, $P = 0.0003$, respectively), and Pink ($\beta = 0.004$, $P = 0.037$; $\beta = 0.004$, $P = 0.031$, respectively) (Figure 7).

In addition, we observed a positive association between *E. hallii* group and *Dorea* genera abundance and changes in body weight ($\beta = 0.158$, $P = 0.004$; $\beta = 0.173$, $P = 0.001$, respectively), waist circumference ($\beta = 0.150$, $P = 0.003$; $\beta = 0.119$, $P = 0.020$, respectively), BMI ($\beta = 0.156$, $P = 0.005$; $\beta = 0.171$, $P = 0.002$, respectively), triglycerides ($\beta = 0.077$, $P = 0.043$; $\beta = 0.121$, $P = 0.002$, respectively), insulin ($\beta = 0.104$, $P = 0.007$; $\beta = 0.111$, $P = 0.006$, respectively), and the HOMA-IR index ($\beta = 0.089$, $P = 0.024$; $\beta = 0.103$, $P = 0.012$, respectively) (Figure 8). No association was observed between calculated alpha diversity indexes and changes in cardiovascular disease risk factors (Supplemental Figure 7).

Discussion

Diet can affect the gut microbiome and interacts with the host [21]. The fecal metabolome has been proposed as a functional readout of the gut microbiome [22]. Thus, studies with both analyses, fecal metabolome and microbiome, are essential for the understanding of how dietary interventions influence the metabolism of the host. In this study, we demonstrated that an intensive intervention based on an er-MedDiet and physical activity promotion, compared with a control ad libitum MedDiet, significantly affects both gut microbiota and fecal

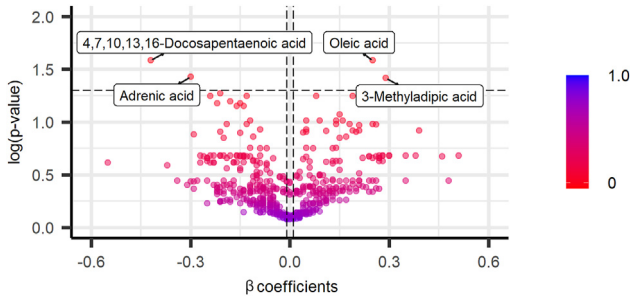

FIGURE 2. Volcano plot showing the 1-y effect of the PREDIMED-Plus intervention on fecal metabolites. Multivariable linear regression models were adjusted for recruiting center (Alicante, Barcelona, Reus, and Valencia), smoking status (former smoker, never smoker, and smoker), type 2 diabetes status, sex, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI > 30 kg/m^2); age categories (below the median, ≤ 65 y old; above the median, >65 y old), alcohol intake (g/d^2), and hypertension status. A false discovery rate of <0.05 was considered statistically significant (up dash line).

metabolites with important relationships between them, indicating a possible interplay.

As previously described in the context of the PREDIMED-Plus study [23], in this analysis, we observed that participants allocated to the IG, after 1 y of intervention, showed a greater reduction in adiposity and improvements in lipid profile and markers of glucose metabolism. Within this context, the fecal metabolome analysis has established that 2 metabolites (DPA and adrenic acid) and 3 subnetworks (Black, Midnight blue, and Pink) were decreased in the IG compared with the CG, whereas 2 metabolites (oleic acid and 3-MAA) and 1 subnetwork (Salmon) were increased in the IG and over time. These differences in gut metabolite changes may reflect the differential effect of the interventions that could result directly from food or its digestion or be ascribed to endogenous host secretions or changes in gut microbiota metabolism.

The Black subnetwork was mainly constituted by sphingolipids, the Pink subnetwork was mainly constituted by PUFAs and cholesterol esters, and the Midnight blue subnetwork was mainly constituted by metabolites from purine metabolism, the Krebs cycle, and nucleotides. These subnetworks were reduced in the IG compared with the CG. Interestingly, 2 components of the Pink subnetwork, DPA, a ω3-PUFA, and adrenic acid, a ω6-PUFA, also significantly decreased in the IG. DPA acts as an intermediate between eicosapentaenoic acid and DPA and can be found in high concentrations in marine foods (typically included in MedDied), red meat, and milk [24]. Plasma and erythrocyte levels of DPA have been reported to decrease after weight loss [25], but we cannot discard that changes in this stool metabolite could be produced by intervention-related changes in the gut microbiota. Adrenic acid is a nondietary ω6-PUFA derived from arachidonic acid that to the best of our knowledge has not previously related to changes in gut microbiota. The gut microbiota plays an important role in fatty acid metabolism. Several studies have shown that PUFA can be produced and modulated by the intestinal microbiota and, in turn, the concentration of PUFA can modify the functionality of the microbiota after high-fat diets, such as the MedDiet [26,27]. Similarly, sphingolipids can be produced endogenously, come directly from food, or be the end-products of microbial metabolism [28]. Endogenous sphingolipids are restricted by the breakdown of ceramides [29], so their content in the digestive tract hardly comes from the host. Because the dietary origin of sphingolipids vary considerably [30], and their intestinal absorption change depending on their origin [31], the differences observed between our study groups could be explained by higher consumption of certain foods, such as refined wheat or whole milk, in the CG compared with the IG. In addition, some bacteria from the *Bacteroides* genus can assimilate and produce sphingolipids that can be absorbed by intestinal epithelial cells and modify the sphingolipids plasma pool [32]. Although plasma sphingolipids have been related to impaired glucose metabolism and insulin resistance [33], in our study, no such association between this sphingolipid subnetwork and the *Bacteroides* genus was shown. Nevertheless, changes in the Pink subnetwork were positively related to 1-y changes in body weight, HOMA-IR, insulin, and glucose, and inversely associated with changes in LDL cholesterol, suggesting that this network may play an important role in the metabolism of the host.

