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Mediterranean Diet, Energy Restriction, Physical Activity, and Atherogenicity of Very-Low Density Lipoproteins: Findings from Two Randomized Controlled Trials

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Scope: Some very-low density lipoprotein (VLDL) properties may render them more pro-atherogenic. We aimed to assess whether a Mediterranean diet (MedDiet) or an energy-reduced MedDiet with increased physical activity improves them.

Methods and results: In a sample of the PREvención con DIeta MEDiterránea (PREDIMED) study, a 1-year intervention with MedDiet with extra-virgin olive oil ($n = 89$) or nuts (MedDiet-Nuts; $n = 79$) is compared with a low-fat diet ($n = 90$). In the PREDIMED-Plus study, a 1-year intervention with energy-reduced MedDiet and physical activity ($n = 103$) is compared with an ad libitum MedDiet ($n = 101$). VLDL levels of apolipoprotein C-I, C-III, triglycerides, and cholesterol; the apolipoprotein E-/C-I ratio; and VLDL ex-vivo triglyceride transfer are measured. In PREDIMED participants in both MedDiet groups combined, VLDL apolipoprotein C-III levels are nominally reduced (−0.023 SD units, 95% CI −0.44 to −0.014, $p = 0.037$). VLDL triglyceride transfer is nominally increased in the MedDiet-Nuts group (+0.39 SD units, 95% CI 0.012–0.78, $p = 0.045$). In PREDIMED-Plus, no inter-group differences are detected.

Conclusions: In older adults at high cardiovascular risk, MedDiet is associated with lower VLDL atherogenicity versus a low-fat diet. No differences are seen after an energy-reduced MedDiet with physical activity.

1. Introduction

High triglyceride levels cause cardiovascular disease.[1] Triglycerides are mainly found in the bloodstream in very-low density lipoproteins (VLDL; synthesized in the liver) at fasting, and in intestinal-derived lipoproteins such as chylomicrons at postprandial state. Both particles transfer triglyceride to peripheral tissues via their interaction with lipoprotein lipase.[2] One of the mechanisms explaining the pathogenic role of triglycerides on cardiovascular disease is the pro-atherogenic potential of VLDLs. Some VLDLs can cross the endothelial barrier, trigger the development of atherosclerotic plaques, and contribute to the local activation of pro-inflammatory immune cells. In addition, VLDLs that remain too long in circulation are likely to be transformed into highly atherogenic, small, dense low-density lipoproteins (LDLs).[2] As observed in other lipoproteins such as high-density lipoproteins (HDL)[3,4] and LDLs,[5] some qualitative characteristics

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may increase VLDL atherogenicity or their time in circulation. For example, VLDLs may become richer in apolipoprotein C-I (ApoC-I) and C-III (ApoC-III), both of which are inhibitors of lipoprotein lipase and the VLDL binding to their hepatic receptors. This hinders the transfer of VLDL fatty acids to peripheral cells and their withdrawal from circulation, which may increase VLDL concentrations and half-life.[6,7] VLDLs might become poorer in apolipoprotein C-II (ApoC-II, an activator of the lipolytic action of lipoprotein lipase),[8] which contributes to greater VLDL concentrations, or in apolipoprotein E (ApoE, an essential mediator of VLDL return to hepatocytes), which may increase their time in circulation.[9] Lipid-rich VLDLs (with higher levels of triglycerides or cholesterol) are also present in dyslipidemias that are linked to greater cardiovascular risk such as familial hypertriglyceridemia.[10]

There is evidence that lifestyle modifications can improve quality-related traits of lipoproteins. Adhering to a Mediterranean diet (MedDiet) can improve several HDL functional properties[11] and decrease LDL atherogenicity.[12] An extended intervention with an energy-reduced MedDiet intervention plus physical activity further improved some HDL properties related to triglyceride metabolism.[13] Regarding VLDL, a short-term intervention with MedDiet decreased VLDL lipids in a small sub-sample of the PREDIMED (PREvención con DIeta MEDiterránea) study.[14] Thus, we hypothesize that MedDiet-related lifestyle interventions may improve further VLDL pro-atherogenic characteristics.

