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Original Research Article

Adherence to the Mediterranean diet and nuclear magnetic resonance spectroscopy biomarkers in older individuals at high cardiovascular disease risk: cross-sectional and longitudinal analyses

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A B S T R A C T

Background: The Mediterranean diet (MedDiet) has been related to a decreased risk of cardiovascular disease (CVD) and diabetes.

Objectives: We aimed to prospectively assess the relationship between adherence to the MedDiet and advanced lipoprotein subclass profiles and glucose metabolism and inflammation markers, as determined by nuclear magnetic resonance (NMR) spectroscopy.

Design: We conducted cross-sectional and longitudinal analyses within the framework of the PREvención con DIeta MEDiterránea study in 196 participants from the Reus-Tarragona center. Adherence to the MedDiet was assessed using a 14-item validated questionnaire [Mediterranean Diet Adherence Score (MEDAS)]. Plasma lipoprotein subclasses and molecular metabolite profiles were determined using NMR spectra collected on a Vantera Clinical Analyzer at baseline and after 1 y of follow-up. Baseline and 1-y categories of MEDAS were related to measures of lipoprotein atherogenicity and diabetes risk using multivariable-adjusted analysis of covariance models.

Results: Compared with participants in the lowest category of baseline MEDAS, those in the highest category showed higher concentrations of total high-density lipoprotein (HDL) particles and H1P HDL, lower concentrations of very low-density lipoprotein (VLDL)-triglyceride, smaller size of VLDL, and lower concentrations of very large VLDL, as well as lower concentrations of branched-chain amino acids, leucine, and GlycA and reduced Diabetes Risk Index (DRI) scores. In addition, participants who increased by 3 or more points in their 1-y MEDAS showed an increase in concentrations of H7P-HDL, H5P-HDL, and citrate, and reduced acetone and DRI scores compared with those with lesser adherence increases.

Conclusions: In older adults at high cardiometabolic risk, higher MEDAS was associated with modest beneficial changes in lipoprotein and glucose metabolism. The results suggest that lipoprotein subclass distribution and glycemic control are potential mechanisms behind the well-known salutary effects of MedDiet on CVD and diabetes risk. Future clinical trials exploring the effects of the MedDiet on advanced lipoprotein subclass profiles and glucose metabolism markers are needed to confirm the results of our study.

Trial registration number: This trial was registered at controlled-trials.com as ISRCTN35739639.

Keywords: Mediterranean diet, NMR lipoproteins, T2DM, insulin resistance, atherogenic lipids

Abbreviations: BCAA, branched-chain amino acid; CVD, cardiovascular disease; DRI, Diabetes Risk Index; EVOO, extra virgin olive oil; FFQ, food frequency questionnaire; LP-IR, Lipoprotein Insulin Resistance Index; MEDAS, Mediterranean Diet Adherence Score; MedDiet, Mediterranean diet; NMR, nuclear magnetic resonance; PREDIMED, PREvención con DIeta MEDiterránea; T2DM, type-2 diabetes mellitus.

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Introduction

There is strong evidence regarding the role of the Mediterranean diet (MedDiet) in health maintenance and prevention of non-communicable diseases such as type-2 diabetes mellitus (T2DM), dyslipidemia, cardiovascular disease (CVD), neurodegenerative disorders, and some types of cancer, as well as a reduction of all-cause mortality [1].

The MedDiet is a plant-based dietary pattern characterized by a high content of vegetables, fruits, legumes, whole grains, and nuts; abundant use of extra virgin olive oil (EVOO); low consumption of animal and processed foods; and moderate intake of wine with meals [2]. Previous investigations have shown that the MedDiet provides several nutrients and bioactive compounds that might be implicated in the proposed biological mechanisms behind the reported observations, such as reduction of oxidative stress and inflammation, improvement of endothelial function, and modulation of gut microbiota, among others [3]. Many controlled trials have demonstrated that the MedDiet has a beneficial effect on the lipid profile, with reductions of total cholesterol (TC), LDL cholesterol, and triglycerides (TGs), and increased HDL cholesterol [4]. In addition, studies have shown that the MedDiet might have additional antiatherogenic benefits by promoting improvement in HDL functional properties [5] and decreasing the atherogenicity of VLDL and LDL particles [6,7], as well as improving insulin resistance [8]. However, the precise mechanisms behind the reduction of cardiometabolic risk by the MedDiet are not well understood.

In recent years, in addition to traditional plasma lipid markers (TC, LDL, HDL, etc.), lipoprotein subclasses and changes in particle size, especially large VLDL, small LDL, and small HDL particles, as assessed by nuclear magnetic resonance (NMR) spectroscopy, have been associated with an increased risk of atherosclerotic CVD [9–13]. However, to date, few studies have examined the effects of the MedDiet on lipoproteins subclass number, size, and composition, measurements that are more relevant to the atherosclerotic process [9,10,13,14]. Similarly, in the last years, the MedDiet has been inversely associated with glucose-related risk markers of diabetes, such as branched-chain amino acid (BCAA) concentrations, Lipoprotein Insulin Resistance Index (LP-IR), and the Diabetes Risk Index (DRI), assessed via a targeted NMR platform [8,15], thus providing insight into the mechanisms behind its beneficial effects on glucose metabolism and T2DM risk [16,17]. Nonetheless, knowledge of the long-term impact of the MedDiet on these biomarkers remains limited.

In this study, conducted in older Spanish individuals at increased risk of cardiometabolic disorders, we aimed to prospectively assess the relationship between adherence to the MedDiet and the lipoprotein subclass profile, and glucose metabolism and inflammation markers determined by NMR spectroscopy. We hypothesized that high adherence to the MedDiet would have a beneficial effect on the lipoprotein and glucose-related metabolic profiles in this population. We aimed to prospectively assess the relationship between adherence to the MedDiet and advanced lipoprotein subclass profiles, and glucose metabolism and inflammation markers, as determined by NMR spectroscopy.

