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Journal of Nutritional Biochemistry 21 (2010) 892–898
Journal of Nutritional Biochemistry

Reduction in systemic and VLDL triacylglycerol concentration after a 3-month Mediterranean-style diet in high-cardiovascular-risk subjects ☆

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Received 22 April 2009; received in revised form 3 July 2009; accepted 6 July 2009

Abstract

The first results of the PREDIMED (PREvencion con Dieta MEDiterranea) randomized trial, after 3-month intervention, showed that the Mediterranean Diet (MD), supplemented with either virgin olive oil (VOO) or nuts, reduced systolic blood pressure, serum cholesterol and triacylglycerol (TG) concentrations and increased high-density lipoprotein (HDL)-cholesterol when compared to a control (low-fat diet) group. Serum TG levels are an independent risk factor for coronary heart disease and are strongly determined by very low-density lipoprotein (VLDL) composition, which can be specifically modified by dietary lipid source. Within the context of the PREDIMED study, we assessed the VLDL composition in 50 participants after 3 months of intake of two MD, supplemented with VOO or nuts, compared with a low-fat diet. Total and low-density lipoprotein cholesterol concentrations were reduced in subjects on the MD+nuts, whereas HDL-cholesterol increased after consumption of the MD+VOO. Serum TG concentrations were significantly lowered in both intervention groups (either the MD+nuts or MD+VOO). However, only the MD+VOO reduced the VLDL-cholesterol and VLDL-TG content and the TG/apolipoprotein B ratio in VLDL, which was used to estimate particle size. Although VLDL-TG fatty acids were very slightly modified, VLDL-TG molecular species in VLDL after consumption of the MD+nuts were characterized by a higher presence of linoleic acid (18:2, n-6), whereas after the intake of MD+VOO, they were rich in oleic acid (18:1, n-9). Therefore, we conclude that the reduction in systemic TG concentrations observed after consumption of the MD may be explained by reduction of the lipid core of VLDL and a selective modification of the molecular species composition in the particle.

Keywords: Mediterranean Diet; VLDL; Triacylglycerol; Virgin olive oil; Nuts

1. Introduction

Serum triacylglycerol (TG) concentration is an independent risk factor of coronary heart disease (CHD), often with stronger correlation than serum cholesterol with future CHD incidence in multivariate analyses [1,2]. TG levels are strongly determined by VLDL concentrations, which are precursors of low-density lipoprotein (LDL). The transformation of VLDL into LDL is dependent upon TG hydrolysis by lipoprotein lipase (LPL), which is attached to the surface of the vascular endothelium [3,4]. This enzyme can differentiate between substrates and exhibits specificity with respect to fatty acid chain length and unsaturation [5,6]. Therefore, the fatty acid composition of VLDL-TG is decisive for the activity of LPL and the formation of proatherogenic LDL and VLDL remnants.

A meta-analysis of 60 strictly controlled feeding trials [7] indicates that replacement of carbohydrates with any class of fatty acids decreases fasting serum TG concentrations. However, as recently reviewed [8], when carbohydrate was exchanged for monounsaturated fatty acid (MUFA)-rich olive oil in studies using natural diets in free-living individuals, no consistent TG lowering occurred. One study showed that substituting carbohydrate for olive oil in patients with

☆ Sources of financial support: This study was funded, in part, by the Instituto de Salud Carlos III (Thematic Network G03/140 and RD06/0045, and PI051839), Spain and AGL2008-02285 FEDER. Monstserrat Fito CP06/00100 SNS contract.
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doi:10.1016/j.jnutbio.2009.07.005

Type 2 diabetes had no effect on the serum total TG level but decreased the TG content of VLDL [9]. Similarly, a recent review of 25 feeding trials of diets enriched with different types of nuts versus control diets [10] shows an inconsistent TG-lowering effect of nut-rich diets in face of consistent cholesterol reduction. Also, changes in VLDL lipids in response to nut-enriched diets, measured in two studies [11,12], have shown discordant results. Nevertheless, recent studies carried out in our laboratory showed that slight but non-significant differences in fatty acid composition of dietary virgin olive oil (VOO) can lead to differences in the molecular species composition of VLDL-TG of elderly individuals, with significant increases in polyunsaturated fatty acid (PUFA) content [13].

There is evidence that VOO consumption can modify the lipid composition of VLDL [13,14], mainly by modulating the hepatocyte incorporation of lipids into newly formed VLDL particles [15]. Furthermore, cell culture experiments using rat primary hepatocytes have shown that minor components of the unsaponifiable fraction of VOO incorporated into human lipoproteins can influence both uptake of TG-rich lipoproteins (TRL) via regulation of LDL receptor-related protein expression [16] and VLDL secretion through modulation of the expression of enzymes involved in lipoprotein synthesis and secretion [17].