Our aims were to assess whether a 1-year intervention with a traditional MedDiet (relative to a low-fat control diet) improve the atherogenic properties of VLDL particles, and whether an intervention with an energy-reduced MedDiet with physical activity (relative to a traditional, ad libitum MedDiet) further enhanced these properties in individuals at high cardiovascular risk.

2. Experimental Section

2.1. Study Design and Population

The study populations were two samples of the PREDIMED (PREvención con DIeta MEDiterránea; clinical trial number ISRCTN35739639) and PREDIMED-Plus (clinical trial number ISRCTN89898870) studies.

In PREDIMED, participants were randomized to: a) a MedDiet enriched with extra-virgin olive oil (MedDiet-EVOO), b) a MedDiet enriched with nuts (MedDiet-Nuts), or c) a low-fat control diet. Eligible individuals were women aged 60–80 years and men aged 55–80 years who presented at enrollment: a) type 2 diabetes mellitus or b) three or more cardiovascular risk factors: current smoking, hypertension (blood pressure ≥140/90 mmHg or use of antihypertensive drugs), LDL cholesterol levels ≥160 mg dL^{-1}, low HDL cholesterol (≤40 mg dL^{-1}), independently of lipid-lowering therapy, body mass index (BMI) ≥25 kg m^{-2}, or a family history of premature coronary heart disease.[15,16] More details of the design and main outcomes were previously published.[15]

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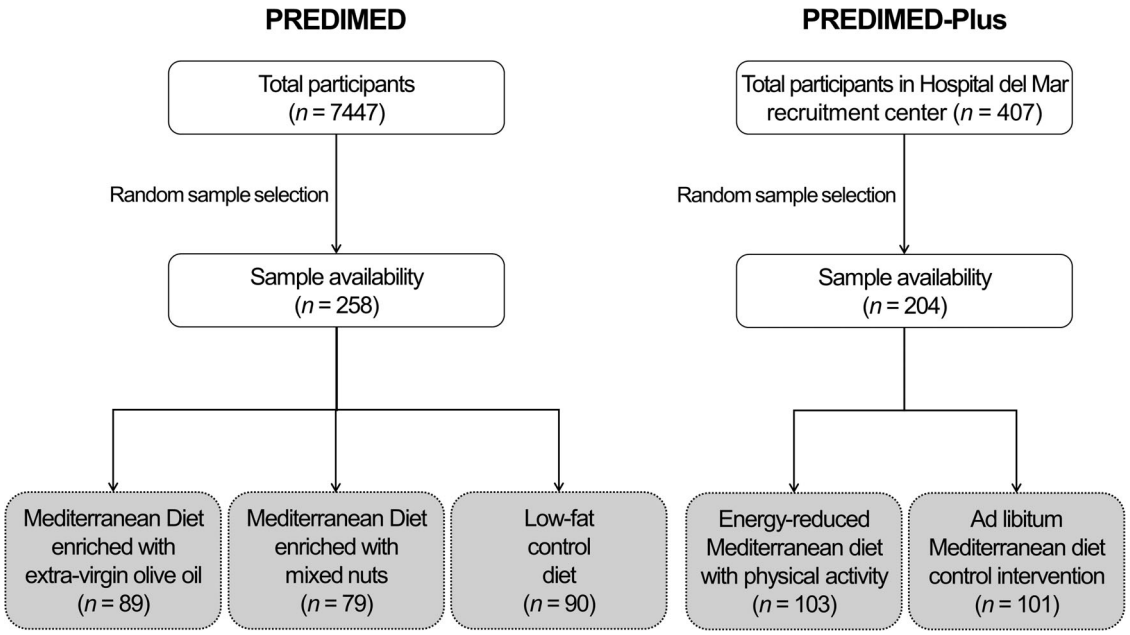

Figure 1. Flow charts.