Materials

Study design

This analysis was designed as an observational cohort study, in which cross-sectional and longitudinal analyses were conducted in 196 participants from the PREvención con DIeta MEDiterránea (PREDIMED)-Reus center (Spain) with both stored plasma samples collected at the moment of a glucose tolerance test and data on the MedDiet adherence score (MEDAS) at baseline and 1 y of follow-up (Figure 1).

The PREDIMED study was a large randomized, parallel, controlled multicenter clinical trial designed to assess the effect of MedDiet on the primary prevention of CVD in an older population at high risk but free of CVD at baseline. Participants were females and males aged 55–80 y. Inclusion criteria included: a diagnosis of diabetes or meeting ≥3 risk factors for coronary artery disease (smoking, hypertension, hyperlipidemia, low HDL cholesterol concentration, overweight/obesity, or family history of premature coronary artery disease). Candidates were excluded in case of severe chronic illness, drug or alcohol addiction, or allergy/intolerance to olive oil or nuts. In the main study, participants were randomly assigned to 3 intervention groups: a MedDiet supplemented with EVOO, a MedDiet supplemented with mixed nuts, or a low-fat diet according to the American Heart Association guidelines (control group). The PREDIMED study protocol was registered in http://www.controlled-trials.com as ISRCTN35739639 and a detailed version has been published [18]. Enrollment began on June 25, 2003, and the last participant was recruited on June 30, 2009. The intervention was terminated in 2010 with an extended follow-up to August 2015. The protocol of the PREDIMED trial followed the Helsinki Declaration and was approved by the institutional review boards of all centers (for the Reus center, the protocol was approved by Hospital Universitari Sant Joan de Reus). Participants agreed and gave their written informed consent to authorize the use of samples for biochemical measurements.

Dietary assessment

Trained dietitians assessed dietary intake in face-to-face interviews at baseline and yearly using a validated semiquantitative 137-item food frequency questionnaire (FFQ) [19]. For each item, the serving size was established, and 9 consumption frequencies were available, ranging from "never or rarely" to "≥6 times/day." Energy and nutrient intakes were obtained using data from Spanish food composition tables and by multiplying the frequency by the portion size and accounting for the duration of the period assessed [20]. Adherence to the MedDiet was assessed using a validated 14-item questionnaire (MEDAS) [21,22], collected yearly by qualified dietitians in a face-to-face interview with each participant.

Covariates

Information about sociodemographic and lifestyle variables, including smoking status, medical conditions, family history of the disease, and medication use, were collected at baseline. Physical activity was estimated with the validated Spanish version of the Minnesota Leisure-Time Physical Activity Questionnaire [23]. Height and body weight were measured without shoes and wearing light clothing.

Lipidomic profiling by NMR spectroscopy

Fasting blood samples were collected at the baseline and 1-y visits. Plasma was obtained and aliquots were stored at −80°C until metabolomics analyses. NMR spectra were acquired on a Vantera Clinical Analyzer, a 400-MHz NMR instrument, from EDTA plasma samples as described for the NMR LipoProfile test (Labcorp) [24,25]. The LP4 deconvolution algorithm was used to report lipoprotein particle concentrations and sizes, as well as concentrations of metabolites such as

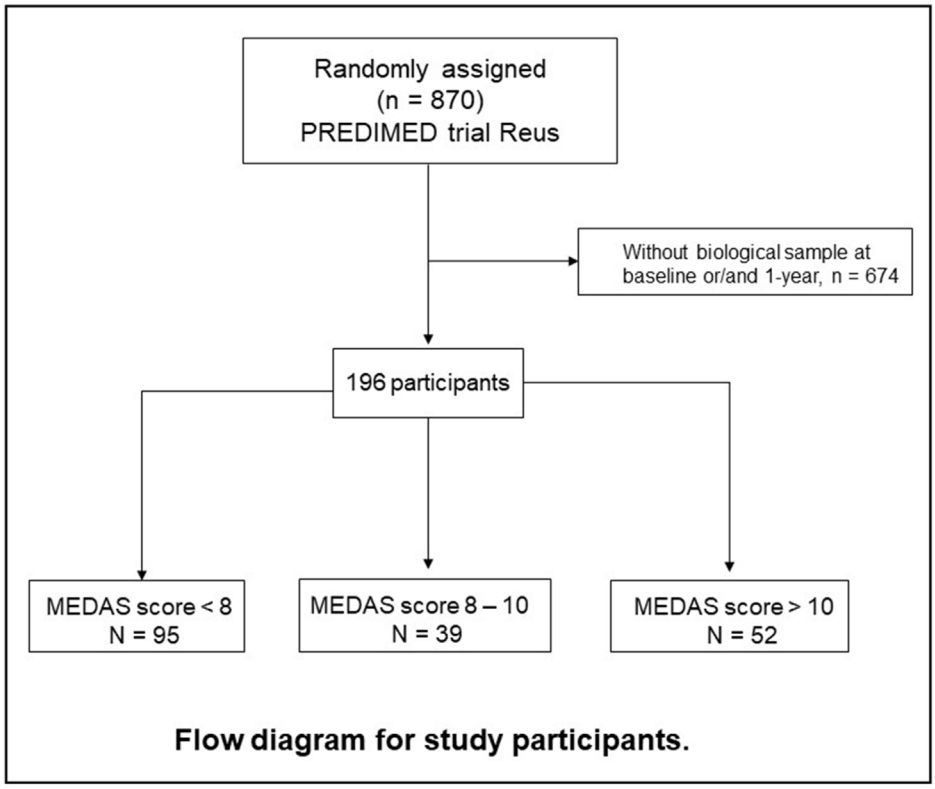

FIGURE 1. Flow diagram for study participants. MEDAS, Mediterranean Diet Adherence Score; PREDIMED, PREvención con DIeta MEDiterránea study.