VOO and nuts are key ingredients of the standard Mediterranean Diet (MD), which is also rich in vegetables, legumes, fruits and fish. The PREDIMED study is a large randomized trial allocating asymptomatic participants who are initially free of cardiovascular disease but are at high risk for cardiovascular events to three dietary intervention groups. Changes in cardiovascular risk factors in the first 772 patients after 3 months showed that, compared with a low-fat diet, the MD supplemented with VOO (MD+VOO) or supplemented with nuts (MD+nuts) reduced systolic blood pressure, serum total cholesterol and TG concentrations and increased serum high-density lipoprotein (HDL)-cholesterol concentration [18]. Our hypothesis is that modifications of the molecular species composition of TG in VLDL may explain the decrease in serum TG, and so as to address this endeavor, apolipoprotein B, lipid and TG molecular species of VLDL were analyzed in a subset of participants of the PREDIMED trial.

2. Materials and Methods

2.1. Study design and participants

The PREDIMED (PREvencion con Dieta MEDiterranea) study is a large, parallel-group, multicenter, randomized, controlled, 5-year clinical trial aimed at assessing the effects of the MD on the primary prevention of cardiovascular disease [18]. The trial is currently taking place, with an estimated number of 7300 participants at high risk for CHD to be assigned to two intervention groups: MD+VOO and MD+nuts and a control (low-fat diet) group. The present study was performed to assess the 3-month effects of the dietary interventions on VLDL lipid composition in a subgroup of 50 participants. The institutional review boards approved the study protocol, and participants signed an informed consent. Before and after 3 months of intervention, biological samples were obtained after an overnight fast, coded, frozen at −80°C and shipped to central laboratories until the assay. Detailed description of the intervention can be found in Estruch et al. [18] and Zazpe et al. [19].

2.2. Participants

The first 50 hypertensive participants entering in the PREDIMED study from three nodes (Vitoria, Barcelona and Valencia) were divided into three groups and assigned to the MD+VOO group, the MD+nuts group or the control group. Eligible participants were community-dwelling people (55–80 years of age for men; 60–80 years of age for women) who fulfilled at least one of two criteria: type 2 diabetes; ≥3 cardiovascular risk factors (current smoking, hypertension, hypercholesterolemia, body mass index (BMI) ≥25 kg/m^2 or a family history of premature cardiovascular disease). Exclusion criteria were cardiovascular disease, any severe chronic illness, drug or alcohol addiction, history of allergy, or intolerance to olive oil or nuts.

2.3. Intervention

The baseline examination included assessment of standard cardiovascular disease factors, medications and sociodemographic factors. A 137-item food validated frequency questionnaire [20] and a 14-item questionnaire, an extension of a questionnaire designed to assess the degree of adherence to the traditional Mediterranean Diet was used [18]. On the basis of the baseline 14-item questionnaire, each participant was given personalized dietary advice by a dietitian during a 30-min session. Participants allocated to a low-fat diet were advised to reduce all types of fat and were given written recommendations according to the American Heart Association guidelines. Participants in the MD groups received instructions directed to upscale the 14-item score, including the use of VOO for cooking and dressing; increased consumption of vegetables, nuts and fish products; consumption of white meat instead of red or processed meat; preparation of home-made sauce by simmering tomato, garlic, onion and aromatic herbs with VOO to dress vegetables pasta, rice and other dishes and for alcohol drinkers and to follow a moderate pattern of red wine consumption. No energy restrictions were suggested for any intervention group. Participants in the MD groups were given 3-month allotments of free VOO (1 L/week) or mixed nuts (30 g/day, as 15 g walnuts, 7.5 g hazelnuts and 7.5 g almonds). All participants had free access to their dietitian throughout the study. The fatty acid and minor components composition of the virgin olive oil and nuts employed in the study was published elsewhere [18].

2.4. Evaluation of the intervention

After the 3-month intervention the general, food-frequency and 14-item questionnaires were repeated. Biological assessment of the intervention compliance was performed by measuring tyrosol and hydroxytyrosol levels in urine by gas chromatography-mass spectrometry (GC-MS) [21] to assess the compliance of the MD rich in VOO group and α-linolenic (18:3, n-3) acid in serum by GC [22] as a biomarker of compliance of the MD rich in nuts.

2.5. Outcome measures

Anthropometric data (height, weight and waist circumference) were obtained by standardized methods. Total serum and lipoprotein cholesterol and TG concentrations were measured using standard enzymatic automated methods (Trinder; Bayer Diagnostics, Tarrytown, NY, USA). HDL-cholesterol was measured as soluble HDL-cholesterol determined by an accelerator selective detergent method (ABXHoriba Diagnostics, Montpellier, France). Analyses were performed in a PENTRA-400 autoanalyzer (ABXHoriba Diagnostics), and LDL-cholesterol was calculated by the Friedewald formula. Insulin was measured by immunoassay (Abbott Laboratories, Maidenhead, UK) and glucose using an enzymatic colorimetric slide assay (ABXHoriba Diagnostics, Montpellier, France). Apolipoprotein A1 and B were measured by immunoturbidimetric methods (Biosystems, Barcelona, Spain).