In PREDIMED-Plus, participants were randomized to: a) an intensive intervention with an energy-restricted MedDiet combined with physical activity, or b) an ad libitum MedDiet and without physical activity recommendations. Eligible subjects were women aged 60–75 years and men aged 55–75 years with BMI between 27 and 40 kg m^{-2} and at least three criteria for metabolic syndrome: 1) triglycerides ≥150 mg dL^{-1} or triglyceride-lowering medication; 2) fasting glucose ≥100 mg dL^{-1} or glucose-lowering medication; 3) systolic/diastolic blood pressure ≥130/85 mmHg or antihypertensive medication; 4) low HDL cholesterol levels <50 mg dL^{-1} in women and <40 mg dL^{-1} in men or medication; and/or 5) waist circumference ≥88 cm in women and ≥102 cm in men.[13,17] Details of the study were available elsewhere.[17]

A traditional MedDiet was the dietary pattern underlying both interventions. It was rich in extra-virgin olive oil as main culinary fat, seasonal vegetables and fruits, and legumes; presented moderate intakes of fish and red wine; showed low consumption of red and processed meats, refined grains, whole-fat dairy, and ultra-processed foods (sweets, bakery goods, and sugary beverages); and was based on traditional cooking methods such as "sofrito."[17] In PREDIMED, the consumption of mixed nuts was particularly encouraged in the MedDiet intervention enriched with nuts, whereas in PREDIMED-Plus it was promoted in both arms. Further details of lifestyle interventions in PREDIMED and PREDIMED-Plus were available in the Supporting information. Due to the nature of interventions, blinding only affected data analysts.

For this study, random selections of participants with blood samples at baseline and 1-year post intervention were made. The study population was a subsample of 258 participants from the PREDIMED Study (3.5% of total participants, from seven recruiting centers) and 204 from the PREDIMED-Plus Study (2.9% of total participants, from the Hospital del Mar recruiting center). Flow charts were available in **Figure 1**.

2.2. Ethical Aspects

In both studies each recruiting center had the protocol approved by local institutional ethics committees. All participants gave written informed consent before enrolling in the studies. The two protocols were registered in the ISRCTN Registry (PREDIMED: ISRCTN35739639; PREDIMED-Plus: ISRCTN89898870).

2.3. Biological Samples and Plasma Determinations

Fasting ethylenediaminetetraacetic acid plasma were collected at baseline and post-intervention and stored at −80 °C until use. In these, glucose (Glucose HK CP, Horiba ABX), total cholesterol (Cholesterol CP, Horiba ABX), triglycerides (Triglycerides CP, Horiba ABX), and HDL cholesterol (HDL Direct CP, Horiba ABX) in an autoanalyzer ABX Pentra were measured. LDL cholesterol with the Friedewald equation when triglycerides were <300 mg dL^{-1} was calculated. VLDL particles from these samples by sequential density gradient ultracentrifugation as previously described were isolated.[11,12]

2.4. VLDL Atherogenicity Properties

In isolated VLDL samples, their content of triglycerides (Triglycerides CP, Horiba ABX), cholesterol (Spinreact), apolipoprotein B (ApoB, Spinreact), ApoC-II (Spinreact), ApoC-III (Spinreact), and ApoE (Spinreact) in an ABX Pentra autoanalyzer were measured. Triglyceride, cholesterol, ApoC-I, and ApoC-III concentrations in VLDL were expressed per μg of ApoB in the VLDL sample.[13] The study also calculated the ratio ApoE/ApoC-I (the main player vs the main inhibitor of VLDL hepatic clearance),[9] an indicator of the VLDL uptake by hepatocytes.

Ex vivo triglyceride transfer from VLDL in a human THP-1 monocyte-derived macrophage cell line was measured. After growing the cells as previously described,[13] macrophages were incubated with isolated VLDL samples of the participants for 48 h, and finally measured triglyceride levels in the supernatant (Triglycerides CP, Horiba ABX). Triglyceride transfer was calculated as the difference between the initial quantity of triglycerides in the VLDL sample and the concentration after the incubation (expressed as percent change). Samples in duplicate were ran and allowed no intra-repetition coefficient of variation >50%. The study divided this result by the amount of ApoC-II in the VLDL sample to assess this functional capacity per unit of the main apolipoprotein driving this phenomenon.[8]

Inter-assay variability was controlled by: 1) analyzing pre- and post-intervention samples of the same participant in the same experimental run; 2) randomizing the order in which the samples were analyzed (in the PREDIMED samples, the study analyzed one sample pair from the MedDiet-EVOO group followed by one from the MedDiet-Nuts group and one from the low-fat control group; in the PREDIMED-Plus study, the study alternated sample pairs from the intervention and control groups); and 3) including in each experiment a VLDL sample pool from 20 healthy volunteers to calculate inter-assay coefficients of variation. Laboratory analyzes for both studies were performed consecutively to use same the methodology and reagent batch.

