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Crossover study of diets enriched with virgin olive oil, walnuts or almonds. Effects on lipids and other cardiovascular risk markers☆

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Abstract *Background and aims:* Virgin olive oil (VOO) and nuts are basic components of the Mediterranean diet, a heart-healthy dietary pattern. Nuts have well known cholesterol lowering effects, while evidence is unclear for VOO. We designed a study in hypercholesterolemic patients to assess the effects on serum lipids and other intermediate markers of cardiovascular risk of replacing 40% of the fat in the background diet with VOO, walnuts or almonds. *Methods and Results:* After a 4 week run-in period with a healthy diet, eligible candidates were randomized into three diet sequences in a crossover design, with a common background diet enriched with VOO, walnuts or almonds, lasting 4 weeks each. Outcomes were changes of serum lipids and oxidation and inflammation markers, measured by standard methods. Plasma fatty acids were determined by gas chromatography to assess compliance.

In 18 participants completing the study (9 women, mean age 56 y, BMI 25.7 kg/m^2), LDL-cholesterol was reduced from baseline by 7.3%, 10.8% and 13.4% after the VOO, walnut and almond diets, respectively ($P = 0.001$, Friedman test). Total cholesterol and LDL/HDL ratios decreased in parallel. LDL-cholesterol decreases were greater than predicted from dietary fatty acid and cholesterol exchanges among diets. No changes of other lipid fractions, oxidation analytes or inflammatory biomarkers were observed. Plasma fatty acid changes after each diet sequence supported good compliance.

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Conclusion: The results confirm the cholesterol lowering properties of nut-enriched diets. They also suggest that phenolic-rich VOO has a cholesterol lowering effect independently of its fatty acid content, which clearly deserves further study.

Introduction

Dietary habits are potent factors influencing coronary heart disease (CHD) risk at a global level [1]. Consistent evidence from epidemiological studies suggests that the dietary pattern known as Mediterranean diet might be particularly beneficial in CHD prevention [2,3]. Among components of the Mediterranean diet, the frequent intake of two foods that are rich in unsaturated fatty acids and other bioactive nutrients, olive oil [4] and nuts [5,6], has shown a beneficial impact on intermediate markers of cardiovascular risk. Nut consumption has also been associated with reduced CHD outcomes in prospective studies [7].

Early results from the PREDIMED study, a clinical trial in persons at high cardiovascular risk testing two Mediterranean diets, enriched with either virgin olive oil (VOO) or mixed nuts, versus a low-fat control diet for outcomes on cardiovascular disease events, showed both LDL-cholesterol lowering and HDL-cholesterol raising effects of the two Mediterranean diets after intervention for 3 months in 772 participants [8]. In this study there were no among-group differences in consumption of saturated fatty acids, cholesterol or fiber, suggesting that the supplemental foods (VOO and nuts) were influential in the LDL-cholesterol response. Many clinical trials using different types of nuts have consistently reported a cholesterol lowering effect of nut-enriched diets when compared to similar diets without nuts [5], while the few studies that have tested VOO (rich in polyphenols) versus oils with a lower phenolic content but with similar monounsaturated fatty acid (MUFA) composition have reported both an HDL-cholesterol rise and improved oxidative status, but no LDL-cholesterol changes [4,9].

Walnuts and almonds, the two nut types most frequently studied for effects on intermediate markers of cardiovascular risk [5,6], are also part of the PREDIMED intervention [8]. These nuts differ in fatty acid and micronutrient composition. Walnuts have a high content in polyunsaturated fatty acids (PUFAs), particularly linoleic acid and α-linolenic acid, the vegetable n$-$3 fatty acid, while almonds are rich in MUFA [10]. Whereas both nut types contain sizable amounts of phenolic compounds, walnuts are particularly rich in γ-tocopherol, while almonds contain abundant α-tocopherol and are richer in phytosterols [11]. An earlier study that compared walnut and almond diets for lipid outcomes found similar LDL-cholesterol responses [12]. However, the differential effects of these two nut types on markers of oxidation, inflammation and vascular reactivity have not been directly compared [6].