2.6. Lipid analyses in VLDL

VLDL were isolated from 4 ml of serum layered with 6 ml of a NaCl solution (density 1.006 g/ml) by a single ultracentrifugation spin (40000 rpm, 18 h, 15°C). Ultracentrifugation was performed using a SW 41 Ti rotor in a BECKMAN L8-70M preparative ultracentrifuge (Beckman Instruments, Inc, Palo Alto, CA, USA). Total lipids were extracted from VLDL following a modification of the method of Folch et al. [23], using butyl-hydroxytoluene (BHT) as antioxidant. The lipids extracted were preserved under −20°C until used. Lipid classes and TG molecular species were analyzed as described previously by Perona et al. [24,25] using a high-performance liquid chromatography system (2690 separations module, Waters, Milford, MA, USA) coupled to a light-scattering detector (W2420, Waters). For fatty acid analysis, lipids were transmethylated using sodium metoxide in methanol (.5%) and the resulting FAME analyzed by GC, using a model 5890 series II gas chromatograph (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector and a capillary silica column Supelcowax 10 (Sulpelco, Bellefonte, PA, USA) of 60 m length and .25 mm internal diameter as previously described [26].

2.7. Statistical analyses

Results are expressed as mean±S.D., unless otherwise stated. One-factor analysis of variance (ANOVA) or Kruskal–Wallis test (when the data were not normally distributed) were used to assess differences in baseline characteristics among the three interventions. To compare 3-month changes in VLDL lipids, multiple groups were compared by ANOVA with repeated measures, followed by Tukey's post hoc tests. SPSS 15.0 for Windows (SPSS, Chicago, IL, USA) statistical package was used for all the analyses. Differences were considered significant at $P<.05$.

3. Results

3.1. Subjects and diets

The first 20-five men and 25 women from three nodes were randomly distributed among the 3 intervention groups, and selected for the study of VLDL composition. No significant baseline differences

Table 1
Baseline characteristics of the study participants

Characteristic	Low-fat diet (n=15)	MD+nuts (n=17)	MD+VOO (n=18)	P
Age, years	67.7±5.5	68.8±5.3	68.7±5.8	.820
Women %	40	58.8	44.4	.529
Tobacco consumption, %	13	18	17	.216
Family history of CHD, %	53	30	41	.284
Diabetes, %	47	65	71	.358
Hypertension, %	93	76	83	.470
Systolic blood pressure, mmHG	156.9±14.2	153.9±18.0	156.6±18.2	.859
Diastolic blood pressure, mmHG	85±7.1	83.2±9.9	82.5±9.3	.745
BMI, kg/m^2	30.8±4.8	29.5±3.5	29.9±4.6	.668
Waist circumference, cm	107.1±13.7	98.1±8.5	103.3±11.9	.100
EEPA leisure time, kcal/day*	84 (23-192)	143 (60-340)	168 (55-493)	.232
Antihypertensive agents, %	77	70	83	.759
Lipid-lowering agents, %	53	47	18	.084
Insulin, %	0	13	6	.326
Oral hypoglycemic agents, %	33	40	35	.926
Aspirin or other antiplatelet agents, %	33	20	18	.539
Disposition to dietary change, %	100	100	100	-
Education level, %				.141
Primary school	93	59	83	.050
High school	7	6	0	.553
University	0	12	0	.132

Data are presented as the percentage of participants or mean±S.D. unless otherwise indicated. EEPA, daily energy expenditure in leisure-time physical activity.
*Median (interquartile range).

were found among participants in each group regarding age, CHD risk factors, medication, disposition to change dietary habits or educational level (Table 1).

Adherence to supplemental foods was good. Urinary tyrosol was increased in all groups compared to baseline, whereas hydroxytyrosol was only increased in urine in the group allocated to the MD +VOO (Fig. 1A and B). α -Linolenic acid was increased in serum in both groups receiving the MD, but the level of significance (P<.001) was greater in the group receiving nuts (Fig. 1C). No significant change in energy and nutrient intake was observed at baseline among the experimental groups (Supplemental Table 1), including fatty acid intake, except for a lower saturated fatty acid (SFA) intake in the group receiving nuts. Consumption of olive oil, pulses, vegetables, fruits, cereals, fiber, meat fish and alcohol was similar in all groups, but the 14-point Mediterranean score showed a higher adherence in the groups receiving recommendations for a MD plus VOO or nuts.

