Mediterranean Diet and atherothrombosis biomarkers: a randomized controlled trial

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Abbreviations:

MedDiet: Mediterranean Diet

MedDiet-Nuts: Mediterranean Diet enriched with mixed nuts

MedDiet-VOO: Mediterranean Diet enriched with virgin olive oil

NEFA: non-esterified fatty acids

PAF-AH: platelet activating factor-acetylhydrolase

PAI-1: plasminogen activator inhibitor-1

PREDIMED: PREvención con DIeta MEDiterránea

Keywords: Mediterranean diet, atherothrombosis, biomarker, clinical trial, cardiovascular risk

ABSTRACT

Scope. To assess whether following a Mediterranean diet (MedDiet) improved atherothrombosis biomarkers in high cardiovascular risk individuals.

Methods and results. In 358 random volunteers from the *PREvención con DIeta MEDiterránea* trial, we assessed the 1-year effects on atherothrombosis markers of an intervention with MedDiet, enriched with virgin olive oil (MedDiet-VOO; *n*=120) or nuts (MedDiet-Nuts; *n*=119) versus a low-fat control diet (*n*=119). We also studied whether large increments in MedDiet adherence (≥3 score points, relative to compliance decreases) and intake changes in key food items were associated with 1-year differences in biomarkers. We observed differences between 1-year changes in the MedDiet-VOO intervention and control diet on the activity of platelet activating factor acetylhydrolase in HDLs (+7.5% [95% confidence interval: 0.17; 14.8]) and HDL-bound α_1-antitrypsin levels (-6.1% [-11.8; -0.29]), and between the MedDiet-Nuts intervention and the control arm on non-esterified fatty acid concentrations (-9.3% [-18.1; -0.53]). Large MedDiet adherence increments were associated with less fibrinogen (-9.5% [-18.3; -0.60]) and non-esterified fatty acid concentrations (-16.7% [-31.7; -1.74]). Increases in nut, fruit, vegetable, and fatty fish consumption, and decreases in processed meat intake were linked to beneficial changes in atherothrombosis biomarkers.

Conclusion. Following a MedDiet improved atherothrombosis biomarkers in high cardiovascular risk individuals.

INTRODUCTION

Better adherence to a traditional Mediterranean Diet (MedDiet) prevents major cardiovascular clinical outcomes[1–3]. These beneficial effects may be mediated by improvements in risk factors related to glucose metabolism, endothelial function, lipid profile, oxidative stress, and low-grade inflammation[4]. Nevertheless, little evidence related to MedDiet effects on atherothrombosis mechanisms is available, although the antithrombotic properties of some of its individual components are known[5,6].

Increased levels of biomarkers of platelet aggregation (P-selectin[7], platelet factor-4[8]) and coagulation (fibrinogen[9], prothrombin fragment 1+2 –proportional to thrombin formation–[10], antithrombin[11]), and decreased concentrations of fibrinolysis indicators (plasminogen activator inhibitor-1 –PAI-1–[12], D-dimer[13]) have been linked to increased incidence of major atherosclerotic events in prospective human studies. In addition, high levels of non-esterified fatty acids (NEFAs) and dysfunctional high-density lipoproteins (HDLs) have also been attributed pro-thrombotic properties[14,15]. In the context of the PREDIMED (*PREvención con DIeta MEDiterránea)* trial, following a MedDiet decreased P-selectin levels[16]. Beyond this study, only a small-scale prospective analysis with 21 young, healthy male volunteers indicated that following a MedDiet-like dietary pattern was related to lower fibrinogen levels and an attenuation of the coagulation response[17], and two cross-sectional studies have reported associations between MedDiet adherence and less D-dimer and fibrinogen values[18,19]. However, no intervention trial has studied to date the long-term effects of this healthy dietary pattern on a comprehensive set of biomarkers of atherothrombosis.

Our main aim was to assess whether a 1-year intervention with MedDiet improved a set of atherothrombosis biomarkers in high cardiovascular risk individuals. Our secondary aim was to determine whether 1-year changes in MedDiet adherence and in the

consumption of key food items of the MedDiet were associated with 1-year differences in these indicators.

EXPERIMENTAL SECTION

Study design

Study subjects were participants of the PREDIMED Study. It was a large-scale, parallel, multicenter, randomized controlled trial assessing the long-term effects of following a MedDiet on the primary prevention of cardiovascular disease in high cardiovascular risk individuals[1]. Volunteers were men (aged 55-80) and women (aged 60-80) free of cardiovascular disease at enrolment but presenting type 2 diabetes mellitus or at least three of these factors: high levels of low-density lipoprotein cholesterol, low HDL cholesterol concentrations, hypertension, overweight/obesity, current smoking, or family history of premature heart disease. Further details of inclusion/exclusion criteria are available in **Supplemental Methods**. The trial protocol complied with the Declaration of Helsinki, was approved by local institutional ethic committees, registered under the International Standard Randomized Controlled Trial Number ISRCTN35739639 (http://www.isrctn.com/ISRCTN35739639), and described in previous publications[1,20]. An institutional ethic committee (CEIC-PSMAR) also approved the protocol of the present sub-study. All participants provided written informed consent before joining the trial.

Individuals were randomly assigned to one of three interventions (in a 1:1:1 ratio): 1) a MedDiet enriched with virgin olive oil (MedDiet-VOO); 2) a MedDiet enriched with mixed nuts (MedDiet-Nuts); and 3) a low-fat control diet. MedDiet interventions promoted: 1) the consumption of fruits, vegetables, legumes, nuts, and fish; 2) the use of virgin olive oil as main culinary fat; 3) a decrease in the intake of ultra-processed foods/drinks and

animal fats and the substitution of red/processed meats for poultry; and 4) the consumption of foods cooked by home-made methods (such as the traditional “sofrito”). To promote compliance and account for family needs, volunteers allocated in the MedDiet-VOO intervention received 1L/week of virgin olive oil and those in the MedDiet-Nuts group 210 g/week of mixed nuts. Volunteers allocated to the low-fat control group were advised: 1) to promote the intake of fruits, vegetables, and legumes; 2) to decrease the consumption of high-fat dairy products, butter/margarine, meat, and ultra-processed foods/drinks, and 3) to moderate their intake of other fatty foods (olive oil, nuts, and fatty fish). Further details of the dietary intervention are available in **Supplemental Methods**.

For the present analyses, we selected a random subsample of 358 subjects (4.8% of the total PREDIMED population) recruited in Hospital Clinic (Barcelona) and Hospital del Mar Medical Research Institute (Barcelona) study sites, with fasting citrate plasma samples collected at baseline and after 1 year of intervention. 120 volunteers were allocated to the MedDiet-VOO intervention group, 119 to MedDiet-Nuts, and 119 to the control diet. Samples were stored at -80ºC until the analyses. The CONSORT checklist regarding our study is available in **Supplemental Table 1**.

Mediterranean diet adherence, intake of key food items, and covariates

MedDiet adherence was estimated at baseline and after 1 year of intervention using the MedDiet adherence score. It was a validated short screener interrogating whether the volunteer followed 14 essential dietary characteristics associated with a MedDiet (positively scoring: intake of virgin olive oil, nuts, fruits, vegetables, legumes, fish, and wine in moderation; and the substitution or avoidance of animal fats, red and processed meats, processed foods, and sugary drinks)[21]. 1-year increments in MedDiet adherence were calculated by subtracting the baseline adherence to the 1-year value.