total BCAAs, valine, leucine, and isoleucine, alanine, glucose, citrate, glycine, total ketone bodies, β-hydroxybutyrate, acetoacetate, and acetone [26]. The diameters of the various lipoprotein classes and subclasses are total TG-rich lipoprotein particles (TRL-P) also known as very low density lipoprotein particles (VLDL-P) (24–240 nm), very large VLDL-P (90–240 nm), large VLDL-P (50–89 nm), medium VLDL-P (37–49 nm), small VLDL-P (30–36 nm), very small VLDL-P (24–29 nm), total LDL-P (19–23 nm), large LDL-P (21.5–23 nm), medium LDL-P (20.5–21.4 nm), small LDL-P (19–20.4 nm), total HDL-P (7.4–12.0 nm), large HDL-P (10.3–12.0 nm), medium HDL-P (8.7–9.5 nm), and small HDL-P (7.4–7.8 nm). The peak diameters for the largest (H7) to the smallest (H1) HDL subspecies are 12.0, 10.8, 10.3, 9.5, 8.7, 7.8, and 7.4 nm. Mean VLDL, LDL, and HDL particle sizes are weighted averages derived from the sum of the diameters of each of the subclasses multiplied by the relative mass percentage. Linear regression against serum lipids measured chemically in a healthy study population ($n = 698$) provided the conversion factors to generate NMR-derived concentrations of TC, TG, VLDL-TG, VLDL-C, LDL-C, and HDL-C. NMR-derived concentrations of these parameters are highly correlated with those measured by standard chemistry methods. Details regarding the performance of the assays that quantify BCAA, alanine, citrate, and ketone bodies have been reported [27–29]. The development of the LP-IR (0–100; least to most insulin resistant), the DRI (1–100; the lowest to the highest risk of type 2 diabetes), and GlycA, as well as their analytic and clinical validation, has been previously published [30–32].

Statistical analyses

The baseline characteristics of study subjects are presented as means ± SD for quantitative variables and percentages for categorical variables. Baseline individual lipoprotein, lipid, apolipoprotein, amino acid, ketone body, and other molecule values were normalized and scaled using Blom's rank-based inverse normal transformation to improve normality [33]. In addition, 1-y changes of these molecules were estimated, and the differences were normalized and scaled using Blom's rank-based inverse normal transformation. Participants were grouped into 3 categories of the baseline 14-point MEDAS adherence screener (<8, 8–10, and >10), using the lowest category (<8) as the reference group. Three categories of 1-y changes in MEDAS were also constructed (<1, 1–3, and >3).

First, we assessed the associations at baseline and 1-y changes between MEDAS categories and lipoprotein values. For cross-sectional analyses, ANCOVA models between categories of baseline MEDAS and lipoprotein values adjusted by age, sex, BMI (kg/m^2), smoking status (ever smoker/never smoker), physical activity (met/d), diabetes (yes/no), dyslipidemia (yes/no), hypertension (yes/no), and statin treatment (yes/no) were used. For 1-y changes, the ANCOVA models between MEDAS 1-y change categories and 1-y changes in lipoprotein values were additionally adjusted by the baseline lipid value, baseline MEDAS, and intervention group (MedDiet + EVOO, MedDiet + nuts, low-fat diet). We used the Tukey test to make multiple comparisons between MEDAS tertiles. The same analyses were performed with apolipoproteins, small-molecule metabolites, and markers of diabetes risk and inflammation. Finally, Spearman correlation coefficients were computed between baseline and 1-y changes in MEDAS and each individual lipoprotein and different molecule measurements.

The assumptions of the ANCOVA models were checked using visual or quantitative methods. All graphs and tests (Shapiro–Wilk test and Levene's tests) yielded models that met the independence of observations, homogeneity of variance (all Levene's test P values >0.05), and normality of residuals (all Shapiro–Wilk test P values >0.05)

criteria. All analyses were performed with R software v4.2.2 (www.r-project.org) (The R Foundation for Statistical Computing, 2022).

Results

Participants were 57% females, aged 67 ± 6 y, with a mean BMI of 29.5 ± 3.3 kg/m^2, who reported a mean physical activity expenditure of 263 ± 242 MET/d. A total of 62% of the participants never smoked and the prevalence of T2D was 2%. The mean MEDAS score was 8 ± 2. Participants involved in this analysis did not differ from the rest of the participants enrolled in the PREDIMED trial in terms of age, sex, and BMI. As expected, differences were observed for hypertension, dyslipidemia, and T2D prevalence ($P < 0.05$), as most of the individuals in this substudy did not have diabetes.

Table 1 shows the baseline characteristics of the study population by categories of the MEDAS score. Participants with the highest adherence to MedDiet had higher educational levels, physical activity, and total energy intake than those with the lowest adherence.

The differences between the lowest and highest categories of baseline MEDAS and 1-y changes of mean normalized values of lipoprotein particles at baseline and 1-y changes are displayed in Table 2. The differences between the lowest and highest categories of baseline

TABLE 1
Baseline characteristics of participants in a subsample of the PREDIMED-Reus trial by categories of adherence to the Mediterranean diet[1]

	Baseline MedDiet Adherence Score (MEDAS)		
	<8	8–10	>10
N	95	39	62
Females (%)	60	59	52
Age (y)	67 ± 6	68 ± 7	66 ± 6
Education (%)			
≥Secondary	19	21	40
Allocation arm (%)			
MedDiet + EVOO	33	46	44
MedDiet + nuts	32	31	40
Low-fat diet	35	23	16
Hypertension (%)	97	90	97
Smoking status (%)			
Never	65	62	57
Current	17	15	16
Dyslipidemia (%)	78	85	86
Type 2 diabetes (%)	2	3	2
Waist circumference (cm)	101 ± 9	100 ± 9	100 ± 9
BMI (kg/m^2)	29.8 ± 3.5	29.5 ± 3.4	29.1 ± 3.4
Leisure-time physical activity (MET-min/d)	226.5 ± 216.8	265.3 ± 231.5	318.9 ± 276
Total energy intake (kcal/d)	2325 ± 564	2284 ± 496	2425 ± 585
Lipid profile (mg/dL)			
Total cholesterol	225.8 ± 32.3	223.1 ± 33.9	223.9 ± 36.6
HDL	57.8 ± 14.3	59.2 ± 14.3	59.7 ± 12.8
LDL	141.3 ± 29.7	139.9 ± 27.7	143.1 ± 30.2
Triglycerides	137.4 ± 56.5	127.2 ± 57.1	114.3 ± 38.0

Data are means ± SDs unless otherwise stated.
Abbreviations: EVOO, extra virgin olive oil; MedDiet, Mediterranean diet; PREDIMED, Prevención con DIeta MEDiterránea.
[1] On the basis of the 14-item screener (MEDAS).