2.5. Other Variables

Healthcare professionals collected at baseline data of age, sex, prevalence of diabetes, hypercholesterolemia, and hypertension, and BMI.[16,17] Obesity was defined as having BMI $\geq$30 kg m^{-2}, and atherogenic dyslipidemia as presenting triglycerides >150 mg dL^{-1} and low HDL cholesterol levels (<40 mg L^{-1} in men, <46 mg dL^{-1} in women).[18] Adherence to the PREDIMED Study intervention (traditional MedDiet) was assessed with a 14-item questionnaire[19] and compliance with the PREDIMED-Plus intervention (energy-reduced MedDiet) with a 17-item questionnaire.[20] Physical activity levels were estimated using the long (PREDIMED) and short version (PREDIMED-Plus) of the Minnesota Leisure-Time Physical Activity Questionnaire validated for Spanish adult population.[16,17] Intakes of energy and food group were estimated by semiquantitative validated food frequency questionnaires.[21]

2.6. Sample Size

As there was no previous randomized controlled trial with diet on VLDL atherogenic properties, the study calculated sample size considering the variability of the differences in plasma triglycerides considering a 2-sided type I error of 0.05, $\geq$80% power, and the standard deviation (SD) of the inter-group differences in PREDIMED[15] and PREDIMED-Plus.[13] In PREDIMED, a sample size of 80 participants per group allowed the detection of differences of 27 mg dL^{-1} among intervention arms. In PREDIMED-Plus, a sample size of 100 participants per group allowed to observe inter-arm differences of 23 mg dL^{-1} in triglycerides.

2.7. Statistical Analyses

The study described categorical variables by proportions, normally distributed continuous variables by means and SDs, and non-normally distributed continuous variables by medians (1st–3rd quartile). The study explored by multivariable linear regressions whether there were differences in the post-intervention values in the MedDiet-EVOO and MedDiet-Nuts intervention groups (individually and combined) relative to the low-fat control diet in PREDIMED participants, and in the intensive intervention group relative to the control group in the PREDIMED-Plus subjects. VLDL-related variables were transformed into *z*-scores prior to the analyses and findings were described in SD units. Models were adjusted for baseline levels of each parameter (continuous), age (continuous), sex, recruiting center (in PREDIMED), and triglycerides (continuous). Given that the study was unable to quantify some biomarkers because some determinations were below the limit of quantification of the technique (ApoC-III levels in VLDL, the ApoE/ApoC-I ratio), inter-group differences were assessed here using an adapted version of survival regression models as described by Helsel.[22] To correct the findings on VLDL characteristics for multiple comparisons (VLDL determinations were highly inter-correlated), the number of independent factors was first identified that correctly defined the set of VLDL-related variables according to Horn's parallel analysis[23] and subsequently used it for the Bonferroni correction. As complementary analysis, the differences in VLDL properties at baseline between participants with and without several cardiovascular risk factors (age <65 or $\geq$65 years, sex, type-2 diabetes, hypercholesterolemia, atherogenic dyslipidemia, hypertension, and obesity) were assessed. Analyses were performed using R Software version 4.0.5.[24]

3. Results

3.1. Study Population

PREDIMED participants were older adults (mean age 65.2 years old, 52.7% women), 89.5% of them were overweight or obese and presented a high prevalence of cardiovascular risk factors (type-2 diabetes mellitus 48.4%, hypercholesterolemia 77.9%, atherogenic dyslipidemia 35.0%, hypertension 82.2%, current smoking 14.3%). PREDIMED-Plus participants were also older adults (mean age 65.7 years old, 51.5% women), by study design all of them were overweight or obese and presented as well as a high prevalence of cardiovascular risk factors (type-2 diabetes mellitus 34.3%, hypercholesterolemia 69.6%, atherogenic dyslipidemia 45.3%, hypertension 85.3%, current smoking 8.33%). There were no apparent inter-group differences in baseline characteristics in both trials (**Table 1**). VLDL properties at baseline were exclusively impaired in participants with atherogenic dyslipidemia (Table S1, Supporting Information).