3.2. Effects of diets on serum glucose and lipid levels

After intervention for 3 months, fasting blood glucose and serum lipids and apolipoproteins were unchanged in the low-fat diet group (Table 2). In contrast, total and LDL-cholesterol levels were lower (P<.05) in the MD with nuts group and HDL-cholesterol was higher (P<.05) in the MD with VOO group. In the two MD groups, fasting blood glucose (P<.05) and serum TG concentrations (P<.01) were significantly lower after intervention. The TG/apo B ratio decreased in both MD groups, but the change was significant (P<.01) only in the VOO group. The insulin and apolipoproteins AI and B levels remained unchanged in all groups.

3.3. VLDL lipid composition

Fig. 2 shows baseline levels and 3-month changes in the lipid and apolipoprotein composition of VLDL after the 3-month consumption of the experimental diets. A significant decrease in VLDL-cholesterol (Fig. 2A), VLDL-TG (Fig. 2B) and the TG/apo B ratio (Fig. 2D) was observed in the MD+VOO group. No changes were observed in the other two groups. In the MD+nuts an increase in α-linolenic acid in VLDL phospholipids (PL) was found. Contrarily, a decrease in α-linolenic acid in VLDL-PL was observed after consumption of the low-fat diet (Table 3). In the MD+nuts group, a significant (P<.05) increase in arachidonic acid (20:4, n-6) and its precursor dihomo-γ-linolenic acid (20:3, n-6) was observed, as well as a decrease in palmitic acid (16:0). Conversely, the MD+VOO was associated with a higher concentrations of oleic acid (18:1, n-9) but lower concentrations of arachidonic and dihomo-γ-linolenic acids.

Differences in the fatty acid composition of VLDL-TG were significant only after the intake of the MD with nuts (Table 4), which was associated to decreases in palmitic acid and increases in linoleic and α-linolenic acids. Although the differences from baseline in the fatty acid profile of VLDL-TG were small, large significant differences in the composition of VLDL molecular species were observed (Table 5). The low-fat diet group showed lower

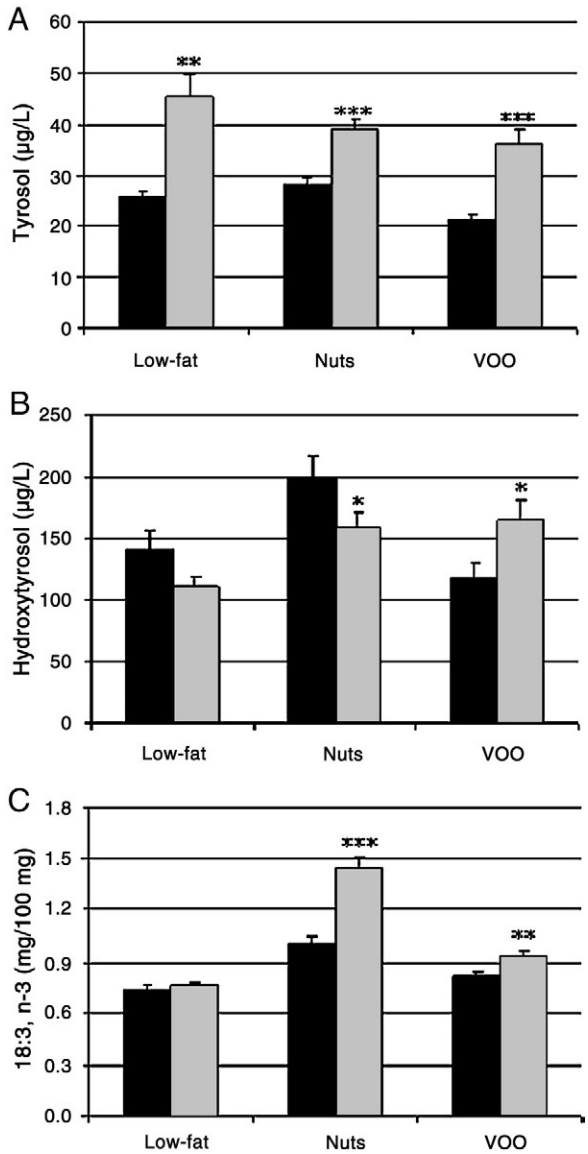

Fig. 1. Tyrosol (A), hydroxytyrosol (B) levels in urine and α-linolenic (18:3, n-3) levels in plasma (C), at baseline (dark bar) and after the 3-month intervention (light bar). Data are presented as mean±S.D. Abbreviations: VOO, virgin olive oil. *P<.05; **P<.01; *P<.001, compared with baseline.