TABLE 2
Lipoprotein particles at baseline and 1-y changes in the highest and lowest categories of Mediterranean diet adherence at baseline and changes at 1 y in a subsample of the PREDIMED-Reus trial

Variables	Baseline visit		1-y changes	
	Lowest MEDAS (<8) (*n* = 95)	Highest MEDAS (>10) (*n* = 62)	Lowest (*n* = 71)	Highest (*n* = 55)
Median (IQ range)	7 [6, 8]	10 [10, 11]	−1 [−1, 0]	4 [3, 5]
Triglyceride-rich lipoprotein (TRL or VLDL) particle concentrations				
Total	0.00 (−0.20. 0.20)	0.03 (−0.23, 0.29)	0.00 (−0.22, 0.22)	0.16 (−0.13, 0.44)
Very large	0.21 (0.00. 0.42)[1]	−0.21 (−0.43, −0.01)[1]	−0.07 (−0.31, 0.18)	0.08 (−0.28, 0.43)
Large	0.14 (−0.06, 0.34)	−0.13 (−0.34, 0.09)	0.10 (−0.12, 0.33)	−0.16 (−0.43, 0.10)
Medium	−0.05 (−0.24, 0.15)	−0.02 (−0.29, 0.26)	0.07 (−0.17, 0.31)	0.03 (−0.23, 0.28)
Small	−0.01 (−0.21, 0.19)	−0.05 (−0.32, 0.22)	−0.01 (−0.24, 0.21)	0.00 (−0.31, 0.32)
Very small	0.01 (−0.20. 0.23)	0.07 (−0.20. 0.34)	0.06 (−0.21, 0.32)	0.11 (−0.21, 0.44)
Calibrated LDL particle concentrations				
Total	0.08 (−0.11, 0.28)	0.08 (−0.15, 0.30)	−0.01 (−0.22, 0.20)	−0.01 (−0.24, 0.22)
Large	−0.02 (−0.22, 0.18)	0.10 (−0.16, 0.35)	−0.06 (−0.23, 0.11)	0.07 (−0.21, 0.35)
Medium	0.02 (−0.20. 0.25)	0.02 (−0.18, 0.22)	−0.01 (−0.31, 0.28)	−0.10 (−0.49, 0.28)
Small	0.04 (−0.16, 0.24)	0.04 (−0.19, 0.27)	0.07 (−0.17, 0.32)	−0.04 (−0.28, 0.20)
Calibrated HDL particle concentrations				
Total	−0.14 (−0.35, 0.01)[1]	0.20 (−0.01, 0.44)[1]	−0.02 (−0.19, 0.15)	0.11 (−0.10. 0.33)
Large	−0.05 (−0.24, 0.13)	0.02 (−0.23, 0.26)	0.01 (−0.13, 0.14)	0.09 (−0.09, 0.27)
Medium	0.05 (−0.14, 0.25)	0.08 (−0.16, 0.33)	0.02 (−0.18, 0.21)	0.07 (−0.15, 0.30)
Small	−0.11 (−0.31, 0.08)	0.14 (−0.12, 0.40)	−0.04 (−0.23, 0.15)	0.03 (−0.17, 0.24)
H7P	−0.09 (−0.29, 0.12)	−0.02 (−0.27, 0.23)	−0.11 (−0.30. 0.00)[1]	0.19 (0.01, 0.47)[1]
H6P	0.04 (−0.16, 0.24)	0.01 (−0.23, 0.25)	0.17 (−0.06, 0.41)	−0.08 (−0.36, 0.19)
H5P	−0.12 (−0.32, 0.07)	0.05 (−0.20. 0.30)	−0.09 (−0.31, 0.00)[1]	0.25 (0.04, 0.53)[1]
H4P	0.09 (−0.11, 0.28)	0.01 (−0.25, 0.26)	−0.08 (−0.31, 0.16)	−0.01 (−0.30. 0.28)

(*continued on next page*)

TABLE 2 (*continued*)