3.2. Adherence to Interventions

After 1-year of follow-up, participants in the intervention arms in the PREDIMED Study increased their adherence to a MedDiet relative to the control group (MedDiet-EVOO: +1.50 points,

Table 1. Baseline characteristics of the participants in PREDIMED and PREDIMED-Plus sub-samples.

	PREDIMED study				PREDIMED-Plus study		
	All participants (n = 259)	MedDiet-EVOO (n = 89)	MedDiet-Nuts (n = 79)	Low-fat diet (n = 90)	All participants (n = 204)	Intensive intervention (n = 101)	Ad libitum MedDiet (n = 103)
Age (years, mean ± SD)	65.2 ± 6.33	65.0 ± 5.72	65.2 ± 6.72	65.4 ± 6.61	65.7 ± 4.70	65.7 ± 4.84	65.7 ± 4.57
Women (n, %)	136 (52.7%)	43 (48.3%)	49 (62.0%)	44 (48.9%)	105 (51.5%)	52 (51.5%)	53 (51.5%)
Type-2 diabetes mellitus (n, %)	125 (48.4%)	43 (48.3%)	41 (51.9%)	41 (45.6%)	70 (34.3%)	34 (33.7%)	36 (35.0%)
Atherogenic dyslipidemia (n, %)	90 (35.0%)	34 (38.2%)	28 (35.9%)	28 (31.1%)	92 (45.3%)	50 (50.0%)	42 (40.8%)
Hypertension (n, %)	212 (82.2%)	74 (83.1%)	64 (81.0%)	74 (82.2%)	174 (85.3%)	83 (82.2%)	91 (88.3%)
Hypercholesterolemia (n, %)	201 (77.9%)	71 (79.8%)	61 (77.2%)	69 (76.7%)	142 (69.6%)	69 (68.3%)	73 (70.9%)
Triglycerides (mg dL^{-1}, median [1^{st}-3^{rd} quartile])	113 [84.4;159]	121 [94.0;163]	113 [78.0;164]	108 [80.9;146]	146 [106;188]	146 [111;183]	145 [106;189]
Body mass index categories:							
<25 kg m^{-2} (n, %)	27 (10.5%)	7 (7.87%)	13 (16.5%)	7 (7.78%)	-	-	-
25–30 kg m^{-2} (n, %)	109 (42.2%)	39 (43.8%)	33 (41.8%)	37 (41.1%)	43 (21.1%)	24 (23.8%)	19 (18.4%)
>30 kg m^{-2} (n, %)	122 (47.3%)	43 (48.3%)	33 (41.8%)	46 (51.1%)	161 (78.9%)	77 (76.2%)	84 (81.6%)
Tobacco use:							
Never smoker (n, %)	143 (55.4%)	50 (56.2%)	40 (50.6%)	53 (58.9%)	90 (44.1%)	46 (45.5%)	44 (42.7%)
Current smoker (n, %)	37 (14.3%)	15 (16.9%)	10 (12.7%)	12 (13.3%)	17 (8.33%)	8 (7.92%)	9 (8.74%)
Former smoker (n, %)	78 (30.2%)	24 (27.0%)	29 (36.7%)	25 (27.8%)	97 (47.5%)	47 (46.5%)	50 (48.5%)

MedDiet, Mediterranean diet; MedDiet-EVOO, Mediterranean diet enriched with extra-virgin olive oil; MedDiet-Nuts, Mediterranean diet enriched with mixed nuts. Atherogenic dyslipidemia is defined as presenting triglycerides >150 mg dL^{-1} and low HDL cholesterol levels (<40 mg L^{-1} in men, <46 mg dL^{-1} in women).