The Salmon subnetwork only included metabolites of bile acids metabolism. Bile acids have a key role in regulating energy metabolism, satiety, and body weight [34], and changes in bile acid

TABLE 3
Effect of 1-y PREDIMED-Plus intervention on metabolomic subnetworks

Metabolomic subnetworks	CG (n = 200) Mean ± SD	IG (n = 200) Mean ± SD	β coefficient	95% CI
Black	0.006 ± 0.056	–0.006 ± 0.055	–0.012	(–0.020, –0.001)
Blue	–0.005 ± 0.062	0.004 ± 0.060	0.008	(0.000, 0.020)
Brown	–0.001 ± 0.040	0.001 ± 0.043	–0.001	(–0.010, 0.010)
Cyan	–0.002 ± 0.052	0.000 ± 0.042	–0.001	(–0.010, 0.010)
Green	–0.002 ± 0.048	0.002 ± 0.052	0.006	(0.000, 0.010)
Green yellow	0.001 ± 0.047	–0.002 ± 0.051	–0.005	(–0.010, 0.000)
Grey60	0.004 ± 0.055	–0.003 ± 0.050	–0.005	(–0.010, 0.000)
Light cyan	–0.002 ± 0.052	0.003 ± 0.053	0.003	(–0.010, 0.010)
Magenta	–0.004 ± 0.059	0.003 ± 0.054	0.002	(–0.010, 0.010)
Midnight blue	0.005 ± 0.052	–0.005 ± 0.052	–0.011	(–0.020, –0.001)
Pink	0.004 ± 0.050	–0.004 ± 0.048	–0.010	(–0.020, –0.001)
Purple	0.005 ± 0.053	–0.004 ± 0.050	–0.007	(–0.020, 0.000)
Red	–0.002 ± 0.039	0.002 ± 0.038	0.005	(0.000, 0.010)
Salmon	–0.005 ± 0.052	0.005 ± 0.060	0.010	(0.001, 0.020)
Tan	0.000 ± 0.061	–0.001 ± 0.053	–0.005	(–0.010, 0.000)
Yellow	0.004 ± 0.050	–0.004 ± 0.046	–0.008	(–0.020, 0.000)

Multivariable linear regression models adjusted for recruiting center (Alicante, Barcelona, Reus, and Valencia), smoking status (former smoker, never smoker, and smoker), type 2 diabetes status, sex, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2); age categories (below the median, ≤65 y old; above the median, >65 y old), alcohol intake (g/d^2), and hypertension status.
Abbreviations: CG, control group; IG, intervention group.

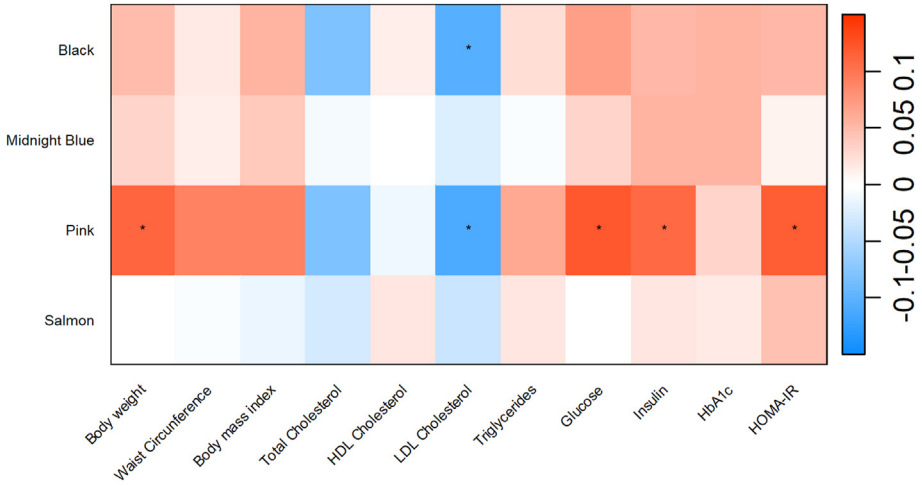

FIGURE 3. Associations between 1-y changes in significant metabolite subnetworks and 1-y changes in cardiovascular disease risk factors. The models were adjusted for recruiting center (Alicante, Barcelona, Reus, and Valencia), smoking status (former smoker, never smoker, and smoker), type 2 diabetes status, sex, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2); age categories (below the median, ≤65 y old; above the median, >65 y old), alcohol intake (g/d^2), and hypertension status. *$P < 0.05$; **$P < 0.01$. HbA1c, glycated hemoglobin.