Variables	Baseline visit		1-y changes	
	Lowest MEDAS (<8) (n = 95)	Highest MEDAS (>10) (n = 62)	Lowest (n = 71)	Highest (n = 55)
H3P	0.03 (−0.16, 0.22)	0.07 (−0.19, 0.33)	0.03 (−0.21, 0.27)	0.10 (−0.13, 0.33)
H2P	0.00 (−0.22, 0.22)	0.05 (−0.16, 0.26)	−0.01 (−0.22, 0.20)	−0.07 (−0.33, 0.20)
H1P	−0.16 (−0.36, 0.01)[1]	0.17 (0.01, 0.40)[1]	−0.02 (−0.20. 0.16)	0.08 (−0.12, 0.28)
Mean lipoprotein particle size				
VLDL	0.18 (0.01, 0.38)[1]	−0.21 (−0.46, 0.00)[1]	0.06 (−0.12, 0.25)	−0.11 (−0.36, 0.14)
LDL	−0.06 (−0.27, 0.15)	0.05 (−0.19, 0.28)	−0.06 (−0.23, 0.10)	0.06 (−0.15, 0.27)
HDL	0.01 (−0.18, 0.21)	−0.03 (−0.26, 0.20)	−0.01 (−0.13, 0.11)	0.04 (−0.13, 0.21)
Derived triglyceride and cholesterol concentrations				
TG	0.12 (−0.01, 0.34)[1]	−0.17 (−0.39, 0.01)[1]	0.05 (−0.14, 0.25)	−0.04 (−0.29, 0.21)
TC	0.04 (−0.16, 0.23)	0.07 (−0.18, 0.33)	−0.01 (−0.23, 0.20)	0.10 (−0.15, 0.36)
VLDL-TG	0.13 (0.01, 0.34)[1]	−0.19 (−0.42, 0.00)[1]	0.06 (−0.13, 0.25)	−0.04 (−0.30. 0.21)
VLDL-C	0.05 (−0.16, 0.25)	−0.06 (−0.32, 0.19)	0.03 (−0.18, 0.24)	0.04 (−0.23, 0.32)
LDL-C	0.07 (−0.13, 0.27)	0.09 (−0.15, 0.33)	−0.03 (−0.24, 0.17)	0.00 (−0.25, 0.25)
HDL-C	−0.08 (−0.29, 0.12)	0.11 (−0.11, 0.32)	−0.02 (−0.14, 0.10)	0.11 (−0.06, 0.28)

Lipid data are means (95% CI) of data normalized and scaled in multiples of 1 SD with Blom's rank-based inverse normal transformation data.

Abbreviations: MedDiet, Mediterranean diet; PREDIMED, PREvención DIeta MEDiterránea; TC, total cholesterol; TG, triglyceride; TRL-P, triglyceride-rich lipoprotein particle.

[1] Indicates adjusted P values <0.05 between high compared with low MedDiet obtained by ANCOVA adjusted by age, sex, BMI (kg/m^2), smoking status (ever smoker/never smoker), physical activity (met/d), diabetes (yes/no), dyslipidemia (yes/no), hypertension (yes/no), statin treatment (yes/no), and adjusted using honestly significant difference Tukey method. In addition, 1-y change ANCOVAs were adjusted by baseline lipid values, baseline MEDAS, and intervention group (MedDiet + EVOO, MedDiet + nuts, low-fat diet).

MEDAS and 1-y changes of mean raw values of lipoprotein particles at baseline and 1-y changes are displayed in Supplemental Table 1. Concentrations of very large VLDL-P (adj P value: 0.005), mean VLDL size (adj P value: 0.013), and concentrations of VLDL-TG (adj P value: 0.042) were lower in the highest baseline MEDAS category compared with the lowest MEDAS category. Concentrations of total HDL-P (adj P value: 0.034) and H1P (adj P value: 0.028) were higher in the highest MEDAS category in comparison with the lowest MEDAS category. After 1 y, participants in the highest category of change showed increases in the concentrations of H7P (adj P value: 0.036) and H5P (adj P value: 0.024) compared with those in the lowest category of change.

Table 3 shows Spearman correlation coefficients between MEDAS and the lipoprotein metabolites at baseline and 1-y changes. Concentrations of very large VLDL-P (rho: −0.22; P value: 0.002; adj P value: 0.012) and mean VLDL size (rho: −0.15; P value: 0.034; adj P value: 0.102) were inversely correlated with MEDAS at baseline. After 1 y, no significant negative associations were observed between MEDAS changes and lipoprotein changes. Concentrations of total HDL-P (rho: 0.15; P value: 0.038; adj P value: 0.152) and H1P (rho: 0.15; P value: 0.030; adj P value: 0.210) were positively correlated with MEDAS at baseline, and 1-y changes of H7P were positively correlated with 1-y changes of MEDAS (rho: 0.15; P value: 0.034; adj P value: 0.238).

Differences between baseline and 1-y MEDAS categories in apolipoproteins, small-molecule metabolites, and markers of diabetes risk and inflammation at baseline and 1-y changes are displayed in Table 4 and Supplemental Table 2. Total BCAAs (adj P value: 0.047), leucine

TABLE 3
Spearman correlations coefficients between the lipoprotein metabolites and Mediterranean diet adherence in a subsample of the PREDIMED-Reus trial (n = 196)

Variables	Baseline visit		1-y changes	
	rho	P value	*Rho*	P value
Triglyceride-rich lipoprotein (TRL or VLDL) particle concentrations				
Total	0.05	0.478	0.09	0.188
Very large	−0.22	0.002	0.03	0.683
Large	−0.13	0.065	−0.10	0.172
Medium	0.04	0.617	−0.02	0.762
Small	0.00	0.950	0.02	0.824
Very small	0.01	0.864	0.03	0.728
Calibrated LDL particle concentrations				
Total	−0.03	0.655	0.05	0.475
Large	0.06	0.375	0.09	0.206
Medium	−0.03	0.724	−0.04	0.548
Small	−0.03	0.723	−0.02	0.772
Calibrated HDL particle concentrations				
Total	0.15	0.038	0.05	0.483
Large	0.10	0.177	0.01	0.931
Medium	0.02	0.823	0.04	0.606
Small	0.12	0.090	0.02	0.757
H7P	0.09	0.220	0.15	0.034
H6P	0.03	0.669	−0.06	0.431
H5P	0.12	0.088	0.09	0.194
H4P	−0.04	0.542	0.01	0.885
H3P	0.01	0.904	0.02	0.762
H2P	0.03	0.700	0.00	0.968
H1P	0.15	0.030	0.03	0.677
Mean lipoprotein particle size				
VLDL	−0.15	0.034	−0.08	0.277
LDL	0.06	0.375	0.08	0.288
HDL	0.04	0.614	0.00	0.945
Derived triglyceride and cholesterol concentrations				
TG	−0.12	0.092	−0.03	0.656
TC	0.04	0.626	0.12	0.093
VLDL-TG	−0.13	0.065	−0.05	0.518
VLDL-C	−0.03	0.716	0.04	0.558
LDL-C	0.00	0.957	0.06	0.375
HDL-C	0.12	0.094	0.05	0.464

Lipid data are normalized and scaled in multiples of 1 SD with Blom's rank-based inverse normal transformation data. Adherence to MedDiet was measured on the basis of the 14-item MEDAS screener.