We estimated at baseline and after 1 year of intervention the intake of eight key food items whose consumption was encouraged or discouraged in the MedDiet intervention (virgin olive oil, mixed nuts, fruits, vegetables, legumes, fresh fatty fish, processed meat, and alcohol) using a semiquantitative, validated 137-item food frequency questionnaire[22]. 1-year changes in their intake were calculated by subtracting the baseline consumption to that after one year of intervention, and expressed in common portion sizes (+10 g/day of virgin olive oil, +30 g/day of nuts, +100 g/d of vegetables or fruits, +25 g/day of legumes or fresh fatty fish, -25 g/day of processed meat, and +10 g/day of alcohol).

Data on age, sex, educational level, body mass index, smoking habit, and presence of type-2 diabetes mellitus, hypercholesterolemia, hypertension, leisure-time physical activity, and use of antithrombotic medications were gathered at baseline by trained clinical personnel[1,20].

Atherothrombosis biomarkers

We used ELISA kits to quantify the levels of: fibrinogen (*Human Fibrinogen SimpleStep ELISA kit*, ref.: ab208036, abcam, UK), PAI-1 (*Human PAI1 SimpleStep ELISA kit (SERPINE1)*, ref.: ab184863, abcam, UK), platelet factor-4 (*PF4 (CXCL4) Human SimpleStep ELISA kit*, ref.:ab189573, abcam, UK), and prothrombin fragment 1+2 (*Human F1+2 (prothrombin fragment 1+2) ELISA kit*, ref.: E-EL-H1793, Elabscience, USA). We assessed the concentrations of antithrombin (*Antithrombin III*, ref.: SR-1102016, Spinreact, Spain) and D-dimer (*D-Dimer*, ref.: SR-1709231, Spinreact, Spain) by immunoturbidimetry, and of NEFAs (*Non-Esterified Fatty Acids*, ref.: FA115, Randox, Spain) using a colorimetric technique in an ABX-Pentra 400 autoanalyzer (Horiba-ABX, France). All the previous analyses were performed in fasting citrate plasma samples. In parallel, in apolipoprotein B-depleted plasma samples (specimen in which all lipoproteins but HDLs were eliminated, also prepared from fasting citrate plasma[23]), we quantified total

cholesterol by colorimetry (*Cholesterol Enzymatic-Colorimetry*, ref.: SR-41022, Spinreact, Spain) and α_1-antitrypsin (*a-1-antitrypsin*, ref.: SR-1102054, Spinreact, Spain) by immunoturbidimetry in an ABX-Pentra 400 autoanalyzer (Horiba-ABX, France). With this data, we calculated the α_1-antitrypsin/cholesterol ratio ("α_1-antitrypsin levels in HDL"). We also determined in these samples the activity of the PAF-AH enzyme by a colorimetric kit (*PAF Acetylhydrolase Activity Assay Kit*, ref.: K765-1000, Cayman Chemical, USA). A detailed explanation of the quality control is available in **Supplemental Methods** and **Supplemental Table 2**.

According to our post-analysis sample sizes and standard deviations of the post- vs. pre-intervention differences for each determination, we estimated the intra- and inter-group differences we were allowed to detect with ≥80% power and assuming a type-I error of 0.05 (**Supplemental Table 3**).

Statistical analyses

We first checked the distribution of continuous variables by normality plots and the Shapiro-Wilk test. We assessed whether there were differences in baseline values among the three intervention groups with one-way ANOVA tests for normally distributed continuous variables, Kruskal-Wallis tests for non-normally distributed continuous ones, and chi-squared tests for categorical parameters.

We first evaluated whether there were differences in the 1-year changes in atherothrombosis biomarkers (calculated by subtracting baseline from post-intervention values) in the MedDiet interventions relative to the control diet by multivariable linear regressions. We studied these differences in the whole population of the study (main approach), in participants with very low adherence to a MedDiet at baseline (<8 score points), and users of antithrombotic drugs[24]. We also analyzed if there were intra-group differences between pre- and post-intervention values in every study arm by paired t tests

in normally distributed variables and Wilcoxon signed-rank tests in non-normally distributed ones. As secondary, observational analyses, we studied whether substantial increments in MedDiet adherence after 1 year of intervention (≥3 score points, in relation to decreases in MedDiet compliance), and whether 1-year changes in the consumption of key food items (virgin olive oil, mixed nuts, fruits, vegetables, legumes, fresh fatty fish, processed meat, and alcohol), were associated with 1-year differences in atherothrombosis biomarkers by multivariable linear regression models.

To help in data interpretation, we present the results as percentage changes relative to baseline values. To calculate them, we divided the adjusted difference coefficients obtained in the regression models by the baseline value of the variable in each of the groups (in the main analyses) or in the 358 volunteers (in the secondary analyses). Models were adjusted for: age (continuous), sex, study site, educational level (primary education/secondary/higher education), baseline value of the atherothrombosis biomarker, diabetes (yes/no), hypercholesterolemia (yes/no), hypertension (yes/no), use of antithrombotic medication (yes/no), tobacco use (never/former/actual smoker), body mass index (continuous), leisure-time physical activity (continuous), alcohol use (continuous), and two propensity scores to correct for the theoretical deviations in the randomization process (calculated from 30 baseline variables)[1]. Secondary, observational analyses were additionally adjusted for the allocated intervention group and MedDiet adherence score at baseline. Models were plotted using the “lme” package in R Software[25].

We performed statistical analyses with R software version 3.5.0[26].

RESULTS

Study population and intervention

Our study sub-sample were elderly adults (mean age 67 years, 63% women) with high prevalence of cardiovascular risk factors at baseline (78% hypertension, 74% hypercholesterolemia, 51% diabetes, 50% overweight, 44% obesity, 14% current smokers, 13% antithrombotic drug users). We found no clinical differences in baseline characteristics among intervention groups (**Table 1**). Individuals in our analytical sample were less likely to be men and users of antithrombotic drugs, and more prone to present hypercholesterolemia than the rest of the PREDIMED study population (**Supplemental Table 4**).

Volunteers' compliance improved during the year of intervention (MedDiet-VOO: +1.14 points [95% confidence interval: 0.79; 1.49]; MedDiet-Nuts: +1.35 [1.00; 1.69]). No changes in physical activity were observed (**Supplemental Table 5**).

MedDiet interventions and 1-year changes in atherothrombosis biomarkers

In the whole study population, we observed significant differences between 1-year changes in the MedDiet-VOO intervention and the control diet on the activity of platelet activating factor acetylhydrolase in HDLs (adjusted difference: +7.5% [0.17; 14.8]; **Figure 1I**) and HDL-bound α_1-antitrypsin levels (adjusted difference: -6.1% [-11.8; -0.29]; **Figure 1H)**. We also detected significant differences between 1-year changes in the MedDiet-Nuts intervention and the control arm on the concentrations of NEFAs (adjusted difference: -9.3% [-18.1; -0.53]; **Figure 1G**). Exact results are available in **Supplemental Table 6**.