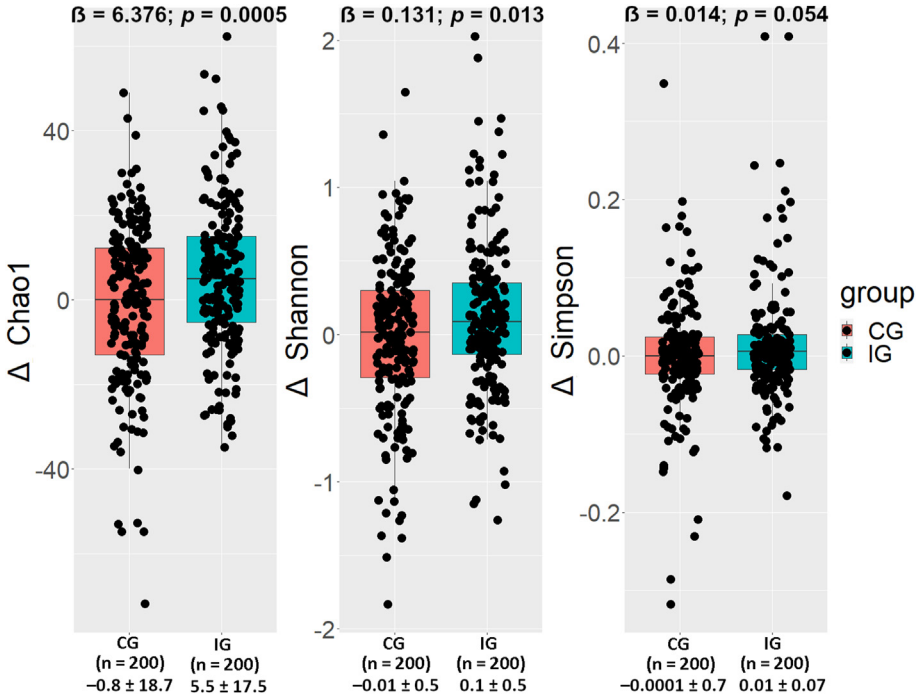

FIGURE 4. Effect of PREDIMED-Plus intervention on 1-y changes in alpha diversity indexes Chao1, Shannon, and Simpson. Effects of PREDIMED-Plus intervention tested with linear regression model adjusted for recruiting center (Alicante, Barcelona, Reus, and Valencia), smoking status (former smoker, never smoker, and smoker), type 2 diabetes status, sex, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2); age categories (below the median, ≤65 y old; above the median, >65 y old), alcohol intake (g/d^2), hypertension status. $P < 0.05$ deemed as significant. CG, control group; IG, intervention group. Values indicated as mean ± SD.

metabolism have been reported in the low-grade inflammation status related to obesity and diabetes [35]. Animal studies have shown that physical activity induces an increase in biliary bile acid secretion and bile acid concentrations in feces [36], but in adults with obesity, higher adherence to the MedDiet was associated with lower concentrations of bile acids in feces [12], and higher BMI was associated with increased levels of bile acids in plasma [37]. Therefore, the effect of interventions on fecal bile acids may reflect higher physical activity and weight loss achieved in the IG. The increase in fecal oleic acid and 3-MAA in the IG could be explained by the higher adherence to the MedDiet achieved by the participants. Oleic acid is a monounsaturated ω9 fatty acid present mainly in olive oil [38], and the 3-MAA, a methyl-branched

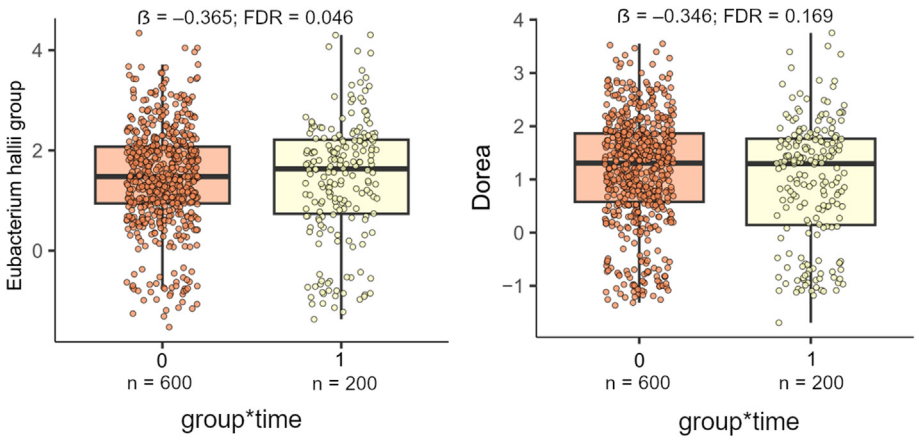

FIGURE 5. Effect of 1-y PREDIMED-Plus interventions on fecal microbiota taxa abundances. Multivariable association between-group*time and fecal microbiota taxonomic features tested with generalized linear models adjusted for recruiting center (Alicante, Barcelona, Reus, and Valencia), smoking status (former smoker, never smoker, and smoker), type 2 diabetes status, sex, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2); age categories (below the median, ≤65 y old; above the median, >65 y old), alcohol intake (g/d^2), hypertension status. Participants' IDs are set as random effect parameters. Multiple testing corrections were performed with the Benjamini–Hochberg procedure. Features with FDR < 0.25 were reported. Label "1" indicates group*time = intervention*1-y, "0" otherwise. FDR, false discovery rate.