Abbreviations: MedDiet, Mediterranean diet; PREDIMED, Prevención con DIeta MEDiterránea; TC, total cholesterol; TG, triglyceride; TRL-P, triglyceride-rich lipoprotein particle.

TABLE 4
Baseline levels and 1-y changes of apolipoproteins, small-molecule metabolites, and markers of diabetes risk by categories of baseline Mediterranean diet adherence and its 1-y changes in a subsample of the PREDIMED-Reus trial

Variables	Baseline visit		1-y changes	
	Lowest MEDAS (<8) (n = 95)	Highest MEDAS (<10) (n = 62)	Lowest (n = 71)	Highest (n = 55)
Median (IQ range)	7 [6, 8]	10 [10, 11]	−1 [−1, 0]	4 [3, 5]
Derived apolipoprotein concentrations				
Apo B	0.06 (−0.13, 0.26)	0.07 (−0.18, 0.31)	−0.03 (−0.24, 0.19)	0.04 (−0.21, 0.28)
Apo A-1	−0.08 (−0.29, 0.14)	0.13 (−0.09, 0.35)	−0.01 (−0.16, 0.14)	0.06 (−0.13, 0.25)
Amino acid concentrations				
BCAA	0.15 (−0.01, 0.37)[1]	−0.12 (−0.34, 0.00)[1]	0.04 (−0.19, 0.28)	0.07 (−0.14, 0.28)
Valine	0.12 (−0.10. 0.34)	−0.11 (−0.33, 0.12)	0.09 (−0.15, 0.32)	0.07 (−0.17, 0.31)
Leucine	0.14 (−0.01, 0.37)[1]	−0.14 (−0.36, 0.01)[1]	0.00 (−0.24, 0.24)	0.12 (−0.11, 0.35)
Isoleucine	0.13 (−0.08, 0.35)	−0.06 (−0.30. 0.17)	0.05 (−0.20. 0.29)	−0.05 (−0.32, 0.21)
Alanine	−0.01 (−0.21, 0.19)	−0.09 (−0.34, 0.16)	0.10 (−0.11, 0.30)	0.08 (−0.16, 0.33)
Glycine	0.02 (−0.19, 0.23)	0.04 (−0.19, 0.27)	0.04 (−0.12, 0.21)	0.05 (−0.13, 0.24)
Small-molecule metabolites				
Glucose	0.07 (−0.14, 0.28)	−0.11 (−0.35, 0.13)	0.02 (−0.17, 0.20)	−0.09 (−0.34, 0.16)
Citrate	−0.11 (−0.31, 0.09)	0.06 (−0.20. 0.31)	−0.30 (−0.56, −0.04)[1]	0.15 (0.04, 0.44)[1]
Ketone body concentrations				
Total KB	−0.02 (−0.22, 0.18)	0.18 (−0.09, 0.44)	−0.14 (−0.43, 0.15)	−0.10 (−0.43, 0.23)
Beta-hydroxy-butyrate	−0.04 (−0.24, 0.16)	0.21 (−0.05, 0.48)	−0.18 (−0.45, 0.08)	−0.05 (−0.40. 0.29)
Acetoacetate	0.05 (−0.13, 0.24)	0.01 (−0.28, 0.30)	−0.10 (−0.45, 0.26)	−0.10 (−0.41, 0.22)
Acetone	−0.04 (−0.25, 0.17)	0.09 (−0.14, 0.32)	0.13 (0.03, 0.45)[1]	−0.29 (−0.68, −0.10)[1]
Diabetes risk multimarkers				
LP-IR score	0.11 (−0.08, 0.31)	−0.14 (−0.36, 0.09)	0.00 (−0.18, 0.17)	−0.15 (−0.38, 0.09)
DRI score	0.16 (0.03, 0.36)[1]	−0.29 (−0.53, −0.06)[1]	0.32 (0.00. 0.63)[1]	−0.24 (−0.56, −0.07)[1]
Other biomarkers				
GlycA	0.18 (0.01, 0.37)[1]	−0.24 (−0.50. 0.00)[1]	−0.03 (−0.21, 0.15)	0.01 (−0.20. 0.22)
TMAO	0.02 (−0.17, 0.21)	−0.07 (−0.35, 0.21)	0.14 (−0.20. 0.48)	−0.01 (−0.34, 0.31)

Data are means ± SD of normalized and scaled in multiples of 1 SD with Blom's rank-based inverse normal transformation data. Adherence to MedDiet was measured based on the 14-item MEDAS screener. These values are presented as median [IQR].
Abbreviations: Apo, apolipoprotein; BCAA, branched-chain amino acid; DRI, Diabetes Risk Index; KB, ketone body; LP-IR, lipoprotein insulin resistance; MedDiet, Mediterranean diet; PREDIMED, PREvención con DIeta MEDiterránea; TMAO, trimethylamine N-oxide.
[1] Indicates adjusted P values <0.05 between high compared with low MedDiet obtained by ANCOVA adjusted by age, sex, BMI (kg/m^2), smoking status (ever smoker/never smoker), physical activity (met/d), diabetes (yes/no), dyslipidemia (yes/no), hypertension (yes/no), statin treatment (yes/no), and adjusted using the honestly significant difference Tukey method. In addition, 1-y change ANCOVAs were adjusted by baseline metabolic values, baseline MEDAS, and intervention group (MedDiet + EVOO, MedDiet + nuts, low-fat diet).