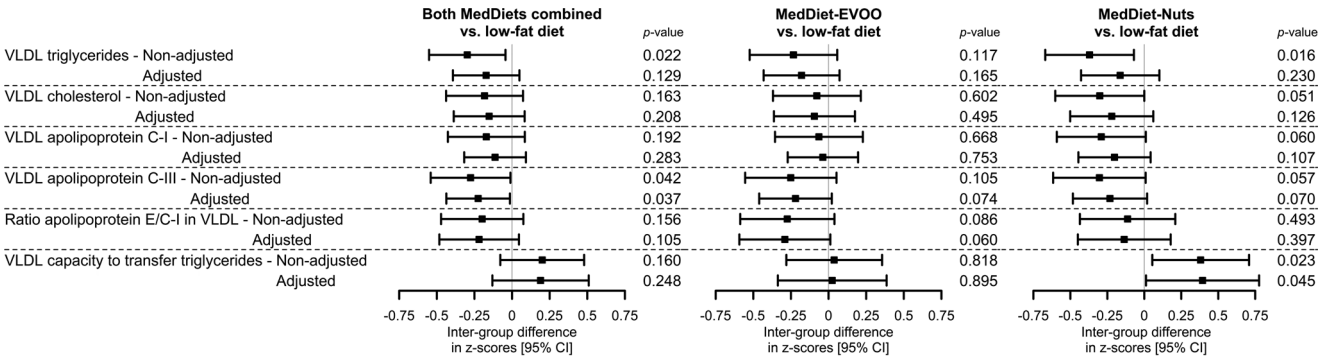

Figure 2. Inter-group differences in VLDL characteristics in the PREDIMED study. Intergroup comparisons in post-intervention values relative to control group were estimated by multivariable linear regression models adjusted for age, sex, recruiting center, baseline levels of the parameters, and triglycerides.

95% confidence interval [CI] 1.05–1.96, p-value < 0.001; MedDiet-Nuts: +1.92 points, 95% CI 1.44–2.39, p-value < 0.001) due to increases in the consumption of olive oil, legumes, and fish, and decreases in the intake of bakery goods (Table S2, Supporting Information). No inter-group differences for changes in physical activity were found (Table S2, Supporting Information).

In PREDIMED-Plus, participants in the intensive group had a higher adherence to the intervention after 1-year of follow-up when compared with the control arm (+1.22 points, 95% CI 0.60–1.84, p-value < 0.001) because of a higher consumption of whole grains, fish, and white meat (Table S3, Supporting Information). We observed higher physical activity in the intensive intervention arm relative to baseline (+700 metabolic equivalents of task-min/wk, 95% CI 254–1100, p-value = 0.003), but not versus the control group (Table S3, Supporting Information).

3.3. Effects of the PREDIMED Intervention

No inter-group differences in post-intervention clinical parameters were observed (Table S4, Supporting Information).

Regarding VLDL properties (**Figure 2**), according to the Horn's parallel analysis, two independent factors correctly defined our set of VLDL-related variables. Thus, following the Bonferroni correction, here we considered as significant results with p-values < 0.05/2 (<0.025). When assessing both MedDiet intervention groups together, we observed nominally lower levels of ApoC-III relative to the low-fat control group (−0.23 SD units, 95% CI −0.44 to −0.014, p-value = 0.037; similar results were seen in the MedDiet-EVOO and MedDiet-Nuts groups individually). There was a nominally significant increase in VLDL triglyceride transfer to cells only in the MedDiet-Nuts arm relative to the control

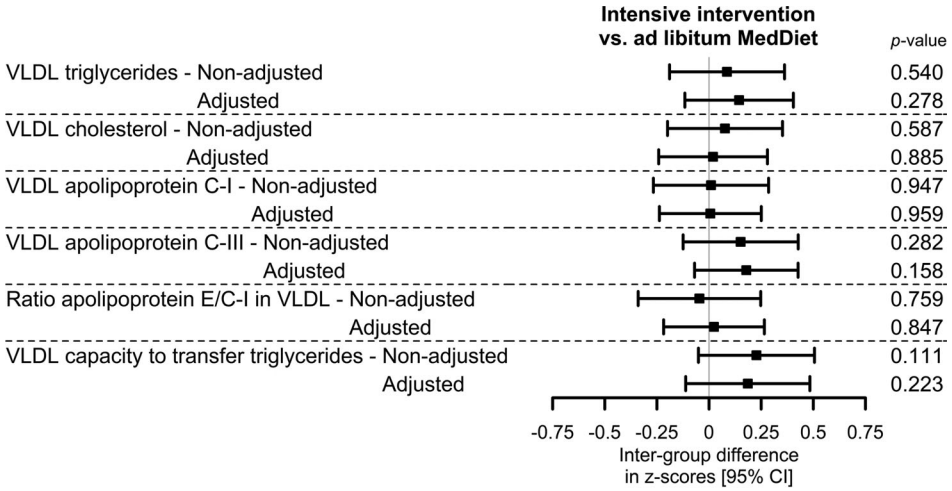

Figure 3. Inter-group differences in VLDL characteristics in the PREDIMED-Plus Study. Intergroup comparisons in post-intervention values relative to control group were estimated by multivariable linear regression models adjusted for age, sex, baseline levels of the parameters, and triglycerides.