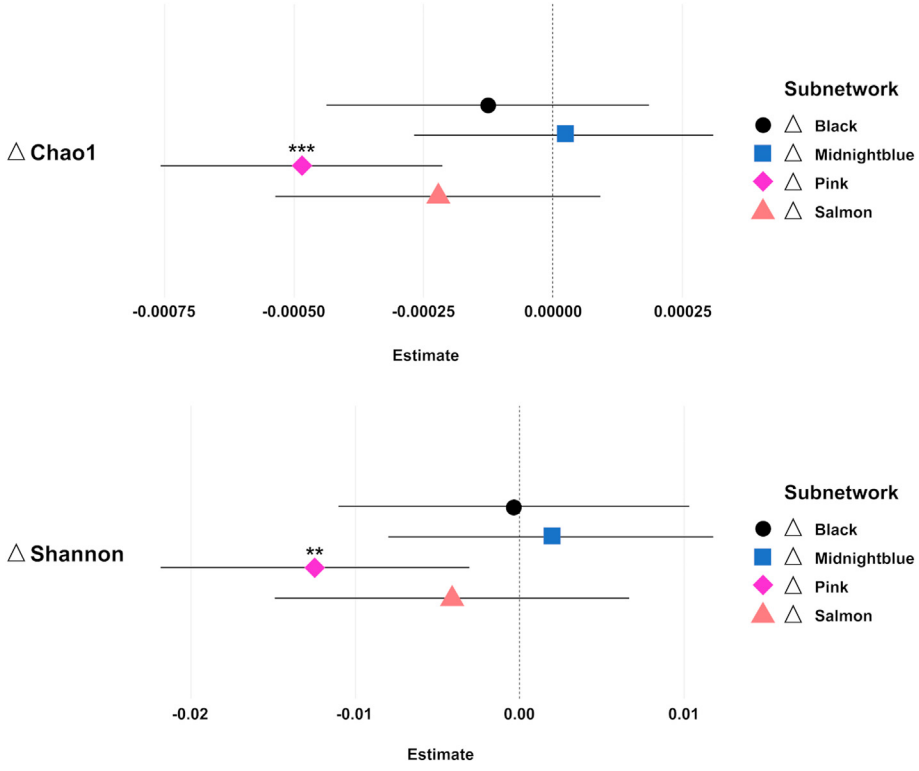

FIGURE 6. Association between 1-y changes in alpha diversity indexes and 1-y changes in fecal metabolites subnetworks. Association tested with linear regression models adjusted for the study group, recruiting center (Alicante, Barcelona, Reus, and Valencia), smoking status (former smoker, never smoker, and smoker), type 2 diabetes status, sex, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2); age categories (below the median, ≤65 y old; above the median, >65 ye old), alcohol intake (g/d^2), and hypertension status. *P < 0.05; **P < 0.01; ***P < 0.001.

fatty acid involved in the catabolism of phytanic acid [39], may be derived from food such as fatty fish or cheese (typical in MedDiet), but also meat.

Low-gut bacteria diversity has been associated with several diseases [40]. A systematic review of observational and randomized clinical trials reported a positive relationship between MedDiet adherence and alpha diversity using data from observational studies [9]. However, they also reported inconclusive findings from randomized trials regarding the effects of MedDiet on gut microbiota diversity. In a small study involving adult volunteers living in a Mediterranean area, those

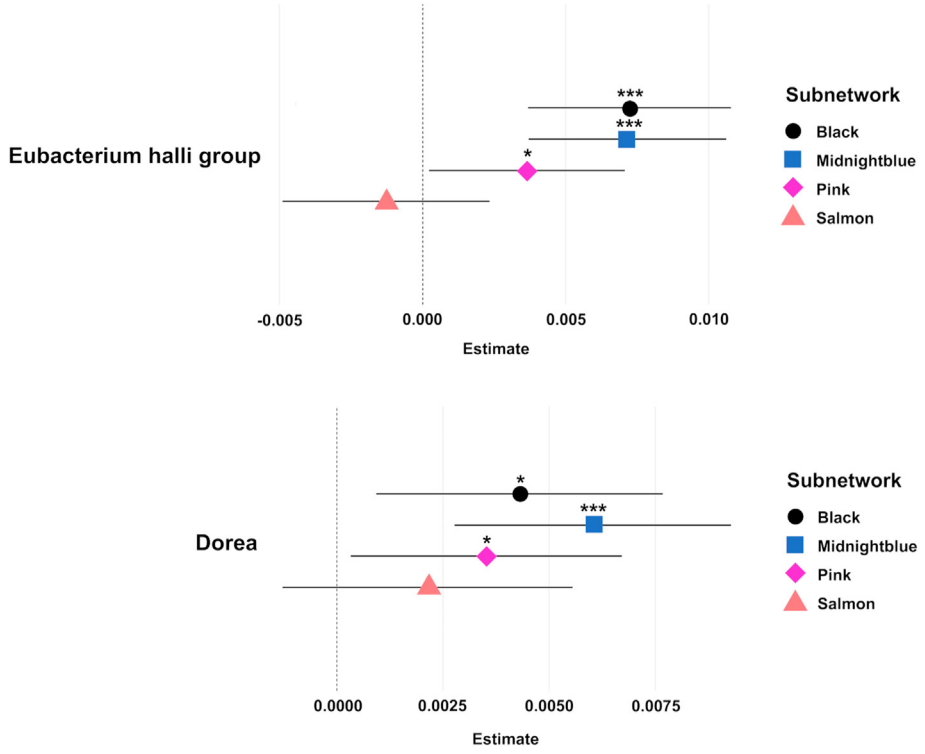

FIGURE 7. Association between differential abundant taxonomic features and metabolites subnetworks. Association tested with linear mixed models adjusted for study group*time, recruiting center (Alicante, Barcelona, Reus, and Valencia), smoking status (former smoker, never smoker, and smoker), type 2 diabetes status, sex, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2); age categories (below the median, ≤ 65 y old; above the median, >65 y old), alcohol intake (g/d^2), and hypertension status. Participants' IDs are set as random effect parameters. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