In volunteers with low MedDiet adherence at enrolment, we observed a significant difference in 1-year changes in antithrombin levels between the MedDiet-VOO intervention and the control diet (adjusted difference: +6.1% [0.76; 11.4]; **Supplemental Figure 1D**). Finally, in users of antithrombotic drugs we also detected significant differences between the MedDiet-VOO intervention and the low-fat control arm in 1-year changes in the

concentrations of D-dimer (adjusted difference: -54.1% [-86.8; -21.3]; **Supplemental Figure 1F**).

When assessing post- vs. pre-intervention changes, and despite these differences were not significant relative to the MedDiet interventions, the low-fat control diet was associated with increases in platelet factor-4 concentrations (*P*=0.012) and prothrombin fragment 1+2 levels (*P*=0.003) relative to baseline values (**Supplemental Table 7**).

Cross-sectional associations of 1-year changes in MedDiet adherence with 1-year differences in atherothrombosis biomarkers

Substantial increments in MedDiet adherence, relative to decreases in compliance, were associated with reduced fibrinogen (adjusted difference: -9.5% [-18.3; -0.60]; **Figure 2B**) and NEFA concentrations (adjusted difference: -16.7% [-31.7; -1.74]; **Figure 2G**). Exact results are available in **Supplemental Table 8**.

Cross-sectional associations of 1-year changes in the intake of key food items with 1-year differences in atherothrombosis biomarkers

1-portion increments of mixed nuts were related to greater antithrombin levels (adjusted difference: +2.2% [0.070; 4.34]). 1-serving increases in the intake of fruits and vegetables were linked to lower prothrombin fragment 1+2 (adjusted difference: adjusted difference: -7.6% [-13.1; -2.09]) and platelet factor-4 concentrations, respectively (adjusted difference: -9.3% [-17.6; -0.90]). 1-portion increments in fatty fish consumption were also associated with lower levels of prothrombin fragment 1+2 levels (adjusted difference: -13.1% [-26.0; -0.20]). Finally, 1-serving decreases in processed meat intake were related to greater antithrombin levels (adjusted difference: +2.0% [0.36; 3.64]), higher PAF-AH activity in HDLs (adjusted difference: +6.1% [1.38; 10.8]), and lower concentrations of PAI-1 in plasma (adjusted difference: -10.4% [-18.2; -2.71]) (**Supplemental Table 9)**.

DISCUSSION

Our results show that following a MedDiet improved several atherothrombosis biomarkers in older individuals at high cardiovascular risk.

Atherothrombosis is strongly affected by inflammation and oxidative stress[27,28]. MedDiet is a dietary pattern known to enhance these risk factors[4,29], possibly explaining its protective effects on thrombosis mechanisms as well. In our data, following the MedDiet-VOO intervention improved HDL antithrombotic properties (and, under certain circumstances, antithrombin and D-dimer levels), whilst the MedDiet-Nuts decreased NEFA levels. We previously reported that the MedDiet-VOO intervention enhanced HDL functionality in the PREDIMED Study[23]. However, these findings extend this protection to HDL antithrombotic functions. Dietary antioxidants in the MedDiet (such as fruit- and vegetable-derived antioxidant vitamins) can bind to the structure of HDLs, contribute to their maintenance in non-oxidized forms, and help explain the reported improved activity of HDL protective enzymes[30]. In addition, following a MedDiet has also been associated with decreased levels of pro-inflammatory molecules in circulation[16]. This fact could contribute to explaining the reduction in the concentrations of pro-inflammatory molecules bound to HDL particles, as well as improvements in other pro-thrombotic signals (the decrease in D-dimer levels after the MedDiet intervention among users of antithrombotic drugs and the association between substantial increments in MedDiet adherence and lower concentrations of fibrinogen, both consistent with previous evidence[18,19]). In parallel, to explain the association between the MedDiet-Nuts intervention and lower NEFA levels, we hypothesize that polyunsaturated fatty acids (whose intake was particularly increased in this group, especially non-marine omega-3 fatty acids –whose intake increased by 1.15 g/day–) bind to GPR120 receptors in adipose tissue, reduce lipolysis, and decrease the circulating levels of free fatty acids[31]. The elevated consumption of dietary fiber in this

dietary pattern could contribute to increasing the circulating levels of short-chain fatty acids (derived from the bacterial fermentation of fiber in the intestine), which are able to bind other GPR family receptors with a similar anti-lipolytic effect[32].

Our study has limitations. First, our analyses were not originally included in the study protocol and, therefore, should be considered as exploratory findings. Second, study volunteers were elderly people at high cardiovascular risk, which does not allow the extrapolation of our findings to other populations. Third, we have only been able to report moderate changes or associations. However, this effect magnitude was expected because our work was based on modest real-life dietary modifications and the control diet was already a well-known healthy dietary pattern. Fourth, some of our inter-group associations (differences in 1-year changes in PAF-AH activity and α_1-antitrypsin levels in apolipoprotein B-depleted plasma between the MedDiet-VOO intervention and the control arm) may rely on non-significant intra-group changes. Fifth, the statistical power of the analyses in particular sub-groups of participants could be low (**Supplemental Table 10**) and, therefore, these results should be interpreted cautiously. Sixth, cross-sectional associations of 1-year changes in the intake of key food items with 1-year differences in atherothrombosis biomarkers were not adjusted for multiple comparisons and, therefore, these findings should be considered as preliminary and verified in future studies. Finally, data on MedDiet adherence (and other covariates such as physical activity and ethanol intake) were self-reported and could be somewhat imprecise. However, the main strengths of our study were its large sample size (*n*=358), randomized design, long-term duration (1 year), and strict quality control for laboratory biomarkers.

In conclusion, 1 year of intervention with MedDiet enhanced HDL antithrombotic properties and decreased NEFA levels in high cardiovascular risk individuals (and at a second level of relevance, increased antithrombin and reduced D-dimer concentrations in certain study subpopulations). As observed in the secondary, observational analyses,

substantial increments in MedDiet adherence were associated with decreases in fibrinogen and NEFA levels. As far as we know, this is the largest, most comprehensive, hypothesis-based analysis to date of the effects of a healthy dietary pattern on atherothrombosis indicators in high cardiovascular risk individuals. Our results support the improvement of thrombosis risk status after following a MedDiet. Further studies are needed to confirm whether these improvements mediate the reported cardioprotective benefits of such lifestyle modifications.

AUTHOR CONTRIBUTIONS

A.H. and R.C. acquired data. A.H. analyzed and interpreted data. X.P., M.Fitó, M.A.M.-G., D.C., J.S.-S., J.L., E.G.-G., F.A., M.Fiol, L.S.-M., X.P., E.R., and R.E. conceived the clinical trial concept and design. A.H., O.C., A.T.-R., X.P., and R.E. obtained funding. A.H. drafted the manuscript. O.C., A.T.-R., X.P., M.Fitó, R.C., M.A.M.-G., D.C., J.S.-S., J.L., E.G.-G., F.A., M.Fiol, L.S.-M., X.P., E.R., and R.E. revised the content of the manuscript and approved its final version. A.H. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the results.