diet (+0.39 SD units, 95% CI 0.012–0.78, *p*-value = 0.045). In the MedDiet-Nuts group in the non-adjusted analyses, we observed a decrease in the VLDL content of triglycerides (−0.37 SD units, 95% CI −0.67 to −0.070, *p*-value = 0.016; similar changes were seen for both MedDiets combined) and trends towards lower concentrations of cholesterol (−0.30 SD units, 95% CI −0.60–2 $\times 10^{-4}$, *p*-value = 0.051), and ApoC-I (−0.29 SD units, 95% CI −0.62–0.009, *p*-value = 0.060). However, these changes were attenuated after adjusting for confounders. No differences in the rest of VLDL properties or in the MedDiet-EVOO intervention group were seen. Pre- and post-intervention values and inter-group differences in physiological units are available in Table S5, Supporting Information.

3.4. Effects of the PREDIMED-Plus Intervention

In participants in the intensive intervention relative to the control arm, we observed lower BMI (−1.79 kg m^{-2}, 95% CI −2.16 to −1.42, *p*-value < 0.001) and waist circumference values (−4.55 cm, 95% CI −5.67 to −3.43, *p*-value < 0.001) (Table S6, Supporting Information). However, we detected no inter-group differences in VLDL-related properties (**Figure 3**, Table S7, Supporting Information).

4. Discussion

A 1-year intervention with a traditional MedDiet pattern, relative to a low-fat diet, tended to decrease VLDL atherogenicity in older adults at high cardiovascular risk. No differences were observed when reducing energy intake and promoting physical activity in this dietary pattern in a second randomized controlled trial in a similar population.

Compared to a control low-fat diet, following a MedDiet pattern tended to modify some VLDL-related properties (we observed a nominally significant decrease in ApoC-III levels, a nominally significant increment in VLDL transfer of triglycerides, and suggestive lower concentrations of VLDL lipids and ApoC-I). These properties were particularly impaired in individuals with atherogenic dyslipidemia (which could in turn be linked to a decrease in cardiovascular risk in this population). Although these differences should be confirmed in studies with larger sample size and other similar dietary interventions, three possible mechanisms may explain them. First, lipoproteins are known to become richer in bioactive food compounds after following MedDiet-related interventions. HDLs and LDLs became richer in antioxidants[25,26] and VLDLs incorporate the fatty acids contained in olive oil and nuts.[14] In HDLs and LDLs, dietary antioxidants are hypothesized to protect HDL active proteins against oxidation and keep them more functional.[11,12,27,28] Something similar may happen in VLDL active proteins such as ApoC-II, which may explain a greater capacity to bind to peripheral cells to transfer triglycerides and a general decrease in circulating levels of VLDL lipids. The known impact of nut-related polyunsaturated fatty acids on triglyceride metabolism[29] may also be partially due to local effects on VLDL. Second, the synthesis of ApoC-III is known to be increased in individuals with impaired glucose tolerance.[30] Following a MedDiet enhances glucose metabolism,[31] which could in turn decrease ApoC-III levels in circulation and bound to VLDL particles. Finally, low-fat diets have been reported to reduce VLDL clearance without increasing de novo VLDL synthesis.[32] This fact may help to explain an inter-group decrease in parameters reflecting VLDL concentrations such as triglycerides and ApoC-III.