We report here the results of a crossover study in which a direct comparison was made among VOO, walnuts and almonds to assess their effects on serum lipids and other intermediate markers of cardiovascular risk.

Methods

Study subjects

Asymptomatic men and women with moderate hypercholesterolemia attending the Lipid Clinic at Hospital Clínic, Barcelona, Spain were recruited into a protocol approved by the institutional review board and gave informed consent. Eligibility criteria were age 25$-$75 years (after menopause in women), serum LDL-cholesterol $\geq$3.36 mmol/L (130 mg/dL), triglycerides $\leq$2.82 mmol/L (300 mg/dL), absence of chronic illnesses or secondary hypercholesterolemia, and no known allergy to nuts. None of the participants took vitamin supplements, hormone replacement therapy, or medications known to affect lipid metabolism. The power of the study was based on the lipid outcomes of a prior study comparing a walnut diet and a Mediterranean diet in a similar patient group [13]. For a crossover design, statistical power calculations indicated that to detect mean differences in LDL-cholesterol of 0.30 mmol/L (12 mg/dL) with an SD of 0.45, 20 patients would need to complete the three treatment periods (α statistic, 0.05; power, 0.8). From a computerized register of clinical records, 26 hypercholesterolemic patients (14 women and 12 men) who initially met the eligibility criteria were selected for screening and were asked to participate in the study. They were offered free VOO, walnuts and almonds, but no monetary compensation.

Study design and dietary intervention

Prior to study, the general recommendations of a Mediterranean-type, cholesterol lowering diet were reinforced in all eligible subjects, and baseline data were collected after 4 weeks. Following this period, participants were recommended to follow a common background Mediterranean-type diet and were individually randomized in a crossover design among three isoenergetic diet sequences lasting 4 weeks each, in which supplements of either VOO, walnuts or almonds were provided. Randomization was simple (not stratified) and was based on a random number table prepared by a biostatistician, resulting in six possible diet sequences, which were coded and introduced into sealed envelopes. Because diet-induced lipoprotein changes stabilize in $<$4 weeks [14], we did not incorporate a washout period between diets. Participants ate on their own, a reason why detailed information was provided to them and, if appropriate, to their partners. The diets were composed of natural foodstuffs. Vegetable products and fish were emphasized, while red and processed meats, whole-fat dairy products and eggs were limited. In the VOO diet the test oil replaced the common, mostly refined olive oil customarily ingested, while in the two nut diets walnuts or almonds, respectively, partially replaced olive oil and other MUFA-rich foods, such as

olives and avocados (no nuts other than those prescribed in the nut diets were allowed during the study). VOO was provided in ½ liter units sufficient to cover daily allowances of 35—50 g, depending on total energy requirements. Raw, shelled Spanish almonds (Marcona variety) and Spanish-grown walnuts (Serr/Chandler variety) were provided also in pre-packaged daily allowances, in amounts varying from 40 to 65 g (walnuts) and 50—75 g (almonds), according to participants' total energy intake. Recipes using nuts were provided to participants, who consumed them with meals in desserts or salads, or as snacks. The supplemental fatty foods used in each diet sequence contributed about 40% of the total fat and 22% of the total energy. All diets were adjusted to individual energy requirements using five diet models (1600, 1800, 2000, 2200 and 2400 kcal/d). The test foods used in the study were analyzed by standard methods in a reference laboratory (IRTA, Generalitat de Catalunya, Monells, Spain) (Table 1).

Compliance was assessed by 3-day diet recalls. The diets were analyzed using the Food Processor Plus software, version 8.0 (ESHA Res., Salem, OR) adapted to nutrient databases of specific Mediterranean foods when appropriate. Changes of specific fatty acids in total plasma were used as biological markers of adherence to the study diets.