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CONFLICT OF INTEREST

X.P. reports being a board member, lecture fees, and grants from Ferrer International; being a board member and grants from the Residual Risk Reduction Initiative Foundation; personal fees from Abbott Laboratories; lecture fees and grants from Merck and Roche; lecture fees from Danone, Esteve, Menarini, Mylan, LACER, and Rubio Laboratories; and grants from Sanofi, Kowa, Unilever, Boehringer Ingelheim, and Karo

Bio. J.S.-S. reports being a board member and personal fees from Instituto Danone Spain; being a board member and grants from the International Nut and Dried Fruit Foundation; personal fees from Aguas Font Vella Lanjarón, and Danone S.A; and grants from Eroski Distributors. F.A. reports personal fees from Menarini and AstraZeneca. L.S.-M. reports being a board member of the Mediterranean Diet Foundation and the Beer and Health Foundation. E.R. reports personal fees, grants, and nonfinancial support from the California Walnut Commission and Alexion; personal fees and nonfinancial support from Danone; and nonfinancial support from the International Nut Council. R.E. reports being a board member of the Research Foundation on Wine and Nutrition, the Beer and Health Foundation, and the European Foundation for Alcohol Research; personal fees from KAO Corporation; lecture fees from Instituto Cerventes, Fundacion Dieta Mediterranea, Cerveceros de España, Lilly Laboratories, AstraZeneca, and Sanofi; and grants from Novartis, Amgen, Bicentury, and Grand Fountaine. The rest of the authors have nothing to disclose.

DATA SHARING STATEMENT

The dataset analyzed during the current study is not publicly available due to national data regulations and for ethical reasons, including that we do not have the explicit written consent of the study volunteers to make their deidentified data available at the end of the study. However, data described in the manuscript, codebook, and analytic code will be made available upon request by sending a letter to the PREDIMED steering Committee (predimed-steering-committe@googlegroups.com). The request will be passed to all the members of the Committee for deliberation.

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FIGURE LEGENDS

Figure 1. Differences in 1-year changes in atherothrombosis biomarkers between Mediterranean diet interventions and the control diet group. Results are shown as adjusted coefficients (percentage changes relative to baseline values) with 95% confidence intervals.

HDL: high-density lipoprotein; *MedDiet-Nuts*: Mediterranean diet enriched with mixed nuts; *MedDiet-VOO*: Mediterranean diet enriched with virgin olive oil; *PAF-AH*: platelet activating factor acetylhydrolase; *PAI-1*: plasminogen activator inhibitor 1.

Figure 2. Associations between substantial increments in MedDiet adherence after 1 year of intervention (≥3 score points, relative to adherence decreases) with 1-year changes in atherothrombosis biomarkers. Results are shown as adjusted coefficients (percentage changes relative to baseline values) with 95% confidence intervals.

HDL: high-density lipoprotein; *PAF-AH*: platelet activating factor acetylhydrolase; *PAI-1*: plasminogen activator inhibitor 1.

TABLES

Table 1. Study population

	All (*n*=358)	MedDiet-VOO (*n*=120)	MedDiet-Nuts (*n*=119)	Low-fat diet (*n*=119)	*P*-value
Age (years), mean ± SD	66.8 ± 5.8	67.3 ± 5.3	66.6 ± 5.8	66.5 ± 6.4	0.530
Sex (female), *n* (%)	227 (63.4)	78 (65.0)	75 (63.0)	74 (62.2)	0.898
Type-2 diabetes mellitus, *n* (%)	183 (51.1)	67 (55.8)	55 (46.2)	61 (51.3)	0.331
Hypercholesterolemia, *n* (%)	264 (73.7)	88 (73.3)	86 (72.3)	90 (75.6)	0.834
Hypertension, *n* (%)	278 (77.7)	95 (79.2)	92 (77.3)	91 (76.5)	0.877
Users of antithrombotic drugs, *n* (%)	46 (12.8)	19 (15.8)	13 (10.9)	14 (11.8)	0.479
Tobacco use:					0.445
Never smoker, *n* (%)	229 (64.0)	83 (69.2)	77 (64.7)	69 (58.0)	
Actual smoker, *n* (%)	51 (14.2)	16 (13.3)	15 (12.6)	20 (16.8)	
Former smoker, *n* (%)	78 (21.8)	21 (17.5)	27 (22.7)	30 (25.2)	
Body mass index:					0.717
<25 kg/m^2, *n* (%)	19 (5.31)	8 (6.67)	4 (3.36)	7 (5.88)	
25.0-29.9 kg/m^2, *n* (%)	180 (50.3)	56 (46.7)	62 (52.1)	62 (52.1)	
≥30.0 kg/m^2, *n* (%)	159 (44.4)	56 (46.7)	53 (44.5)	50 (42.0)	
Mediterranean diet adherence score, mean ± SD	8.76 ± 1.73	8.61 ± 1.68	8.69 ± 1.85	8.99 ± 1.64	0.197
Leisure-time physical activity (metabolic equivalents of task·min/w), median (1st-3rd quartile)	1,376 (555-2,521)	1,505 (724-2,566)	1,253 (538-2,345)	1,410 (416-2,639)	0.447

Alcohol intake (g/week), median (1^{st}-3^{rd} quartile)	15.1 (0.00-80.7)	12.1 (0.00-53.6)	30.7 (0.00-94.3)	10.2 (0.00-79.7)	0.175

MedDiet-Nuts: Mediterranean diet enriched with mixed nuts; *MedDiet-VOO*: Mediterranean diet enriched with virgin olive oil.

SUPPLEMENTAL DATA

SUPPLEMENTAL METHODS

Inclusion and exclusion criteria

Eligible volunteers were 55-80 year old men and 60-80 year old women free of cardiovascular disease antecedents, but diagnosed with type-II diabetes mellitus or presenting three or more of the following six risk factors: 1) tobacco use >1 cigarette/day in the previous month; 2) high blood pressure (any of: systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or antihypertensive drug use); 3) LDL cholesterol ≥160 mg/dL; 4) HDL cholesterol ≤40 mg/dL; 5) overweight/obesity (body mass index ≥25 kg/m^2); 6) family history of premature coronary heart disease (defined as fatal myocardial infarction in father/1st degree male relative under 55 years or in mother/1st degree female relatives under 65 years).

Major exclusion criteria were: 1) documented history of previous cardiovascular disease (coronary heart disease, stroke, and clinical peripheral artery disease); 2) morbid obesity (body mass index >40 kg/m^2); 3) reported difficulties to change dietary habits (religious reasons, chewing/swallowing disorders, a low predicted likelihood to change dietary habits); 4) medical conditions that may compromise the ability of the person to participate in a nutrition intervention study (generalized dietary or fat intolerances; food allergy to olive oil or nuts; cancer; immunodeficiency; major neurological, psychiatric or endocrine diseases; and medical conditions with <1 year of survival); 5) patients with lack of autonomy (institutionalized patients due to chronic care; subjects unable to walk, lacking a stable address, or unable to attend to study visits); 6) illegal drug use or alcoholism; and 7) participation in any drug trial or use of any investigational drug within the last year.