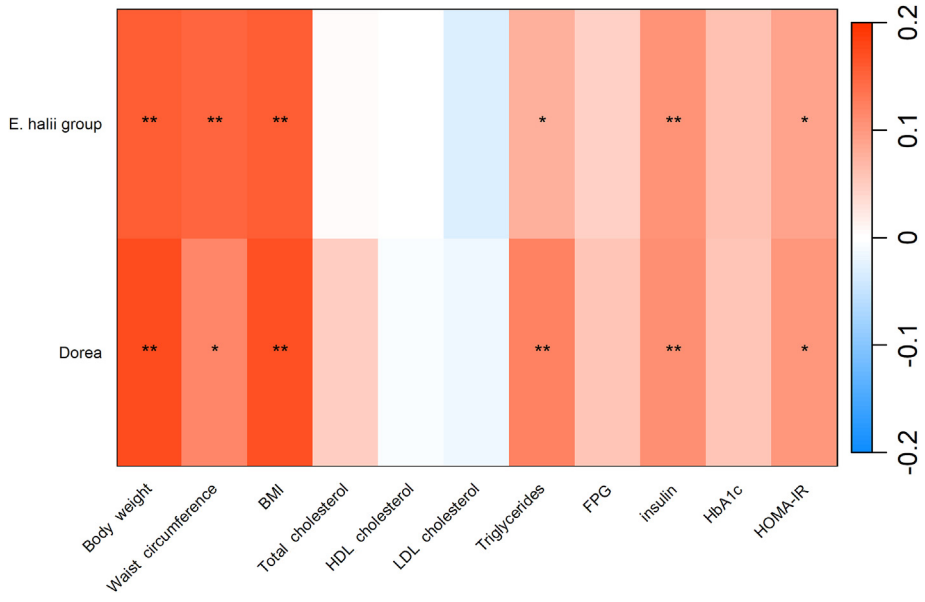

FIGURE 8. Heatmap showing the association between intervention-related differential abundant features and changes in cardiovascular disease risk factors. Association tested with linear mixed models adjusted for study group*time, recruiting center (Alicante, Barcelona, Reus, and Valencia), smoking status (former smoker, never smoker, and smoker), type 2 diabetes status, sex, BMI categories (overweight, BMI = 25–29.9 kg/m^2; obesity, BMI ≥ 30 kg/m^2); age categories (below the median, ≤65 y old; above the median, >65 y old), alcohol intake (g/d^2), and hypertension status. Participants' IDs are set as random effect parameters. For each cell, colors indicate the association coefficient with cardiovascular disease risk factors and asterisks denote significance. *$P < 0.05$; **$P <$ 0.001. FPG, fasting, plasma glucose; HbA1c, glycated hemoglobin.

with higher adherence showed an increase in microbial richness [41]. In line with these findings, we observed an increase in alpha diversity measured using different indexes among the participants in the IG compared with those in the CG. In our study, we observed significant positive effects of the intervention on fecal microbiota richness and diversity after 1 y of follow-up, and the increased alpha diversity secondary to the intervention was associated with one of the fecal metabolite subnetworks identified. Moreover, compared with the CG, we observed a decrease in the abundance of *E. hallii* and *Dorea* spp. after 1 y of intervention. The abundance of these bacterial taxa was directly associated with 4 of the fecal metabolite subnetworks identified and different cardiovascular disease risk factors. These findings are consistent with those from our previous study that assessed the effects of the intervention in a different subsample of individuals and using another genotyping platform [42].

The decrease in the abundance of *E. hallii*, the former name of reclassified *Anaerobutyricum soehngenii* [43], can be partially explained by the ability of this bacterium to produce its fermentation end products from lactate, increased concentrations of which have been described in the small intestine of insulin-resistant subjects, in whom, an increased abundance of lactate-producing bacteria has also been reported [44]. Lactate is an intermediate in the metabolism of glucose that has been implicated in the pathogenesis of insulin resistance in individuals with obesity [45]. Chondronikola et al. [46] conducted a controlled trial in which participants were randomly assigned to a 6-mo weight maintenance or weight-loss intervention, showing the interrelationships among weight loss, glucose metabolism, insulin sensitivity, and lactate concentration. Accordingly, we showed that *E. hallii* was positively associated with body weight, waist circumference, BMI, insulin, and HOMA-IR index. It has also been reported that oral administration of *E. hallii* improved insulin sensitivity, increased energy expenditure, increased fecal butyrate concentrations, and modified bile acid metabolism in mice with obesity and diabetes [47]. Furthermore, we reported a decrease in the abundance of *Dorea* spp. in fecal samples of participants in the er-MedDiet + physical activity intervention. These findings are consistent with our previous findings, which showed an increase in the abundance of *Dorea* within the CG [42]. Western dietary pattern was previously associated with a higher abundance of *Dorea* spp. [48]. *Dorea* spp. has been found consistently elevated in prediabetes and is positively associated with blood glucose concentrations [49]. Consistently, we observed a significant difference in glycated hemoglobin changes between study groups, after 1 y of intervention, and a positive association between this genus, insulin concentrations, and the HOMA-IR index.

These findings have to be interpreted in the context of some limitations. First, given the multifaceted nature of the study intervention, the results cannot be attributed to a single component of the intervention. Second, the participants included in our study are Mediterranean older adults with overweight/obesity and metabolic syndrome. Therefore, the results may not be generalized to other populations outside of this specific context. Third, the nature of 16S sequencing limits taxonomic profiling to genus-level resolution, because the primers used for amplification bind to regions not conserved across all bacteria, not allowing us to differentiate between closely related bacteria at species level. This limitation in taxonomic identification also reduces the possibility to infer the functionality of the microbiome.