Given the nature of the foods provided, which could not be masked, the study was unblinded. Investigators involved in preparation of databases and laboratory determinations, however, were masked with respect to treatment sequence. For each participant the trial's duration was 16 weeks (4 weeks run-in followed by three 4 week periods of active intervention), The study was conducted from the 10th of January to the 20th of December 2007. This clinical trial was registered at International Standard Randomized Controlled Trials with number ISRCTN68210440.

Laboratory measurements

Fasting blood samples were obtained at baseline and at the end of each diet period. Except for immediate lipoprotein determinations, serum and EDTA-plasma samples were stored at −80 °C and analyzed at the end of the study. Cholesterol and triglycerides were measured using enzymatic procedures. HDL-cholesterol was quantified after precipitation with phosphotungstic acid and MgCl. Apolipoproteins Al and B and lipoprotein(a) were determined by using turbidimetry. LDL was isolated from plasma by preparative sequential ultracentrifugation (18 h, 105,000 × g, 4 °C) using a Sorvall ultracentrifuge (Sorvall Ultra Pro80, Sorvall Products, Newtown, CT, USA) equipped with a fixed-angle rotor (T-1270). The LDL fraction was dialyzed against 150 mM sodium chloride, 1.0 mM EDTA and 10.0 mM trizma base (pH 7.2, 4 °C, 12 h) and immediately stored at −80 °C preserved in 100-g/L sucrose solution for use in copper-induced oxidizability studies at the end of the study. Analytes determined by subject in frozen samples of whole serum or plasma as appropriate were malondialdehyde (MDA) by high performance liquid chromatography, oxidized LDL by a monoclonal antibody-based immunoassay (Mercodia AB), homocysteine by fluorescence polarization immunoassay, soluble intercellular adhesion molecule-1(ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) by standard ELISA from DRG Diagnostica (Palex Cormedica), and high-sensitivity C-reactive protein (hsCRP) by particle-enhanced immunonephelometry. LDL susceptibility to oxidation was assessed by measuring conjugated diene kinetics after incubation of 50 mg of LDL protein with

Table 1 Analyzed composition of the supplemental foods used in the study.

Nutrients	Virgin olive oil	Walnuts	Almonds
Total protein (%)	0	15.6 ± 0.2	20.2 ± 0.3
Carbohydrate (%)	0	21.4 ± 0.2	29.6 ± 0.3
Total fiber (% dry matter)	0	19.5 ± 3.2	23.1 ± 2.3
Total fat (%)	100	62.9 ± 0.4	50.2 ± 0.2
Fatty acid (%)[a]			
14:0	< 0.1	< 0.1	< 0.1
16:0	15.1 ± 0.1	7.3 ± 0.1	7.1 ± 0.1
18:0	1.9 ± 0.1	2.5 ± 0.1	1.9 ± 0.0
16:1 (n − 9)	1.9 ± 0.0	0.1 ± 0.0	0.7 ± 0.0
18:1 (n − 7)	3.5 ± 0.0	0.7 ± 0.0	1.2 ± 0.0
18:1 (n − 9)	62.9 ± 0.3	13.8 ± 0.3	62.2 ± 0.3
18:2 (n − 6)	12.5 ± 0.2	62.6 ± 0.4	26.5 ± 0.2
18:3 (n − 3)	0.8 ± 0.0	12.4 ± 0.2	0.1 ± 0.0
α-Tocopherol (mg/100 g)	25.8 ± 1.5	1.8 ± 0.1	68.4 ± 0.9
γ-Tocopherol (mg/100 g)	5.7 ± 0.4	17.9 ± 7.2	4.0 ± 0.2
Total phytosterols (mg/100 g)	155 ± 19	92 ± 2	240 ± 25
β-sitosterol (%)	85 ± 1	88 ± 1	78 ± 1
Δ-5avenasterol(%)	7.9 ± 1.2	5.7 ± 0.9	13.0 ± 0.6
campesterol (%)	4.1 ± 0.2	5.4 ± 0.0	2.7 ± 0.1
Polyphenols (mg/100 g)	34.3 ± 1.5	1.3 ± 0.4	1.1 ± 0.2

Data are means and SD of 3 measurements.