Dietary interventions

Volunteers received personalized dietary advice taking into consideration their previous dietary habits and the diet group to which they had been randomly allocated. A team of trained dietitians provided participants allocated to the traditional Mediterranean diet (TMD) intervention several strategies to increase their adherence to a Mediterranean dietary pattern: 1) to increase their consumption of plant-based products such as vegetables, fruits, legumes, and raw and unsalted nuts; 2) to substitute red/processed meat for lean poultry; 3) to avoid the consumption of butter/margarine, processed meat, carbonated/sugared beverages, pastries, industrial baked goods, industrial desserts, crisps and snacks; 4) to use olive oil for cooking and dressing at all meals; 5) to increase consumption of fish and seafood; 6) to accompany vegetables, pasta, rice and other dishes with the traditional "*sofrito*" (a homemade mix of stir-fried tomato, garlic, onion, and aromatic herbs); and 7) for usual drinkers, to consume wine as essential alcoholic beverage (150 mL/day in men, 100 mL/day in women). Dietitians administered a 14-item questionnaire to assess adherence to a TMD at every visit and used this information to tailor individual dietary advice. Participants allocated to the TMD enriched with virgin olive oil were provided with 1L/week of virgin olive oil and those allocated to the TMD enriched

with nuts group were given 210 g/week of mixed nuts to increase compliance and account for family needs.

Participants allocated to the low-fat control group were as well advised by trained dietitians: 1) to promote the consumption of vegetables, fruits, and legumes; 2) to decrease their intake of non-processed and processed meats, high-fat dairy products, butter/margarine, carbonated/sugared beverages, pastries, industrial baked goods, industrial desserts, and fries/snacks, and 3) to moderate their use of olive oil for cooking and dressing, and their consumption of nuts and fatty fish. Nutritionists administered a 9-item questionnaire assessing the adherence to the low-fat diet and consequently tailored their nutritional counselling. Participants in the control group received small non-food gifts in order to promote adherence to the study.

No specific recommendation was made regarding physical activity in any of the trial groups. All participants were invited to contact their referent dietitian in case of any doubt related to the intervention, and to participate in group sessions in which they received: 1) a detailed description of recommended foods to incorporate into their diets; 2) seasonal shopping lists of advisable products; 3) meal plans and menus adapted to each dietary group; and 4) sets of adapted recipes involving the recommended food items.

Quality control of laboratory data

All laboratory determinations followed a predefined process to control inter-assay variability. First, we analyzed samples from the same volunteer in the same analytical run. Second, the order in which samples were analyzed was randomly assigned before starting the determinations: a random sample of the TMD-VOO group was the first to be assessed, followed by one from the TMD-Nuts group and another one from the low-fat diet group, also randomly selected; this process was repeated 119 times, obtaining a random sequence that minimized any batch effect among analytical runs. Finally, we included a sample pool (isolated from 20 healthy volunteers) in each experiment, and used the values obtained in this determination to calculate an inter-assay coefficient of variation. These sample pool values were also used to correct the batch effect present in those determinations with an excessive inter-assay coefficient of variation (≥20%). In this particular situation (only applicable to the determination of the platelet activating factor-acetylhydrolase activity of isolated HDLs), we provided normalized ratios without units as results.

Inter-assay coefficients of variation and the number of missing values for every determination are available in **Supplemental Table 2.**

SUPPLEMENTAL TABLES

Supplemental Table 1. CONSORT checklist

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomized trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	6, Suppl. Methods
	4b	Settings and locations where the data were collected	6-7, Refs. #1 & #20
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6-7, Suppl. Methods, Refs. #1 & #20
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8-9, Suppl. Methods
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	9, Suppl. Table 3
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomization:			
Sequence generation	8a	Method used to generate the random allocation sequence	Refs. #1 & #20
	8b	Type of randomization; details of any restriction (such as blocking and block size)	Refs. #1 & #20

Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	Refs. #1 & #20
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	Refs. #1 & #20
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	Refs. #1 & #20
	11b	If relevant, description of the similarity of interventions	6-7, Suppl. Methods, Refs. #1 & #20
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9-10
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	9-10
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analyzed for the primary outcome	Table 1
	13b	For each group, losses and exclusions after randomization, together with reasons	-
Recruitment	14a	Dates defining the periods of recruitment and follow-up	Refs. #1 & #20
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analyzed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Suppl. Tables 3
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	11-13, Figures 1 & 2, Suppl. Tables 6 & 7
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	11-13, Suppl. Tables 8 & 9
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	Refs. #1 & #20

Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	14
Generalizability	21	Generalizability (external validity, applicability) of the trial findings	14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13-14
Other information			
Registration	23	Registration number and name of trial registry	6
Protocol	24	Where the full trial protocol can be accessed, if available	6, Refs. #1 & #20
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

Supplemental Table 2. Inter-assay coefficients of variation and number of missing values of atherothrombosis biomarkers

	Coefficient of variation	Missing values
Platelet factor-4	12.8%	4.2% (*n*=15)
Fibrinogen	11.3%	0% (*n*=0)
Prothrombin fragment 1+2	12.1%	3.1% (*n*=11)
Antithrombin	8.68%	0% (*n*=0)
Plasminogen activator inhibitor-1	12.2%	3.1% (*n*=11)
D-dimer	5.44%	2.0% (*n*=7)
Non-esterified fatty acids	2.86%	0.8% (*n*=3)
Platelet activating factor acetylhydrolase activity in HDL	22.7%	0.6% (*n*=2)
Alpha-1 antitrypsin in HDL	4.97% (α_1-antitrypsin), 2.98% (cholesterol)	5.0% (*n*=18)

Supplemental Table 3. Inter- and intra-group differences detectable with ≥80% power and a type-I error of 0.05 according to post-analysis sample sizes and standard deviations

	SD of post- vs. pre-intervention differences	Participants with available data			Capacity to detect differences (α=0.05, β=0.20)	
		Control arm	MedDiet VOO	MedDiet Nuts	Inter-group difference	Intra-group difference
Platelet factor-4 (ng/mL)	387	117	112	114	≥145	≥102
Fibrinogen (mg/dL)	63.4	119	120	119	≥23.0	≥16.3
Prothrombin fragment 1+2 (pg/mL)	397	115	116	116	≥147	≥104
Antithrombin (mg/dL)	3.94	119	120	119	≥1.44	≥1.02
PAI-1 (mg/dL)	2.06	117	115	115	≥0.76	≥0.54
D-dimer (μg/mL)	0.84	117	118	116	≥0.31	≥0.22
Non-esterified fatty acids (mmol/L)	0.21	119	120	117	≥0.076	≥0.054
PAF-AH activity in HDL (ratio units)	0.38	118	120	117	≥0.14	≥0.10
α_1-antitrypsin in HDL (mg/mg of HDL-C)	0.47	114	113	113	≥0.17	≥0.12

MedDiet-Nuts: Mediterranean diet enriched with mixed nuts; *MedDiet-VOO*: Mediterranean diet enriched with virgin olive oil.