Following an energy reduced MedDiet intervention with enhanced physical activity did not modify VLDL quality characteristics relative to an ad libitum MedDiet. Weight loss and physical activity decrease triglyceride concentrations[33] by promoting their uptake by muscle.[34] However, these factors also impact lipid metabolism since the increased lipolysis after energy-reduced diets with physical activity may transiently increase hepatic VLDL synthesis using the fatty acids from adipose tissue.[35] The collection of plasma samples in fasting state after such interventions (also linked to greater adipose lipolysis)[35] may add complexity to the assessment of differences in VLDL

biology. In any case, the PREDIMED-Plus intervention reduced circulating triglycerides[36] and we suggest that this reduction happened without affecting the quality of triglyceride-rich lipoproteins, even though it modified some triglyceride-related properties of other lipoproteins such as HDLs.[13] The smaller size of the PREDIMED-Plus sub-sample, the greater proportion of excess weight and atherogenic dyslipidemia in this population, and the differences in interventions and weight loss objectives may contribute to explaining the divergence in the findings from both studies. While in the PREDIMED subjects a slight improvement in VLDL profile was observed, perhaps a longer steady state in weight loss is required to appreciate an improvement in triglyceride-related markers in VLDL particles.

Our study has some strengths. This is the largest and most comprehensive study addressing the effect of lifestyle interventions on the quality characteristics of VLDL in individuals at high cardiovascular risk. Its randomized design provides high quality scientific evidence and minimizes the risk of potential confounding. However, the study also has some limitations. First, our sample size limited the statistical power in some determinations (e.g., we found no inter-group difference in energy intake and physical activity in the PREDIMED-Plus sub-sample when this difference has been reported in the whole cohort).[36] Therefore, further studies with larger number of participants and involving other kinds of equivalent dietary modifications are warranted. Second, we only found (and expected) moderate differences between intervention groups because the trials are based on modest and realistic lifestyle changes and control groups are active comparators, as observed in similar publications.[11,13] Third, our work followed a hypothesis-driven approach and investigated a comprehensive set of VLDL properties (surrogate outcomes in the PREDIMED and PREDIMED-Plus studies) that are correlated and non-independent. Thus, nominally significant results should be interpreted cautiously. Fourth, participants of both studies were elderly people with high cardiovascular risk, which complicates the extrapolation of our findings to other populations. Finally, samples were collected at fasting state and the increased lipolysis in the adipose tissue in this time frame[35] could add complexity to the interpretation of differences in VLDL qualitative properties. Further studies assessing VLDL biology in non-fasting states are advisable.

5. Concluding Remarks

In older adults at high cardiovascular risk, a traditional MedDiet (relative to a low-fat control diet) seems to be linked to a decrease in VLDL atherogenicity, while an energy reduced MedDiet with increased physical activity did not modify these characteristics. Our results suggest that healthy lifestyle modifications could have a positive impact on VLDL quality characteristics, especially in individuals with atherogenic dyslipidemia. However, our findings should be verified in further studies with similar lifestyle modifications and larger sample sizes.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

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. 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. 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 Cervantes, Fundación Dieta Mediterranea, Cerveceros de España, Lilly Laboratories, AstraZeneca, and Sanofi; and grants from Novartis, Amgen, Bicentury, and Grand Fountaine. X.P. reports being a board member, lecture fees, and grants from Ferrer International; to be 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. L.S.-M. reports being a board member of the Mediterranean Diet Foundation and the Beer and Health Foundation. All other authors report no conflicts of interest.

Author Contributions

K.A.P.V.: formal analysis, methodology, writing – original draft preparation. O.C.: supervision, writing–review & editing. A.S.: supervision, writing–review & editing. C.L.: supervision, writing–review & editing. E.R.: data curation, funding acquisition, project administration, supervision, writing–review & editing. X.P.: data curation, supervision, writing–review & editing. R.E.: data curation, funding acquisition, project administration, supervision, writing–review & editing. J.S.S.: data curation, funding acquisition, project administration, supervision, writing–review & editing. D.C.: data curation, funding acquisition, project administration, supervision, writing–review & editing. A.M.A.G.: data curation, supervision, writing–review & editing. L.S.M: data curation, supervision, writing–review & editing. C.R.: data curation, supervision, writing–review & editing. M.F.: data curation, supervision, writing–review & editing. J.L.: data curation, supervision, writing–review & editing. E.G.G.: data curation, supervision, writing–review & editing. F.J.T.: data curation, funding acquisition, project administration, supervision, writing–review & editing. Á.H.: study design and conceptualization, formal analysis, methodology, writing – original draft preparation. M.F.: study design and conceptualization, formal analysis, methodology, project administration, writing – original draft preparation.

Keywords

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