[a] The number before the colon specifies the number of carbon atoms, and that after the colon the number of double bonds (*n* is the number of carbons counting from the methyl end-group to the first double bond).

$CuSO^4$ (5 μM, 18 h, 37 °C), as described [15]. Protein concentration in LDL was determined by the Lowry method. All analyses were done in duplicate.

Statistical methods

Changes from baseline in LDL-cholesterol for each diet sequence were set as the primary outcome. However, we were equally interested in changes in all end points in our exploratory and nonconfirmatory study. Given the cross-sectional design of the study, end point analyses were made for completers only. Nonparametric tests were used because the study sample was small and deviations from normality existed in the distribution of many variables. Among diet effects on study outcomes were evaluated by the Friedman test. When significant differences were detected, multiple comparisons were made by using the Wilcoxon test for paired samples with Bonferroni correction. To ascertain the extent to which the different fat content of the diets could explain changes in LDL-cholesterol, we applied a predictive model that includes the regression coefficients for percentage energy changes in dietary SFA, PUFA, MUFA and dietary cholesterol [16]. Data are expressed as medians and interquartile ranges. All P values are two-tailed and statistical significance was defined as $P < 0.05$. Analyses were performed using SPSS 16.0 (SPSS Inc, Chicago, IL, USA).

Results

Subjects and diets

We recruited 26 eligible subjects to account for possible losses before completion of the trial. In fact, only 18 participants completed the trial. Six subjects chose not to participate prior to randomization for personal reasons, mainly problems with clinic appointments. After randomization, two participants completed one and two diet sequences, respectively, but left the study because they felt it was too demanding. The clinical characteristics of these subjects did not differ from those of the whole group. Subsequent data refer only to completers, who were 9 men and 9 women, aged 56 ± 13 years with BMI 25.7 ± 2.3 kg/m^2.

Diets were well tolerated by all study subjects. As shown in Table 2, the self-reported nutrient contents of the baseline diet showed good adherence to initial dietary advice. Table 2 also shows the actual nutrient content of the three test diets. Compared to the baseline diet, the intervention diets had a lower protein, saturated fatty acid (SFA) and cholesterol content, and higher total carbohydrate and fiber. The unsaturated fatty acid content of the study diets reflected the composition of the supplemental foods, with higher MUFA in the VOO and almond diets and higher PUFA in the walnut diet, thus supporting good adherence. Plasma fatty acid composition after each diet sequence provided a further measure of compliance by showing increased MUFA, particularly oleic acid, after the VOO diet and to a lesser extent after the almond diet, while linoleic and α-linolenic acids and total PUFA increased after the walnut diet (Table 3). Changes in proportions of other fatty acids were always small, even if significant.

Effects on serum lipids and other cardiovascular risk markers

Table 4 shows that there were no changes of body weight, blood pressure or fasting blood glucose during the trial. On the other hand, the three intervention diets were associated with significant reductions from baseline of total cholesterol, LDL-cholesterol and the LDL/HDL ratio. The mean reduction in LDL-cholesterol was 0.36 mmol/L (7.3%) with the VOO diet, 0.51 mmol/L (10.8%) with the walnut diet, and 0.61 mmol/L (13.4%) with the almond diet. No changes were observed in HDL-cholesterol, triglycerides, apolipoproteins AI and B, or lipoprotein(a). Treatment sequence or gender had no influence on the results. The application of the equations describing the variation of serum LDL-cholesterol according to dietary fatty acid and cholesterol changes predicted decreases of 0.08, 0.21 and 0.14 mmol/L after the VOO, walnut and almond diets, respectively, which are considerably smaller than the average observed decreases and do not cross the lower boundary of the 95% CI of the actual change (Fig. 1).