Table 2
Serum Glucose, insulin, lipid and apolipoproteins at baseline and after the 3-month intervention

	Low-fat (n=15)		MD+nuts (n=17)		MD+VOO (n=18)	
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
mg/dl						
Glucose	129±5	131±5	119±4	114±4*	117±3	112±3*
Insulin (μU/ml)	12.8±0.8	13.9±0.6	16.8±0.5	15.1±0.5	17.6±0.6	16.4±0.6
TC	210±5	212±5	216±4	208±4**	216±4	216±4
TG	157±11	159±13	134±5	122±5**	124±6	115±4**
HDL-c	48.1±1.0	46.3±1.0	45.9±0.9	46.3±0.9	45.2±1.0	47.5±1.0**
Apo A1	142±3	138±3	137±2	137±2	135±2	138.±2
Apo B	102±2	99±2	95±2	94±2	97±2	95±2
LDL-c	148±4	144±4	143±3	138±3*	145±4	145±4
LDL/HDL	3.2±0.1	3.2±0.1	3.2±0.1	3.1±0.1	3.3±0.1	3.2±0.1
TG/Apo B	1.6±0.1	1.6±0.1	1.4±0.1	1.3±0.1	1.3±0.1	1.2±0.0*

Data are presented as mean±S.D. TC, total cholesterol; HDL-c, HDL-cholesterol, Apo, apolipoprotein; LDL-c, LDL-cholesterol.
*P<.05; **P<.01, compared with baseline.

concentrations of two oleic acid-rich TG, linoleoyl-dioleoyl-glycerol (LOO) and dioleoyl-stearoyl-glycerol (OOS), but higher concentrations of tripalmitin. VLDL obtained after 3-month consumption of MD +nuts showed higher concentrations of linoleic acid-containing TG, like dilinoleoyl-oleoyl-glycerol (LLO), dilinoleoyl-palmitoleoyl-glycerol (LLP), LOO, linoleoyl-oleoyl-palmitoyl-glycerol (LOP) and also of linolenoyl-oleoyl-palmitoyl-glycerol (LnOP). In contrast, we found lower levels of SFA-containing TG. In the MD+VOO group, the content of oleic acid-rich TG, like triolein and LOO, but also that of LnOP was increased. Although in the MD+VOO an increment of (PPS) was apparent, decreased concentrations of other SFA-containing TG, like LLP, LOP, oleoyl-dipalmitoyl-glycerol (OOP), and OOS was also observed (P<.001).

4. Discussion

Fasting serum TG levels are strongly determined by VLDL concentrations, which have been shown to actively participate directly and indirectly in the development of atherosclerosis [27]. The present study reports the modifications in VLDL composition of subjects with a high cardiovascular risk after a short-term intake of MD containing VOO or nuts, which are rich in MUFA and n-3 PUFA, respectively. Both supplemental foods are essential constituents of the traditional MD and their regular intake has been associated with beneficial effects on cardiovascular risk factors, including lipid levels, oxidative stress and inflammatory markers, as recently reviewed for VOO [28,29] and nuts [12,30–32]. The PREDIMED study is assessing the effects of the MD enriched in VOO or nuts on a number of cardiovascular outcomes. The first results after 3-month intervention showed that, among other effects, the MD reduced serum total cholesterol and TG concentrations and increased that of serum HDL-cholesterol [18]. The present study, aimed to investigate whether the reduction in serum TG observed in both MD groups is concomitantly associated with modifications in the lipid composition of VLDL with emphasis in the molecular species of TG.

Total and LDL-cholesterol concentrations were reduced in subjects consuming the MD with nuts; the MD with VOO was associated with increased HDL-cholesterol, and no changes occurred after the low-fat diet. The lipid effects of the MD with nuts were predictable, as randomized controlled trials have shown that daily consumption of a