(adj P value: 0.047), DRI (adj P value: 0.005), and GlycA (adj P value: <0.001) were lower in the highest category of baseline MEDAS compared with the lowest category. Only DRI maintained lower 1-y changes (adj P value: 0.002). In addition, participants in the highest category of MEDAS 1-y changes showed higher concentrations of citrate (adj P value: 0.006) and lower concentrations of acetone (adj P value: 0.023) compared with the participants in the lowest category.

Table 5 shows Spearman's correlation coefficients between MEDAS scores and apolipoproteins, small-molecule metabolites, and markers of diabetes risk and inflammation at baseline and 1-y changes. DRI (rho: −0.23; P value: 0.001; adj P value: 0.002) and GlycA (rho: −0.19; P value: 0.007; adj P value: 0.014) had an inverse correlation with baseline MEDAS and only DRI changes (rho: −0.16; P value: 0.024; adj P value: 0.048) maintained an inverse correlation with MEDAS changes. The 1-y citrate changes (rho: 0.18; P value: 0.013; adj P value: 0.026) were positively correlated with the 1-y MEDAS changes.

Discussion

In this study conducted in a population of Spanish older individuals at high cardiometabolic risk, higher adherence to the MedDiet was associated with more favorable profiles of lipoprotein subclasses, small-molecule metabolites, and biomarkers of diabetes risk, as assessed by a targeted NMR spectroscopy platform. At baseline, higher adherence to MedDiet was associated with lower very large VLDL-P, BCAA, leucine, DRI, and GlycA, and lower VLDL size, and higher total HDL-P and H1P. In the 1-y assessment, increased adherence to the MedDiet was associated with decreases in acetone and DRI and increases in H7P, H5P, and citrate. These results suggest that the benefits of the MedDiet not only affect the lipid profile but also the shape, composition, and size of lipoproteins.

In the last decade, interventions with healthy, plant-based dietary patterns have been recognized as the more effective approach to

TABLE 5
Spearman correlations coefficients between other metabolites and Mediterranean diet adherence in a subsample of the PREDIMED-Reus trial ($n = 196$)

Variables	Baseline visit		1-y changes	
	rho	*P* value	*rho*	*P* value
Derived apolipoprotein concentrations				
Apo B	−0.01	0.895	0.10	0.166
Apo A-1	0.12	0.093	0.04	0.533
Amino acid concentrations				
BCAA	−0.12	0.091	−0.02	0.778
Valine	−0.10	0.163	−0.02	0.833
Leucine	−0.12	0.108	0.03	0.709
Isoleucine	−0.05	0.455	−0.07	0.320
Alanine	−0.07	0.297	−0.05	0.515
Glycine	−0.05	0.496	−0.05	0.518
Small-molecule metabolites				
Glucose	−0.05	0.465	−0.02	0.791
Citrate	0.08	0.282	0.18	0.013
Ketone body concentrations				
Total KB	0.09	0.210	0.02	0.769
Beta-hydroxy-butyrate	0.11	0.118	0.05	0.523
Acetoacetate	0.00	0.999	0.00	0.973
Acetone	0.08	0.256	−0.11	0.123
Diabetes risk multimarkers				
LP-IR score	−0.11	0.125	−0.05	0.524
DRI score	−0.23	0.001	−0.16	0.024
Other biomarkers				
GlycA	−0.19	0.007	0.01	0.851
TMAO	−0.02	0.772	−0.06	0.410

Data are normalized and scaled in multiples of 1 SD with Blom's rank-based inverse normal transformation data. Adherence to MedDiet was measured based on the 14-item screener.
Abbreviations: MedDiet, Mediterranean diet; Apo, apolipoprotein; BCAA, branched-chain amino acid; DRI, Diabetes Risk Index; KB, ketone body; LP-IR, lipoprotein insulin resistance; PREDIMED, Prevención con DIeta MEDiterránea; TMAO, Trimethylamine N-oxide.

chronic disease prevention and management [34]. In this work, we aimed to provide an overall view of the MedDiet effects rather than the individual impact of its components over NMR-assessed lipoprotein subfractions.

To date, a handful of studies have examined the relationship between MedDiet and lipoprotein subclasses as assessed by NMR [8–11,14]. In agreement with our results for VLDL and HDL subclasses, an analysis conducted with a large dataset from the Women's Health Study showed that higher adherence to MedDiet was inversely associated with plasma VLDL size and positively associated with HDL particle number and size [8]. In a small randomized controlled trial (RCT) in individuals at risk of metabolic syndrome, Michielsen et al. [14] also found that, compared with a diet rich in saturated fatty acids, a MedDiet decreased large VLDL-P. Another RCT using MedDiet against an average American diet (rich in saturated fat) also reported a reduction of LDL-P with MedDiet [35]. Moreover, in a subsample of individuals from the PREDIMED Barcelona-North site, compared with the participants advised on a low-fat diet, those allocated to MedDiet enriched with EVOO or nuts also increased large HDL concentrations after intervention for 1 y [9]. In addition, participants assigned the MedDiet supplemented with nuts reduced the concentrations of medium-small and very small LDL, decreased LDL-P, and increased LDL size [9]. In our study, MedDiet adherence was unassociated with changes in LDL-P, which contrasts with findings from these 3 studies [9,11,14]. This discrepancy might be explained by the characteristics of the comparator diets in these RCTs, high in saturated fat or carbohydrates, which are known to increase LDL-P [36]. Other epidemiologic studies [10] or clinical trials [12,37] with MedDiet failed to find associations with lipoprotein subfraction profiles.

The beneficial effects of nut consumption within the MedDiet on NMR-assessed lipoprotein subclasses, particularly the LDL fractions [9], have also been reported in RCTs conducted in other populations [35,38], and in an observational study of a similar PREDIMED-Reus subsample [39]. In the older Mediterranean population of that study [9], nut consumption was also associated with lower circulating concentrations of BCAA and decreased insulin resistance.