No significant changes in homocysteine, oxidation analytes, CRP or soluble cell adhesion molecules were observed after the experimental diets (Table 4).

Table 2 Self-reported nutrient intake at baseline and during the three diet sequences.

Nutrients	Baseline	Virgin olive oil	Walnuts	Almonds	P^*
Energy (kcal/day)	1939 (1660–2165)	2009 (1630–2163)	1982 (1676–2137)	1996 (1640–2171)	0.659
Carbohydrates (% En)	44.5 (38.0–51.0)	49.0 (47.5–51.0)	49.5 (47.0–51.0)	48.5 (46.8–50.0)	0.075
Total fiber (g/day)	21.2 (16.9–23.3)^a	25.1 (22.1–27.0)^{a,b}	25.1 (21.2–31.0)^{a,b}	29.0 (24.7–33.9)^b	<0.001
Protein (% En)	20.5 (17.0–21.3)^a	16.0 (16.0–17.3)^b	16.5 (16.0–18.0)^b	17.0 (16.0–18.0)^b	0.002
Total fat (% En)	33.0 (30.8–35.3)	32.5 (32.0–33.3)	32.0 (32.0–33.0)	33.0 (32.0–34.0)	0.205
SFA (% En)	6.9 (5.9–8.9)^a	5.4 (5.3–5.9)^b	4.7 (4.5–5.3)^c	4.9 (4.6–5.1)^c	<0.001
MUFA (% En)	19.3 (16.8–20.5)^a	20.8 (20.3–21.1)^b	14.0 (13.6–14.2)^c	20.3 (19.5–20.6)^a	<0.001
PUFA (% En)	4.0 (3.5–4.9)^a	3.9 (3.6–4.0)^a	11.2 (10.8–11.5)^b	5.6 (5.4–5.7)^c	<0.001
Cholesterol (mg/day)	231 (183–261)^a	138 (101–236)^b	149 (103–197)^{b,c}	117 (86–142)^c	0.003
Alcohol (g/day)	1.2 (0–11.6)	0 (0–10.1)	0 (0–9.1)	0 (0–9.3)	0.311

Data are medians (IQ ranges). En, energy; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

*Friedman test. Means in a row with different superscript letters differ (Wilcoxon test with Bonferroni correction, $P < 0.05$).

Table 3 Plasma fatty acid composition at the end of each dietary period.

Percent	Virgin olive oil	Walnuts	Almonds	P^*
14:0	0.76 (0.64–0.87)[a]	0.55 (0.42–0.70)[b]	0.68 (0.53–0.80)[c]	<0.001
16:0	23.4 (22.3–24.9)[a]	22.3 (20.8–23.6)[b]	22.9 (22.3–24.4)[c]	<0.001
18:0	8.95 (8.61–9.99)[a]	8.81 (8.19–10.73)[a,b]	8.75 (7.99–9.82)[b]	0.056
16:1 (n − 9)	1.38 (1.22–1.68)[a]	1.13 (1.02–1.28)[b]	1.26 (1.13–1.47)[a]	<0.001
18:1 (n − 9)	25.3 (24.0–29.0)[a]	19.9 (18.2–21.4)[b]	21.6 (19.2–23.8)[c]	<0.001
18:2 (n − 6)	21.9 (20.6–24.0)[a]	29.8 (28.4–36.2)[b]	26.1 (23.6–30.5)[c]	<0.001
18:3 (n − 3)	0.33 (0.26–0.45)[a]	0.72 (0.55–1.41)[b]	0.42 (0.33–0.51)[c]	<0.001
20:3 (n − 6)	1.91 (1.50–2.14)[a]	1.53 (1.24–1.78)[b]	1.96 (1.46–2.07)[a]	<0.001
20:4 (n − 6)	6.89 (6.45–7.91)[a]	6.23 (5.71–6.96)[b]	7.80 (6.32–8.95)[a]	<0.001
20:5 (n − 3)	0.99 (0.67–1.37)	0.97 (0.61–1.11)	1.03 (0.80–1.46)	0.024
22:6 (n − 3)	3.37 (2.28–4.31)[a]	2.90 (2.05–3.93)[b]	3.30 (2.69–4.17)[a]	<0.001
Σ SFA	34.3 (32.9–37.1)[a]	32.6 (31.1–35.3)[b]	33.1 (31.6–36.0)[b]	<0.001
Σ MUFA	27.3 (25.5–30.3)[a]	21.1 (19.9–22.8)[b]	23.1 (21.1–25.4)[c]	<0.001
Σ PUFA	37.4 (34.9–38.8)[a]	45.2 (42.8–48.3)[b]	42.1 (39.5–46.9)[c]	<0.001