Supplemental Table 4. Comparison of our subsample and the rest of the PREDIMED Study population

	Study subsample (*n*=358)	Remaining PREDIMED population (*n*=7,089)	*P*-value
Age (years), mean ± SD	66.8 ± 5.8	67.0 ± 6.2	0.454
Sex (female), *n* (%)	227 (63.4)	4,055 (57.2)	0.024
Type-2 diabetes mellitus, *n* (%)	158 (44.1)	2,941 (41.5)	0.349
Hypercholesterolemia, *n* (%)	293 (81.8)	5,286 (74.6)	0.002
Hypertension, *n* (%)	328 (91.6)	6,354 (89.6)	0.263
Users of antithrombotic drugs, *n* (%)	46 (12.8)	1,430 (20.2)	0.001
Tobacco use:			0.423
Never smoker, *n* (%)	229 (64.0)	4,334 (61.1)	
Actual smoker, *n* (%)	51 (14.2)	996 (14.0)	
Former smoker, *n* (%)	78 (21.8)	1,759 (24.8)	
Body mass index:			0.090
<25.0 kg/m^2, *n* (%)	19 (5.31)	537 (7.58)	
25.0-29.9 kg/m^2, *n* (%)	180 (50.3)	3,207 (45.2)	
≥30.0 kg/m^2, *n* (%)	159 (44.4)	3,345 (47.2)	
Mediterranean diet adherence score, mean ± SD	8.76 ± 1.73	8.67 ± 1.91	0.325
PREDIMED Study groups:			0.967
MedDiet-VOO, *n* (%)	120 (33.5)	2,423 (34.2)	
MedDiet-Nuts, *n* (%)	119 (33.2)	2,335 (32.9)	
Low-fat control group, *n* (%)	119 (33.2)	2,331 (32.9)	

MedDiet-Nuts: Mediterranean diet enriched with mixed nuts; *MedDiet-VOO*: Mediterranean diet enriched with virgin olive oil.

Supplemental Table 5. Differences in 1-year changes in dietary and lifestyle variables between Mediterranean diet interventions and the control diet group.

	MedDiet-VOO vs. control diet (adjusted difference, [95% CI])	MedDiet-Nuts vs. control diet (adjusted difference, [95% CI])
Mediterranean diet adherence score	1.14 [0.79; 1.49]	1.35 [1.00; 1.69]
Virgin olive oil (g/week)	248 [217; 279]	6.87 [-24.3; 38.0]
Mixed nuts (g/week)	14.0 [-8.69; 36.6]	246 [224; 269]
Fruits and vegetables (g/week)	569 [124; 1,010]	361 [-84.1; 806]
Legumes (g/week)	31.3 [13.9; 48.8]	14.7 [-2.82; 32.2]
Whole grains (g/week)	0.86 [-90.2; 91.9]	16.7 [-74.9; 108]
Refined grains (g/week)	-16.8 [-173; 140]	113 [-43.8; 271]
Total fish (g/week)	80.9 [29.7; 132]	6.12 [-45.2; 57.4]
Fresh fatty fish (g/week)	53.8 [19.7; 87.9]	42.6 [8.30; 76.9]
Total meat (g/week)	37.4 [-36.5; 111]	-55.2 [-129; 19.0]
White meat (g/week)	42.6 [-8.79; 94.0]	31.7 [-20.0; 83.3]
Red + processed meat (g/week)	-3.50 [-58.6; 51.6]	-84.4 [-140; -29.0]
Fermented dairy products (g/week)	56.0 [-69.4; 181]	11.6 [-114; 137]
Alcohol (g/week)	4.71 [-9.69; 19.1]	3.53 [-11.0; 18.0]
Wine (mL/week)	64.3 [-60.2; 189]	65.8 [-59.4; 191]
Beer (mL/week)	22.5 [-101; 146]	-73.6 [-198; 50.7]
Non-marine ω-3 PUFA intake (g/day)	0.20 [0.063; 0.34]	1.14 [1.00; 1.28]
Marine ω-3 PUFA intake (g/day)	0.19 [0.089; 0.29]	0.10 [$8 \cdot 10^{-4}$; 0.20]
Vitamin C intake (mg/day)	32.0 [12.2; 51.9]	12.2 [-7.72; 32.0]
Vitamin E intake (mg/day)	0.77 [0.048; 1.49]	2.71 [1.99; 3.44]
Phenolic compound intake (mg/day)	2.59 [-62.0; 67.2]	5.48 [-59.4; 70.3]
Leisure-time physical activity (metabolic equivalents of task·min/week)	69.6 [-256; 395]	102 [-225; 429]

MedDiet-Nuts: Mediterranean diet enriched with mixed nuts; *MedDiet-VOO*: Mediterranean diet enriched with virgin olive oil.

Supplemental Table 6. Differences in 1-year changes in atherothrombosis biomarkers between Mediterranean diet interventions and the control diet group

	MedDiet-VOO vs. control diet (adjusted difference [95% CI])	MedDiet-Nuts vs. control diet (adjusted difference [95% CI])
Platelet factor-4 (1-year change, %)	-4.03 [-20.8; 12.7]	-5.13 [-21.5; 11.2]
Fibrinogen (1-year change, %)	-1.84 [-6.95; 3.27]	-1.77 [-6.98; 3.43]
Prothrombin fragment 1+2 (1-year change, %)	-6.66 [-25.6; 12.3]	-0.15 [-19.6; 19.3]
Antithrombin (1-year change, %)	1.49 [-0.95; 3.92]	-0.54 [-2.98; 1.90]
PAI-1 (1-year change, %)	-1.98 [-13.2; 9.24]	1.46 [-9.74; 12.7]
D-dimer (1-year change, %)	-2.77 [-18.5; 12.9]	10.6 [-3.80; 25.0]
Non-esterified fatty acid (1-year change, %)	-1.62 [-10.5; 7.32]	-9.34 [-18.1; -0.53]
PAF-AH activity in HDLs (1-year change, %)	7.48 [0.17; 14.8]	3.39 [-3.64; 10.4]
α_1-antitrypsin in HDLs (1-year change, %)	-6.05 [-11.8; -0.29]	-4.70 [-10.2; 0.81]

HDL: high-density lipoprotein; *MedDiet-Nuts*: Mediterranean diet enriched with nuts; *MedDiet-VOO*: traditional Mediterranean diet enriched with virgin olive oil; *PAF-AH*: platelet activating factor acetylhydrolase; *PAI-1*: plasminogen activator inhibitor 1.