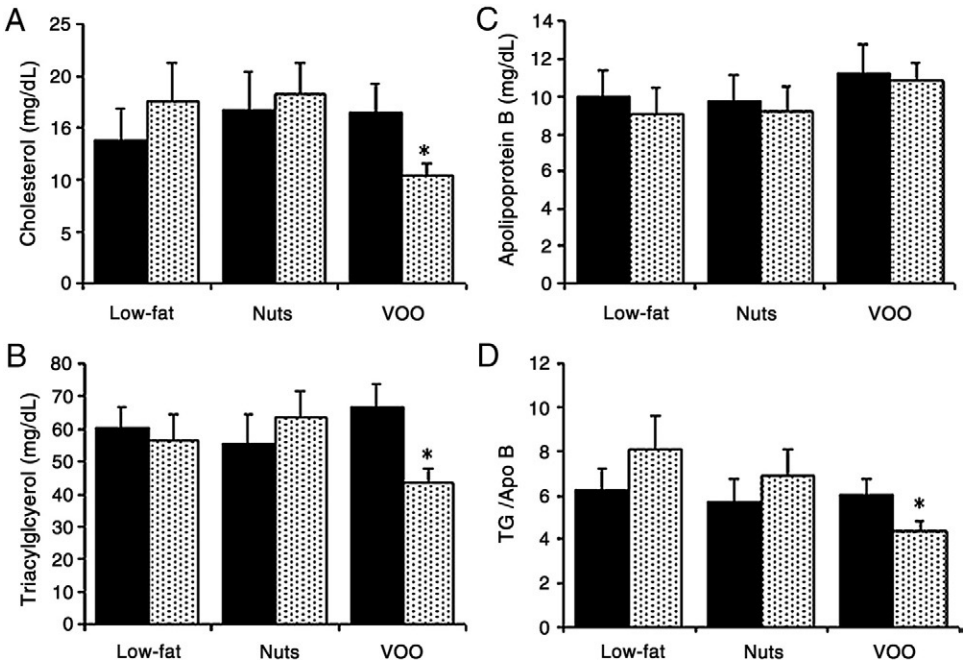

Fig. 2. VLDL cholesterol (A), triacylglycerol (B), apolipoprotein B (C) concentrations and triacylglycerol/apolipoprotein B ratio (D) at baseline (dark bar) and after the 3-month intervention (light bar). Data are presented as mean±S.D. *P<.05, compared with baseline.

Table 3
Fatty acid composition of VLDL phospholipids at baseline and after the 3-month intervention

	Low-fat (n=15)		MD+nuts (n=17)		MD+VOO (n=18)	
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
mg/100 mg						
14:0	0.9±0.6	0.9±0.5	1.0±0.7	0.8±0.3	0.8±0.4	0.9±0.6
16:0	27.4±1.7	28.4±3.0	28.2±4.5	26.7±1.7*	26.9±1.6	26.4±2.0
16:1 n-7	0.6±0.4	0.7±0.4	0.7±0.4	0.5±0.2	0.5±0.4	0.5±0.2
18:0	21.0±4.6	21.8±6.7	19.0±4.7	19.4±2.9	21.5±5.0	20.2±3.4
18:1 n-9	13.7±2.4	14.6±3.0	14.5±4.2	14.4±5.1	14.2±2.8	16.5±4.6*
18:1 n-7	1.3±0.4	1.4±0.3	1.7±1.0	1.4±0.2	1.3±0.2	1.4±0.2
18:2 n-6	17.0±3.2	16.0±3.9	17.8±4.1	18.3±3.0	17.4±4.1	18.2±3.4
18:3 n-3	1.0±0.6	0.3±0.0*	0.7±0.4	1.3±0.5*	0.3±0.2	0.4±0.1
20:1 n-9	ND	0.3±0.2	0.2±0.1	0.2±0.0	0.2±0.2	0.3±0.1
20:3 n-6	3.0 ±0.8	2.6±0.7	2.5±0.8	3.0±0.9*	3.0±0.7	2.7±0.7*
20:4 n-6	8.2 ±1.9	7.7±1.8	8.1±2.2	9.5±1.7*	8.5±2.1	7.6±1.7*
20:5 n-3	1.3 ±1.1	1.1±0.7	0.7±0.3	0.7±0.3	0.9±0.4	0.8±0.3
22:5 n-3	0.6 ±0.2	0.6±0.1	0.5±0.1	0.6±0.1	0.6±0.2	0.5±0.2
22:6 n-3	4.0±1.0	3.6±1.3	3.6±0.8	3.8±1.1	4.1±1.5	3.8±0.8

Data are presented as mean±S.D. *P<.05, compared with baseline.

variety of nuts has a consistent cholesterol-lowering effect [12]. An HDL-cholesterol-raising effect is one of the hallmarks of olive oil-containing diets [33], as confirmed in the present study. Interestingly, both MDs decreased serum TG concentrations. Despite the well-established cholesterol-lowering effect of nut diets, few studies have shown significant TG lowering [12]. Likewise, diets enriched in VOO have shown no consistent effects on plasma TG [8]. Whereas, Perez-Jimenez et al. [34] found no effects on serum TG of a diet rich in VOO compared with a standard low-fat diet, Kris-Etherton et al. [35] observed a significant reduction in serum TG associated with an olive oil-rich diet compared with a low-fat diet. The EUROLIVE Study, a controlled trial of different types of olive oil [33], showed a decrease in serum TG that was related to the MUFA content of the test oils.