Data are medians (IQ ranges). See foot of Table 1 for fatty acid nomenclature. Σ, sum; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
*Friedman test. Means in a row with different superscript letters differ (Wilcoxon test with Bonferroni correction, $P < 0.05$).

Discussion

In this 12-week crossover feeding trial in 18 free-living men and women with moderate hypercholesterolemia we compared the effects of replacing 40% of the fat (22% of energy) in a healthy background Mediterranean diet with VOO, walnuts or almonds. The three test diets were associated with reductions in total cholesterol and LDL-cholesterol, with no change in HDL-cholesterol, thus improving the LDL/HDL ratio. While cholesterol lowering in response to dietary enrichment with walnuts and almonds has been consistently observed in prior studies [5,17], a similar effect of VOO has only been described in a report of the much more powered PREDIMED study [8].

It could be argued that part of the reduction in LDL-cholesterol observed after the test diets was due to changes

Table 4 Body weight, blood pressure, serum lipids, and biomarkers of oxidative stress and inflammation at baseline and at the end of each diet period.

Variables	Baseline	Virgin olive oil	Walnuts	Almonds	P^*
Body weight (kg)	70.7 (63.1–80.3)	68.7 (61.6–79.5)	68.7 (61.9–79.7)	68.9 (61.8–79.2)	0.601
Systolic BP (mm Hg)	140 (118–152)	130 (123–149)	134 (121–142)	133 (120–152)	0.577
Diastolic BP (mm Hg)	85 (79–89)	86 (79–89)	84 (80–88)	83 (79–88)	0.861
Fasting glucose (mmol/L)	4.83 (4.17–5.11)	5.0 (4.61–5.39)	4.89 (4.44–5.33)	4.56 (4.39–5.11)	0.136
Serum lipids (mmol/L)					
Total cholesterol	7.03 (6.71–7.82)[a]	6.71 (6.03–7.33)[a]	6.48 (6.27–6.89)[b]	6.58 (5.78–6.94)[b]	0.012
LDL-cholesterol	5.08 (4.74–5.36)[a]	4.71 (4.25–4.87)[b]	4.53 (4.22–4.87)[b]	4.40 (4.12–4.84)[b]	0.001
HDL-cholesterol	1.63 (1.35–1.86)	1.61 (1.37–1.94)	1.61 (1.35–1.94)	1.61 (1.40–1.86)	0.364
Triglycerides	1.36 (0.84–1.89)	1.22 (1.87–1.57)	1.13 (0.77–1.44)	1.21 (0.87–1.69)	0.121
LDL/HDL ratio	3.2 (2.7–3.6)[a]	3.1 (2.3–3.4)[a]	2.8 (2.4–3.3)[b]	2.9 (2.3–3.2)[b]	0.005
Apolipoprotein A1 (g/L)	1.48 (1.30–1.68)	1.52 (1.21–1.68)	1.41 (1.22–1.57)	1.44 (1.22–1.57)	0.316
Apolipoprotein B (g/L)	1.34 (1.25–1.57)	1.35 (1.20–1.44)	1.29 (1.13–1.40)	1.27 (1.13–1.39)	0.162
Lipoprotein(a) (g/L)	0.38 (0.25–0.58)	0.42 (0.20–0.65)	0.36 (0.17–0.61)	0.38 (0.20–0.63)	0.121
Homocysteine (umol/L)	11.1 (9.2–12.5)	10.2 (8.7–11.7)	10.8 (9.3–12.3)	9.8 (8.1–12.2)	0.081
Oxidation analytes					
Lag time CD production (min)	47 (39–57)	50 (36–59)	45 (33–54)	47 (39–53)	0.483
Malondialdehyde (nmol/L)	109 (93–127)	107 (89–120)	92 (79–118)	95 (80–129)	0.926
Oxidized LDL (U/L)	80 (62–92)	76 (65–114)	84 (70–109)	78 (71–103)	0.136
Inflammation markers					
C-reactive protein (mg/L)	2.1 (1.2–3.1)	1.7 (0.9–2.4)	1.9 (0.9–3.0)	1.7 (1.0–2.5)	0.241
Soluble ICAM-1 (ng/ml)	291 (198–408)	272 (146–382)	238 (174–403)	260 (145–417)	0.182
Soluble VCAM-1 (ng/ml)	670 (639–826)	730 (591–857)	824 (714–1000)	760 (668–1034)	0.180