Our results of advanced metabolomic analyses concerning ketone bodies and glucose-related risk markers of diabetes are in line with those of Ahmad et al. [8], who reported an inverse association between adherence to the MedDiet and lower BCAA (valine, leucine, and isoleucine) and DRI concentrations in a large United States cohort of females. Likewise, in a nested case–cohort study within the PREDIMED trial, Ruiz-Canela et al. [15] reported an inverse association between adherence to a MedDiet enriched with EVOO and plasma BCAA concentrations after 1-y of intervention, whereas elevated BCAAs were associated with higher T2DM risk. Our findings regarding the association between MedDiet, BCAAs, DRI, and GlycA are of special interest, as they provide a novel approach in relation to the mechanisms underlying the health effects of MedDiet, while supporting the potential role of these biomarkers as predictors of insulin sensitivity and diabetes [40–42]. In fact, in different studies, adherence to the MedDiet has been inversely related to diabetes risk [16] and, in individuals with diabetes, to a better metabolic status [4,17]. In addition, reports from the PREDIMED study indicate that diabetes can be prevented in individuals at high risk of CVD with MedDiets enriched with EVOO or nuts even in the absence of weight loss [43,44].

The MedDiet has a high content of unsaturated fatty acids, fiber, and polyphenols, which can act synergistically to counteract inflammatory and oxidative stimuli, thus attenuating the atherosclerotic process and the progression of diabetes; the MedDiet also provides sizable amounts of micronutrients, such as vitamins E and C and minerals like magnesium and potassium, which have been associated with blood pressure lowering, improved endothelial function and insulin sensitivity and further reduce inflammation and oxidative stress [3]. Altogether, these mechanisms could potentially result in a less atherogenic profile and a decreased risk of diabetes.

Our study has limitations. First, study participants were enrolled in a nutrition intervention RCT for the primary prevention of CVD, which might have influenced the results, even though all analyses were controlled for the intervention arm. Second, because of the observational nature of our study and study population (older individuals at high cardiometabolic risk), we cannot establish a cause–effect relationship, and the generalizability of our findings to other populations may be limited. Furthermore, the targeted NMR platform that we used provided a large set of analyses entailing many comparisons, which made necessary the introduction of a constraint on the *P* values, such as the Tukey test. This resulted in an attenuation of several associations. Finally, a larger sample size might be needed to detect other significant correlations between MedDiet adherence and metabolite changes at follow-up. The study also has strengths, such as its prospective design and the use of a robust technique (NMR spectroscopy) of lipoprotein subfractionation and small-molecule determination. Likewise, we have carried out different types of statistical analyses (using ANOVA and correlations, and adjusting by FWER) to confirm the results from various statistical approaches despite the small sample size.

In conclusion, our results suggest that, in older adults at high cardiometabolic risk, higher adherence to the MedDiet is associated with

the modest beneficial features of lipoprotein subfractions and glucose metabolism profiles. The observed associations of the MedDiet with antiatherogenic lipoprotein changes and improvement of glycemic and inflammation markers provide potential novel mechanistic insight into the benefits of the MedDiet on CVD and diabetes risk. If these associations are proven, our findings could also contribute to the identification of novel biomarkers in the prevention and risk prediction of noncommunicable chronic diseases.

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Author contributions

The authors' responsibilities were as follows – CSM, ER, JS-S: conceptualized the research; IP-G, JFG-G, JS-S: designed the work; JFG-G, MAC, CSM: acquired and analyzed the data; IP-G, JFG-G, ER, JS-S: interpreted the results; IP-G, JFG-G, JS-S, ER: drafted the work; MAC, NB, CSM: substantively revised it; and all authors: read and approved the final manuscript.

Conflict of interest

MAC is an employee and owns stock in Labcorp. CSM reports grants through his institution from Merck; has been a shareholder of and has received grants through his institution and personal consulting fees from Coherus Inc. and AltrixBio; reports personal consulting fees from Novo Nordisk, reports personal consulting fees and support with research reagents from Ansh Inc., collaborative research support from LabCorp Inc., reports personal consulting fees from Genfit, Lumos, Amgen, Corcept, Intercept, and Regeneron, reports support (educational activity meals through his institution or national conferences) from Amarin, Novo Nordisk, Astra Zeneca, Boehringer Ingelheim and travel support and fees from TMIOA, Elsevier, the California Walnut Commission, Sacramento, CA, USA (CWC), College Internationale Researche Servier and the Cardio Metabolic Health Conference. None is related to the work presented herein. ER reports receiving grant support through his institution from the CWC, in addition to personal funds for project supervision and advice, and serving as non-paid member of its Scientific Advisory Committee; funds for travel and accommodation from the International Nut and Dried Fruit Council (INC); and personal funds from Alexion for serving in advisory committee. JS-S reports serving on the boards of the INC and receiving grant support from these entities through his institution. He has received research funding from the Instituto de Salud Carlos III, Spain; Ministerio de Educación y Ciencia, Spain; Departament de Salut Pública de la Generalitat de Catalunya, Catalonia, Spain; and the European Commission. He has also received research funding (nuts for free to the PREDIMED participants) from the CWC; La Morella Nuts, Spain; and Borges SA, Spain. He has also received research funding (nuts for free to the PREDIMED-Plus participants) from the Almond Board of California, United States and Pistachio Growers of California, United States. He is on the Clinical Practice Guidelines Expert Committee of the European Association for the Study of Diabetes (EASD), and has served on the Scientific Committee of the Spanish Food and Safety Agency, and the Spanish Federation of the Scientific Societies of Food, Nutrition, and Dietetics. He is a member of the International Carbohydrate Quality Consortium (ICQC), and an Executive Board Member of the Diabetes and Nutrition Study Group (DNSG) of the EASD.

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Data availability

The datasets generated and/or analyzed during the current study are not publicly available because of the lack of authorization from PREDIMED participants but are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate

The Research Ethic Committee of the Institut d'Investigacions Sanitàries Pere i Virgili approved the PREDIMED study protocol and all participants provided written informed consent.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajcnut.2023.11.003.

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