This study also has some strengths. Although the analysis was conducted in a sample that is not representative of the overall population, it is important to mention that individuals at high risk of cardiometabolic diseases represent an important proportion of the global population and hence our findings are relevant to similar community-dwelling older adults who may benefit from approaches to support good health. In addition, the randomized controlled study design, and the significant differences between the components of the intervention (weight loss, adherence to the MedDiet, and physical activity) allowed us to establish causality and assess the potential effects of the intervention. In addition, as we adjusted for major potential confounders when conducting our analyses, residual confounding is highly reduced. Finally, despite the limitations of 16S sequencing, it is important to mention that it is a very suitable technique to analyze a large number of samples.

In conclusion, in this lifestyle intervention-based study, we observed that an energy-reduced MedDiet and physical activity promotion, compared with an ad libitum MedDiet, produced significant changes in gut metabolomics and microbiota in a Mediterranean population of older adults with overweight/obesity and metabolic syndrome and these changes were related to changes in several cardiovascular disease risk factors. These findings highlight that even with similar healthy dietary patterns, the high intensity of the dietary intervention and weight-loss intervention components, such as caloric restriction and physical activity, could have significant benefits on CVD risk factors, potentially through modulation of the fecal microbiota and metabolome.

The impact of our findings extends beyond individual health outcomes. Investigating the effects of MedDiet and physical activity interventions on the gut microbiome provides insights into the underlying mechanisms by which these interventions improve cardiometabolic biomarkers. Understanding the role of the gut microbiome in mediating the health benefits of these interventions can inform more targeted and effective public health strategies. Elucidating the relationship between diet, physical activity, and the gut microbiome can contribute to the development of personalized health recommendations. Public health policies and interventions can be tailored to individual microbiome profiles, allowing for more precise and effective strategies for preventing and managing cardiometabolic diseases.

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Author contributions

The authors' responsibilities were as follows – All the principal PREDIMED-Plus investigators contributed to the study concept and design and to data extraction from the participants; JFG-G, AA: performed the statistical analyses and drafted the manuscript; and all

authors: reviewed the manuscript for important intellectual content and approved the final version to be published.

Conflict of interest

JS-S reports receiving travel expenses from Instituto Danone Spain and International, receiving nonfinancial support from Patrimonio Comunal Olivarero, the Almond Board of California, Pistachio Growers and Borges S.A (tree nuts for free for the PREDIMED-Plus participants); serving on the board of and receiving grant support through his institution from the International Nut and Dried Foundation; and personal fees from Instituto Danone Spain; Serving in the Board of Danone Institute International. The rest of the authors have declared that no competing interests exist.

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Data availability

The datasets generated and analyzed during this study are not publicly available because of data regulations and for ethical reasons, considering that this information might compromise research participants' consent because our participants only gave their consent for the use of their data by the original team of investigators. However, collaboration for data analyses can be requested by sending a letter to the PREDIMED-Plus steering Committee (predimed_plus_scommittee@googlegroups.com). The request will then be passed to all the members of the PREDIMED-Plus Steering Committee for deliberation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2024.02.021.

References

[1] A. Bach-Faig, E.M. Berry, D. Lairon, J. Reguant, A. Trichopoulou, S. Dernini, et al., Mediterranean diet pyramid today. Science and cultural updates, Public Health Nutr 14 (2011) 2274–2284.

[2] R. Estruch, E. Ros, J. Salas-Salvadó, M.I. Covas, D. Corella, F. Arós, et al., Primary prevention of cardiovascular disease with a Mediterranean diet supplemented with extra-virgin olive oil or nuts, N. Engl. J. Med. 378 (2018) e34.

[3] C.L. Bendall, H.L. Mayr, R.S. Opie, M. Bes-Rastrollo, C. Itsiopoulos, CJ. Thomas, Central obesity and the Mediterranean diet: a systematic review of intervention trials, Crit. Rev. Food Sci. Nutr. 58 (2018) 3070–3084, https://doi.org/10.1080/10408398.2017.1351917.

[4] M. Franquesa, G. Pujol-Busquets, E. García-Fernández, L. Rico, L. Shamirian-Pulido, A. Aguilar-Martínez, et al., Mediterranean diet and cardiodiabesity: a systematic review through evidence-based answers to key clinical questions, Nutrients 11 (2019) 655.

[5] S. Soltani, A. Jayedi, S. Shab-Bidar, N. Becerra-Tomás, J. Salas-Salvadó, Adherence to the Mediterranean diet in relation to all-cause mortality: a systematic review and dose-response meta-analysis of prospective cohort studies, Adv. Nutr. 10 (2019) 1029–1039.

[6] F. De Filippis, N. Pellegrini, L. Vannini, I.B. Jeffery, A. La Storia, L. Laghi, et al., High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome, Gut 65 (2016) 1812–1821.

[7] S. Tagliamonte, M. Laiola, R. Ferracane, M. Vitale, M.A. Gallo, V. Meslier, et al., Mediterranean diet consumption affects the endocannabinoid system in overweight and obese subjects: possible links with gut microbiome, insulin resistance and inflammation, Eur. J. Nutr. 60 (2021) 3703–3716.

[8] K. Deng, J.J. Xu, L. Shen, H. Zhao, W. Gou, F. Xu, et al., Comparison of fecal and blood metabolome reveals inconsistent associations of the gut microbiota with cardiometabolic diseases, Nat. Commun. 14 (2023) 571.

[9] R. Kimble, P. Gouinguenet, A. Ashor, C. Stewart, K. Deighton, J. Matu, et al., Effects of a Mediterranean diet on the gut microbiota and microbial metabolites: a systematic review of randomized controlled trials and observational studies, Crit. Rev. Food Sci. Nutr. 63 (27) (2023) 8698–8719, https://doi.org/10.1080/10408398.2022.2057416.