Supplemental Table 7. Intra-group differences in atherothrombosis biomarkers

	Low-fat control diet (*N*=119)			MedDiet-VOO (*N*=120)			MedDiet-Nuts (*N*=119)		
Normally distributed variables (*paired t-tests*)									
	Post-int. value	Difference	*P*-value	Post-int. value	Difference	*P*-value	Post-int. value	Difference	*P*-value
Fibrinogen (mg/dL)	275 (65.1)	7.40 (64.2)	0.211	277 (63.5)	-0.094 (68.0)	0.988	272 (62.1)	1.92 (57.8)	0.718
Antithrombin (mg/dL)	36.1 (4.44)	0.38 (4.14)	0.321	37.0 (4.37)	0.33 (3.19)	0.254	36.1 (5.29)	-0.15 (4.40)	0.718
PAI-1 (mg/dL)	3.68 (1.56)	0.080 (2.12)	0.683	3.60 (1.66)	0.009 (1.97)	0.962	3.68 (1.73)	0.098 (2.10)	0.619
D-dimer (µg/mL)	1.12 (0.54)	0.057 (0.67)	0.360	1.13 (0.59)	0.063 (0.77)	0.378	1.25 (0.70)	0.089 (0.83)	0.251
α_1-antitrypsin in HDLs (per mg of HDL cholesterol)	2.09 (0.65)	0.073 (0.51)	0.130	1.98 (0.68)	0.028 (0.60)	0.615	2.00 (0.61)	-0.009 (0.43)	0.825
PAF-AH activity in HDLs (normalized units)	1.00 (0.35)	-0.076 (0.33)	0.016	1.06 (0.38)	-0.036 (0.38)	0.303	1.05 (0.36)	-0.10 (0.42)	0.010
Non-esterified fatty acids (mmol/L)	0.53 (0.20)	-0.007 (0.24)	0.736	0.51 (0.19)	0.012 (0.19)	0.495	0.47 (0.18)	-0.036 (0.19)	0.038
Non-normally distributed variables (*Wilcoxon signed rank tests*)									
	Pre-int. value	Post-int. value	*P*-value	Pre-int. value	Post-int. value	*P*-value	Pre-int. value	Post-int. value	*P*-value
Platelet factor-4 (ng/mL)	268 [183; 431]	351 [211; 565]	0.012	287 [164; 498]	372 [202; 563]	0.168	288 [186; 486]	323 [205; 514]	0.901
Prothrombin fragment 1+2 (pg/mL)	261 [149; 433]	319 [209; 538]	0.005	246 [162; 415]	312 [223; 487]	0.114	275 [165; 439]	289 [194; 443]	0.573

HDL: high-density lipoprotein; *MedDiet-Nuts*: Mediterranean diet enriched with mixed nuts; *MedDiet-VOO*: Mediterranean diet enriched with virgin olive oil; *PAF-AH*: platelet activating factor acetylhydrolase; *PAI-1*: plasminogen activator inhibitor 1; *Pre-int.*: pre-intervention; *Post-int.*: post-intervention.

Supplemental Table 8. Differences in 1-year changes in atherothrombosis biomarkers between participants with substantial increments in Mediterranean diet adherence and those with decreases in compliance

	Substantial increments in MedDiet adherence vs. decreases in compliance (adjusted difference, [95% CI])
Platelet factor-4 (1-year change, %)	-9.80 [-48.9; 29.3]
Fibrinogen (1-year change, %)	-9.47 [-18.3; -0.60]
Prothrombin fragment 1+2 (1-year change, %)	-3.77 [-50.1; 42.5]
Antithrombin (1-year change, %)	-1.97 [-6.04; 2.11]
PAI-1 (1-year change, %)	3.82 [-18.1; 25.8]
D-dimer (1-year change, %)	19.0 [-8.06; 46.0]
Non-esterified fatty acid (1-year change, %)	-16.7 [-31.7; -1.74]
PAF-AH activity in HDLs (1-year change, %)	2.86 [-9.34; 15.1]
α_1-antitrypsin in HDLs (1-year change, %)	-3.38 [-14.2; 7.41]

HDL: high-density lipoprotein; *MedDiet*: Mediterranean diet; *PAF-AH*: platelet activating factor acetylhydrolase; *PAI-1*: plasminogen activator inhibitor 1.

Supplemental Table 9. Associations between 1-year changes in the consumption of key food groups (virgin olive oil, nuts, vegetables, fruits, legumes, fresh fatty fish, processed meat, and alcohol) and 1-year differences in atherothrombosis biomarkers

	+10 g/day of virgin olive oil (adjusted diff., [95% CI])	+30 g/day of mixed nuts (adjusted diff., [95% CI])	+100 g/day of vegetables (adjusted diff., [95% CI])	+100 g/day of fruits (adjusted diff., [95% CI])	+25 g/day of legumes (adjusted diff., [95% CI])	+25 g/day of fresh fatty fish (adjusted diff., [95% CI])	-25 g/day of processed meat (adjusted diff., [95% CI])	+10 g/day of alcohol (adjusted diff., [95% CI])
Platelet factor-4 (1-year change, %)	-0.22 [-6.87; 6.42]	18.9 [-9.29; 47.1]	-9.25 [-17.6; -0.90]	-6.38 [-13.5; 0.74]	-6.02 [-38.6; 26.6]	-10.9 [-27.7; 5.94]	-15.9 [-36.6; 4.86]	7.64 [-18.6; 33.9]
Fibrinogen (1-year change, %)	0.43 [-0.62; 1.48]	2.54 [-1.89; 6.97]	-0.52 [-1.88; 0.83]	-0.30 [-1.42; 0.83]	-4.29 [-9.50; 0.92]	0.80 [-1.85; 3.45]	1.59 [-1.85; 5.03]	0.50 [-3.48; 4.48]
Prothrombin fragment 1+2 (1-year change, %)	1.42 [-3.75; 6.58]	7.28 [-14.2; 28.8]	0.17 [-6.75; 7.09]	-7.57 [-13.1; -2.09]	0.25 [-25.1; 25.6]	-13.1 [-26.0; -0.20]	-9.29 [-25.8; 7.19]	0.39 [-20.2; 21.0]
Antithrombin (1-year change, %)	0.27 [-0.24; 0.78]	2.21 [0.073; 4.34]	-0.015 [-0.67; 0.64]	-0.23 [-0.77; 0.31]	-0.52 [-3.05; 2.01]	-0.51 [-1.80; 0.77]	2.00 [0.36; 3.64]	-0.67 [-2.60; 1.25]
PAI-1 (1-year change, %)	-1.80 [-4.24; 0.65]	4.53 [-5.71; 14.8]	-1.15 [-4.35; 2.04]	-2.11 [-4.75; 0.52]	1.05 [-10.9; 13.0]	-4.08 [-10.2; 2.06]	-10.4 [-18.2; -2.71]	3.07 [-6.73; 12.9]
D-dimer (1-year change, %)	0.047 [-3.97; 4.07]	-2.86 [-20.0; 14.3]	-1.91 [-7.02; 3.19]	-0.18 [-4.49; 4.13]	14.7 [-4.98; 34.3]	1.63 [-8.39; 11.7]	1.42 [-11.7; 14.6]	-5.39 [-20.3; 9.55]
Non-esterified fatty acid (1-year change, %)	0.55 [-1.23; 2.34]	-2.76 [-10.3; 4.81]	-1.02 [-3.31; 1.28]	0.063 [-1.87; 2.00]	6.42 [-2.43; 15.3]	2.33 [-2.15; 6.82]	-2.91 [-8.71; 2.90]	-1.98 [-8.72; 4.75]
PAF-AH activity in HDLs (1-year change, %)	-0.072 [-1.52; 1.37]	2.17 [-3.95; 8.29]	-0.42 [-2.28; 1.44]	-0.033 [-1.62; 1.56]	-4.70 [-11.9; 2.52]	-0.19 [-3.83; 3.45]	6.07 [1.38; 10.8]	-1.87 [-7.31; 3.57]
α_1-antitrypsin in HDLs (1-year change, %)	-0.049 [-1.16; 1.07]	0.24 [-4.42; 4.90]	-0.53 [-2.02; 0.97]	-0.88 [-2.06; 0.30]	-1.70 [-7.26; 3.85]	1.04 [-1.74; 3.82]	0.45 [-3.10; 3.99]	-2.97 [-7.23; 1.28]

HDL: high-density lipoprotein; *PAF-AH*: platelet activating factor acetylhydrolase; *PAI-1*: plasminogen activator inhibitor 1.