The size of TRL is determined by the amount of TG that are incorporated into nascent particles from the bulk stored in the intestine (chylomicrons) or the liver (VLDL) or by the rate of hydrolysis by LPL in plasma. In the process of VLDL assembly in the liver, a small amount of TG becomes associated with a single apo B molecule. In a second stage, the bulk of VLDL-TG is incorporated into the particle from a pool of hepatic TG [36]. The well-known TG-reducing effect of n-3 fatty acids has been attributed to a lower TG secretion in VLDL from the liver and not to a lower secretion of apo B [37]. Although no similar studies have compared the effect of VOO and nuts on VLDL secretion, the lower TG concentration found in the present study in VLDL formed after VOO consumption agrees with these observations. Liver adipophilin concentrations inversely correlate with plasma TG levels in apolipoprotein E knockout mice as measured by a proteomic approach [38]. When VOO is administered to mice, liver adipohilin concentration is increased in the liver compared with palm oil. Adipophilin overexpression in primary liver cells increases the size of cytosolic lipid droplets and reduces the secretion of VLDL, thereby selectively decreasing VLDL assembly and secretion [39]. Therefore, up-regulation of hepatic adipophilin levels by dietary VOO may be a possible mechanism for decreasing plasma TG levels.

Another possible explanation for the reduced TG content of VLDL with unchanged apo B concentration after VOO consumption is an enhanced activity of LPL, the enzyme responsible for the lipolysis of circulating lipoproteins [5]. The activity of LPL, together with the exchange of lipids and apolipoproteins with HDL, leads to the transformation of VLDL first into intermediate-density lipoproteins (IDL) and VLDL remnants and then, with the participation of hepatic lipase, into LDL [3,4]. Dietary VOO is capable of increasing LPL activity in rat adipose tissue compared with high-oleic sunflower oil [40], but results in humans are inconsistent [40,41]. LPL can differentiate between substrates and exhibits specificity with respect to fatty acid chain length and unsaturation [5,6]. Therefore, the composition of VLDL lipids is decisive for LPL activity and the formation of LDL and VLDL remnants [13].

VLDL-TG fatty acids were slightly modified after the three diets. Nut consumption enriched VLDL with linoleic and α-linolenic acids, as reported for LDL after walnut consumption [42]. Although the presence of n-3 PUFA in the *sn*-2 position of VLDL-TG has been

Table 4
Fatty acid composition of VLDL triacylglycerols at baseline and after the 3-month intervention

	Low-fat (n=15)		MD+nuts (n=17)		MD+VOO (n=18)	
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
mg/100 mg						
14:0	0.4±0.2	0.3±0.1	0.4±0.2	0.4±0.1	0.5±0.2	0.4±0.1
16:0	26.3±2.2	26.5±3.1	25.7±3.1	24.3±2.5*	26.2±2.8	25.2±2.5
16:1 n-9	0.7±0.2	0.9±0.2	0.8±0.2	0.9±0.4	0.8±0.2	0.7±0.3
16:1 n-7	2.5±0.9	2.2±0.8	1.9±0.8	2.0±0.9	2.2±1.0	2.0±0.7
18:0	26.5±9.6	24.9±8.9	25.4±10.5	22.2±11.7	25.7±13.0	27.1±8.9
18:1 n-9	26.4±7.7	27.0±7.8	27.9±9.8	29.8±9.1	27.8±10.6	26.7±6.4
18:1 n-7	1.2±0.4	1.3±0.6	1.4±0.6	1.4±0.5	1.3±0.5	1.1±0.3
18:2 n-6	12.9±4.5	13.3±5.6	13.1±5.5	15.5±5.1**	12.4±4.7	13.3±4.6
18:3 n-3	0.6±0.3	0.6±0.4	0.6±0.3	0.8±0.3*	0.6±0.2	0.6±0.2
20:2 n-6	0.4 ±0.2	0.4±0.1	0.4 ±0.1	0.4±0.2	0.5±0.2	0.4±0.1
20:3 n-6	0.8 ±0.5	1.1±0.8	0.9±0.6	0.7±0.4	0.7±0.4	0.8±0.3
20:4 n-6	1.4 ±0.5	1.3±0.7	1.6±0.9	1.7±0.8	1.5±0.5	1.3±0.4

Data are presented as mean±S.D. *P<.05; **P<.01, compared with baseline.