[10] S. Galié, J. García-Gavilán, C. Papandreou, L. Camacho-Barcía, P. Arcelin, A. Palau-Galindo, et al., Effects of Mediterranean Diet on plasma metabolites and their relationship with insulin resistance and gut microbiota composition in a crossover randomized clinical trial, Clin. Nutr. 40 (2021) 3798–3806.

[11] M. Vitale, R. Giacco, M. Laiola, G. Della Pepa, D. Luongo, A. Mangione, et al., Acute and chronic improvement in postprandial glucose metabolism by a diet resembling the traditional Mediterranean dietary pattern: can SCFAs play a role? Clin. Nutr. 40 (2021) 428–437.

[12] V. Meslier, M. Laiola, H.M. Roager, F. De Filippis, H. Roume, B. Quinquis, et al., Mediterranean diet intervention in overweight and obese subjects lowers plasma cholesterol and causes changes in the gut microbiome and metabolome independently of energy intake, Gut 69 (2020) 1258–1268.

[13] S. Galié, J. García-Gavilán, L. Camacho-Barcía, A. Atzeni, J. Muralidharan, C. Papandreou, et al., Effects of the Mediterranean Diet or nut consumption on gut microbiota composition and fecal metabolites and their relationship with cardiometabolic risk factors, Mol. Nutr. Food Res. 65 (2021) e2000982.

[14] M.A. Martínez-González, P. Buil-Cosiales, D. Corella, M. Bulló, M. Fitó, J. Vioque, et al., Cohort profile: design and methods of the PREDIMED-Plus randomized trial, Int. J. Epidemiol. 48 (2019) 387–388o.

[15] H. Schröder, M.D. Zomeño, M.A. Martínez-González, J. Salas-Salvadó, D. Corella, J. Vioque, et al., Validity of the energy-restricted Mediterranean Diet Adherence Screener, Clin. Nutr. 40 (2021) 4971–4979.

[16] H. Schröder, M. Fitó, R. Estruch, M.A. Martínez-González, D. Corella, J. Salas-Salvadó, et al., A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women, J. Nutr. 141 (2011) 1140–1145.

[17] L. Molina, M. Sarmiento, J. Peñafiel, D. Donaire, J. Garcia-Aymerich, M. Gomez, et al., Validation of the REGICOR short physical activity questionnaire for the adult population, PLOS ONE 12 (2017) e0168148.

[18] A.M. Galmes-Panades, J. Konieczna, I. Abete, A. Colom, N. Rosique-Esteban, M.A. Zulet, et al., Lifestyle factors and visceral adipose tissue: results from the PREDIMED-PLUS study, PLOS ONE 14 (2019) e0210726.

[19] P. Langfelder, S. Horvath, WGCNA: an R package for weighted correlation network analysis, BMC Bioinformatics 9 (2008) 559.

[20] H. Mallick, A. Rahnavard, L.J. McIver, S. Ma, Y. Zhang, L.H. Nguyen, et al., Multivariable association discovery in population-scale meta-omics studies, PLOS Comput. Biol. 17 (2021) e1009442.

[21] I. Bourdeau-Julien, S. Castonguay-Paradis, G. Rochefort, J. Perron, B. Lamarche, N. Flamand, et al., The diet rapidly and differentially affects the gut microbiota and host lipid mediators in a healthy population, Microbiome 11 (2023) 26.

[22] J. Zierer, M.A. Jackson, G. Kastenmüller, M. Mangino, T. Long, A. Telenti, et al., The fecal metabolome as a functional readout of the gut microbiome, Nat. Genet. 50 (2018) 790–795.

[23] J. Salas-Salvadó, A. Díaz-López, M. Ruiz-Canela, J. Basora, M. Fitó, D. Corella, et al., Effect of a lifestyle intervention program with energy-restricted Mediterranean diet and exercise on weight loss and cardiovascular