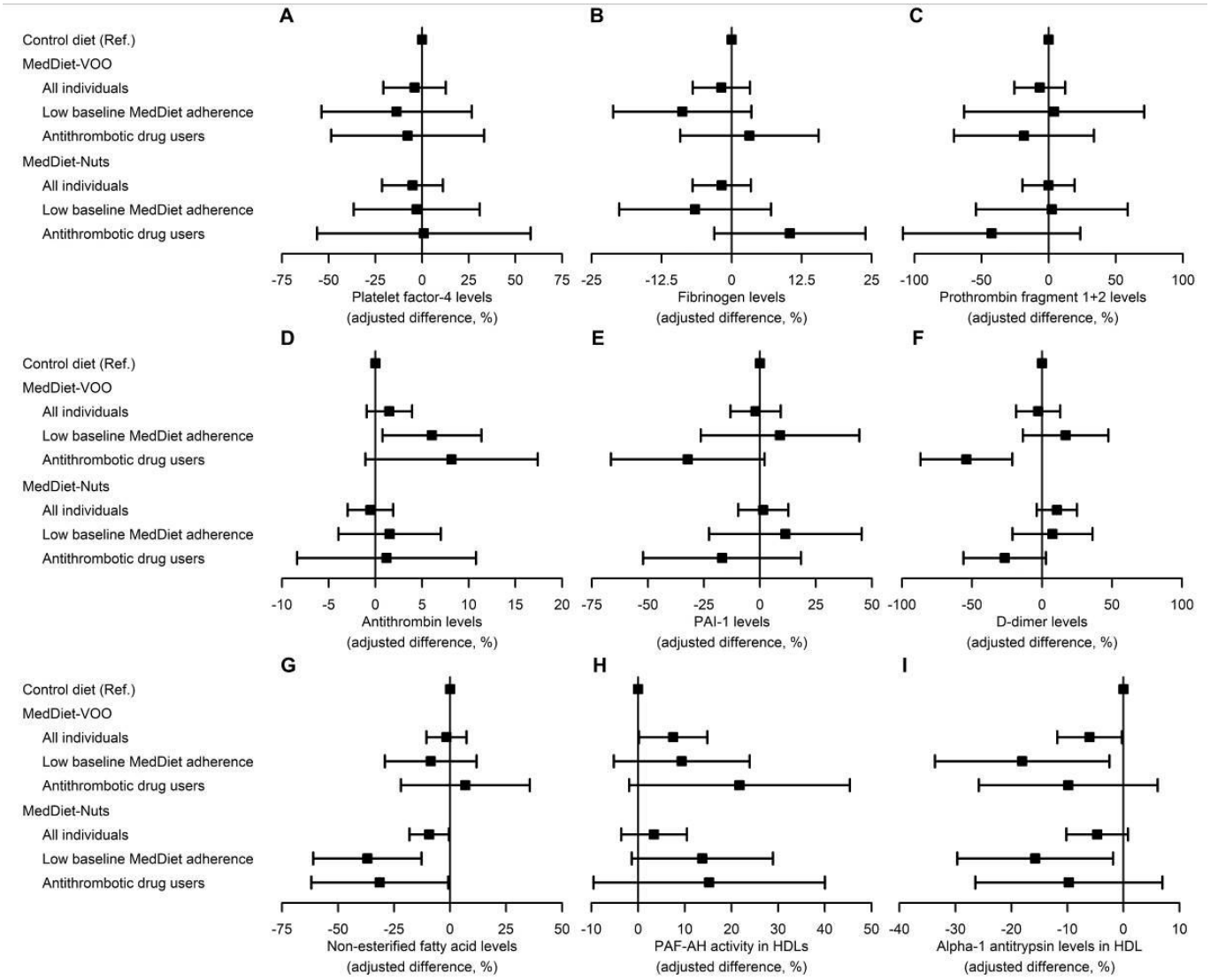
Supplemental Table 10. Power calculation for the analyses in participant subgroups

	SD of post- vs. pre-intervention differences	Mean post- vs. pre-intervention differences			Number of volunteers			Statistical power	
		MedDiet-VOO	MedDiet-Nuts	Control diet	MedDiet-VOO	MedDiet-Nuts	Control diet	MedDiet-VOO vs. control	MedDiet-Nuts vs. control
Participants with low adherence to a MedDiet at baseline									
Platelet factor-4	323	30.9	-28.3	95.9	30	32	24	12%	30%
Fibrinogen	65.5	-13.7	4.96	14.1	31	35	24	35%	8%
Prothrombin fragment 1+2	380	67.0	52.1	104	30	34	23	6%	8%
Antithrombin	3.75	0.95	-0.86	-1.40	31	35	24	64%	8%
PAI-1	2.26	0.55	0.66	0.58	30	35	24	3%	4%
D-dimer	0.80	0.25	0.080	0.033	31	34	24	17%	5%
Non-esterified fatty acids	0.25	0	-0.037	0.051	31	34	24	12%	27%
PAF-AH activity in HDL	0.37	-0.11	-0.097	-0.097	31	35	24	4%	3%
α_1-antitrypsin in HDL	0.51	0.019	-0.014	0.28	30	35	23	46%	58%
Antithrombotic drug users									
Platelet factor-4	292	-11.5	9.37	125	18	11	13	25%	4%
Fibrinogen	54.0	8.36	32.9	7.15	19	13	14	3%	22%
Prothrombin fragment 1+2	351	-71.8	-45.9	163	19	12	14	48%	33%
Antithrombin	4.16	1.22	-0.98	-2.47	19	13	14	72%	16%
PAI-1	1.97	-0.61	0.27	1.13	19	12	14	71%	20%
D-dimer	0.84	-0.23	-0.16	0.40	19	12	13	55%	39%
Non-esterified fatty acids	0.24	0.072	-0.082	-0.028	19	13	14	22%	9%
PAF-AH activity in HDL	0.37	0.011	-0.045	-0.05	19	13	13	7%	3%
α_1-antitrypsin in HDL	0.49	0.068	0.018	0.24	19	12	13	17%	21%

HDL: high-density lipoprotein; *MedDiet-Nuts*: Mediterranean diet enriched with mixed nuts; *MedDiet-VOO*: Mediterranean diet enriched with virgin olive oil; *PAF-AH*: platelet activating factor acetylhydrolase; *PAI-1*: plasminogen activator inhibitor 1.

SUPPLEMENTAL FIGURES

Supplemental Figure 1. Differences in 1-year changes in atherothrombosis biomarkers between Mediterranean diet interventions and the control diet group in study sub-populations



HDL: high-density lipoprotein; *MedDiet-Nuts*: Mediterranean diet enriched with nuts; *MedDiet-VOO*: traditional Mediterranean diet enriched with virgin olive oil; *PAF-AH*: platelet activating factor acetylhydrolase; *PAI-1*: plasminogen activator inhibitor 1.