Table 5
Molecular species composition (mg/100 mg) of VLDL triacylglycerols at baseline and after the 3-month intervention

	Low-fat (n=15)		MD+nuts (n=17)		MD+VOO (n=18)	
	Baseline	Intervention	Baseline	Intervention	Baseline	Intervention
mg/100 mg						
LnLP	ND	ND	1.1±0.0	ND	ND	1.2±0.8
LLO	2.5±1.1	1.8±0.9	2.2±0.4	4.6±2.2***	2.2±0.6	2.7±2.0
LLP	3.7±0.5	4.0±1.4	2.8±1.8	5.8±3.6**	4.2±1.1	3.3±0.2***
LnOO	2.0±0.8	1.1±0.5**	3.0±2.3	1.7±0.9*	1.7±1.2	1.5±0.2
LnOP	2.3±0.5	1.9±0.8	1.9±0.2	2.7±0.6***	1.5±0.6	2.2±1.1*
LOO	3.4±0.9	3.6±0.9	4.7±1.6	6.0±1.8*	4.1±0.8	6.9±4.8*
LOP	16.3±2.0	14.8±1.8	11.0±6.1	16.7±4.0**	15.2±4.0	13.1±1.7*
LPP	8.2±3.3	7.4±2.8	14.9±2.6	15.6±3.5	13.1±4.2	10.7±5.1
OOO	17.6±5.6	19.8±5.4	14.4±6.0	12.5±9.0	9.4±4.3	12.6±2.3**
OOP	26.8±1.3	24.9±3.8	21.7 ±3.7	19.1±4.4	22.5±3.8	25.1±7.1
OPP	4.2±1.7	4.0±0.8	3.6 ±1.9	3.0±1.0	6.1±2.2	3.0±0.4***
PPP	6.6±0.7	9.1±1.6***	9.1 ±2.9	6.6±1.3**	8.0±1.7	7.4±2.7
OOS	2.8±0.6	3.7±1.5*	3.3 ±1.4	2.1±0.4***	2.7±0.4	2.1±0.7***
OPS	2.0±0.9	2.0±0.7	1.9 ±0.4	1.1±0.1***	1.5±0.7	1.8±0.7
PPS	1.2±0.2	1.1±0.4	1.6 ±1.5	1.1±0.2	0.5±0.1	3.9±2.1**
PSS	0.7±0.3	0.7±0.5	1.2±0.2	0.7±0.1***	0.9±0.2	0.9±0.1
SSS	ND	ND	1.7±1.7	0.7±0.0	1.4±0.8	1.8±0.7

Data are presented as mean±S.D. A, arachidonic acid (20:4); Ln, Linolenic acid (18:3); L, linoleic acid (18:2); O, oleic acid (18:1); P, palmitic acid (16:0); S, stearic acid (18:0). OOO, trioleoyl-glycerol; OOP: dioleoyl-palmitoyl-glycerol; ND, not detected. *P<.05; **P<.01; ***P<.001 compared with baseline.

suggested to favor hydrolysis [43], the serum TG lowering effect of these fatty acids is believed to be due to reduced VLDL secretion by the liver and not to accelerated VLDL catabolism [44].

We have recently shown that VOO TG molecular species can be determinants of VLDL lipid composition [13]. Slight differences in the fatty acid composition of VOO, yielding significant differences in TG, caused a different VLDL-TG profile in elderly subjects. This was also observed in the present study in old subjects at high cardiovascular risk, since differences among dietary patterns were more significant in TG molecular species than in fatty acids. Whereas after the MD with nuts TG in VLDL were characterized by a higher presence of linoleic acid, the MD with VOO led to TG molecular species rich in oleic acid. Sato et al. [45] prepared VLDL enriched in palmitic, oleic, linoleic or α-linolenic acids, formed from rats fed palm, olive, safflower and linseed oils, respectively. They found that the LPL specificity for VLDL enriched in oleic acid was higher than that for linoleic acid and was correlated to a reduction in lipoprotein fluidity, suggesting that this effect might enhance the affinity of the particles for the enzyme. PL also play a role in lipoprotein fluidity [46]. Conversely to PUFA, incorporation of SFA into PL stabilizes lipoprotein particles and enhances the access of LPL to core TG [6,41]. Thus, the reduction in PL-PUFA after the MD with VOO might also have favored TG hydrolysis from VLDL. At present, there is no clear evidence of an enzyme catalyzing the hydrolysis of PL in TRL. The phospholipase activity of LPL is low [5], and despite the evidence that endothelial lipase can efficiently hydrolyze PL from HDL, its efficiency on TRL is also very low.

In conclusion, the results of the present study in a subsample of participants in the PREDIMED study suggest that the reduction in TG concentrations associated with the MD observed in a larger study [18] may be explained by a reduction of the lipid core of VLDL. Although only very slight differences were found in VLDL-TG fatty acid composition, the different sizes of VLDL particles among participants receiving the low-fat diet or the MD supplemented with VOO or nuts may be due to selective rates of hydrolysis by LPL on TG molecular species.

Acknowledgments

Fundacion Patrimonio Comunal Olivarero and Hojiblanca SA, the California Walnut Commission, Borges SA, and Morella Nuts SA generously donated the olive oil, walnuts, almonds and hazelnuts, respectively, used in the study. The CIBEROBN is an initiative of the Instituto de Salud Carlos III, Madrid, Spain.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jnutbio.2009.07.005.

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