risk factors: one-year results of the PREDIMED-Plus trial, Diabetes Care 42 (2019) 777–788.
[24] I. de Bus, R. Witkamp, H. Zuilhof, B. Albada, M. Balvers, The role of n-3 PUFA-derived fatty acid derivatives and their oxygenated metabolites in the modulation of inflammation, Prostaglandins Other Lipid Mediat 144 (2019) 106351.
[25] Y.J. Lee, A. Lee, H.J. Yoo, M. Kim, M. Kim, S.H. Jee, et al., Effect of weight loss on circulating fatty acid profiles in overweight subjects with high visceral fat area: a 12-week randomized controlled trial, Nutr. J. 17 (2018) 28, https://doi.org/10.1186/s12937-018-0323-4.
[26] Y. Fu, Y. Wang, H. Gao, D. Li, R. Jiang, L. Ge, et al., Associations among dietary omega-3 polyunsaturated fatty acids, the gut microbiota, and intestinal immunity, Mediators Inflamm 2021 (2021) 8879227.
[27] Y.Y. Lam, C.W.Y. Ha, J.M.A. Hoffmann, J. Oscarsson, A. Dinudom, T.J. Mather, et al., Effects of dietary fat profile on gut permeability and microbiota and their relationships with metabolic changes in mice, Obesity (Silver Spring) 23 (2015) 1429–1439.
[28] I. Olsen, E. Jantzen, Sphingolipids in bacteria and fungi, Anaerobe 7 (2001) 103–112.
[29] G.H. Norris, C.N. Blesso, Dietary and endogenous sphingolipid metabolism in chronic inflammation, Nutrients 9 (2017) 1180.
[30] H. Vesper, E.M. Schmelz, M.N. Nikolova-Karakashian, D.L. Dillehay, DV. Lynch, A.H. Merrill, Sphingolipids in food and the emerging importance of sphingolipids to nutrition, J. Nutr. 129 (1999) 1239–1250.
[31] A. Fujii, Y. Manabe, K. Aida, T. Tsuduki, T. Hirata, T. Sugawara, Selective absorption of dietary sphingoid bases from the intestine via efflux by P-glycoprotein in rats, J. Nutr. Sci. Vitaminol. (Tokyo). 63 (2017) 44–50.
[32] M.-T. Lee, H.H. Le, E.L. Johnson, Dietary sphinganine is selectively assimilated by members of the mammalian gut microbiome, J. Lipid Res. 62 (2021) 100034.
[33] A.E. Rigamonti, M. Dei Cas, D. Caroli, A. De Col, S.G. Cella, R. Paroni, et al., Identification of a specific plasma sphingolipid profile in a group of normal-weight and obese subjects: a novel approach for a "biochemical" diagnosis of metabolic syndrome? Int. J. Mol. Sci. 24 (2023) 7451.
[34] N.C. Penney, J. Kinross, R.C. Newton, S. Purkayastha, The role of bile acids in reducing the metabolic complications of obesity after bariatric surgery: a systematic review, Int. J. Obes (Lond). 39 (2015) 1565–1574.
[35] O. Chávez-Talavera, A. Tailleux, P. Lefebvre, B. Staels, Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease, Gastroenterology 152 (2017) 1679–1694.e3.
[36] M. Meissner, E. Lombardo, R. Havinga, U.J.F. Tietge, F. Kuipers, A.K. Groen, Voluntary wheel running increases bile acid as well as cholesterol excretion and decreases atherosclerosis in hypercholesterolemic mice, Atherosclerosis 218 (2011) 323–329.
[37] P. Prinz, T. Hofmann, A. Ahnis, U. Elbelt, M. Goebel-Stengel, B.F. Klapp, et al., Plasma bile acids show a positive correlation with body mass index and are negatively associated with cognitive restraint of eating in obese patients, Front. Neurosci. 9 (2015) 199.
[38] E. Ros, Olive oil and CVD: accruing evidence of a protective effect, Br. J. Nutr. 108 (2012) 1931–1933.
[39] R.J.A. Wanders, J. Komen, S. Ferdinandusse, Phytanic acid metabolism in health and disease, Biochim. Biophys. Acta. 1811 (2011) 498–507.
[40] Y. Fan, O. Pedersen, Gut microbiota in human metabolic health and disease, Nat. Rev. Microbiol. 19 (2021) 55–71.
[41] I. Garcia-Mantrana, M. Selma-Royo, C. Alcantara, M.C. Collado, Shifts on gut microbiota associated to Mediterranean diet adherence and specific dietary intakes on general adult population, Front. Microbiol. 9 (2018) 890.
[42] J. Muralidharan, I. Moreno-Indias, M. Bulló, J.V. Lopez, D. Corella, O. Castañer, et al., Effect on gut microbiota of a 1-y lifestyle intervention with Mediterranean diet compared with energy-reduced Mediterranean diet and physical activity promotion: PREDIMED-Plus study, Am. J. Clin. Nutr. 114 (2021) 1148–1158.
[43] S.A. Shetty, S. Zuffa, T.P.N. Bui, S. Aalvink, H. Smidt, WM. De Vos, Reclassification of Eubacterium hallii as Anaerobutyricum hallii gen. nov., comb. nov., and description of Anaerobutyricum soehngenii sp. nov., a butyrate and propionate-producing bacterium from infant faeces, Int. J. Syst. Evol. Microbiol. 68 (2018) 3741–3746.
[44] P.W. Gilijamse, A.V. Hartstra, E. Levin, K. Wortelboer, M.J. Serlie, M.T. Ackermans, et al., Treatment with Anaerobutyricum soehngenii: a pilot study of safety and dose–response effects on glucose metabolism in human subjects with metabolic syndrome, NPJ Biofilms Microbiomes 6 (2020) 16.
[45] J. Lovejoy, F.D. Newby, S.S.P. Gebhart, M. DiGirolamo, Insulin resistance in obesity is associated with elevated basal lactate levels and diminished lactate appearance following intravenous glucose and insulin, Metabolism 41 (1992) 22–27.
[46] M. Chondronikola, F. Magkos, J. Yoshino, A.L. Okunade, B.W. Patterson, M.J. Muehlbauer, et al., Effect of progressive weight loss on lactate metabolism: a randomized controlled trial, Obesity (Silver Spring) 26 (2018) 683–688.
[47] S. Udayappan, L. Manneras-Holm, A. Chaplin-Scott, C. Belzer, H. Herrema, G.M. Dallinga-Thie, et al., Oral treatment with *Eubacterium hallii* improves insulin sensitivity in *db/db* mice, NPJ Biofilms Microbiomes 2 (2016) 16009.
[48] J.M. Shikany, R.T. Demmer, A.J. Johnson, N.F. Fino, K. Meyer, K.E. Ensrud, et al., Association of dietary patterns with the gut microbiota in older, community-dwelling men, Am. J. Clin. Nutr. 110 (2019) 1003–1014.
[49] M.S.A. Palmnäs-Bedard, G. Costabile, C. Vetrani, S. Åberg, Y. Hjalmarsson, J. Dicksved, et al., The human gut microbiota and glucose metabolism: a scoping review of key bacteria and the potential role of SCFAs, Am. J. Clin. Nutr. 116 (2022) 862–874.