Values are medians (IQ ranges). BP, blood pressure; CD, conjugated dienes; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule.
*Friedman test. Means in a row with different superscript letters differ (Wilcoxon test with Bonferroni correction, $P < 0.05$).

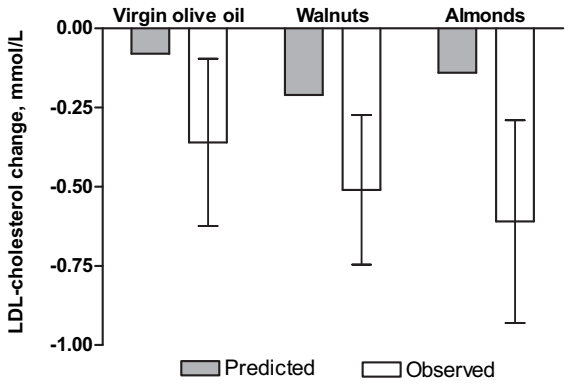

Figure 1 Predicted versus observed mean LDL-cholesterol changes after the three study diets. Error bars represent 95% CIs.

from baseline in other nutrients that are known to influence blood cholesterol, such as SFA, cholesterol and fiber. However, Table 2 shows that such nutrient changes (reductions of 1–2% in saturated fatty acids and ≈100 mg/day cholesterol, and increases of 4–8 g/day in fiber) were of small magnitude. Given that the equations explaining the component of the LDL-cholesterol variation imparted by dietary fatty acid and cholesterol changes [16] predicted decreases that were substantially smaller than those observed (Fig. 1), it can reasonable be concluded that the cholesterol lowering effect of the test diets is explained only to a small extent by their fat content. A larger than predicted hypocholesterolemic effect of nut-enriched diets has been described [14,18], and it has been speculated that nut constituents other than fatty acids, such as fiber, plant protein, tocopherols, phytosterols and possibly other bioactive components could account for this effect [11,18,19]. Our finding that the almond diet achieved a slightly greater LDL-cholesterol reduction than the walnut diet (13.4% versus 10.8%, respectively) might be ascribed in part to the 2.5-fold greater content in phytosterols of almonds compared to walnuts (Table 1). The prior study that directly compared walnuts and almonds for lipid outcomes found a similar LDL-cholesterol lowering effect (9% and 10%, respectively), but no data on nut composition were reported [12]. The VOO used in our study was also rich in phytosterols, which could partly explain the observed LDL-cholesterol reduction beyond fatty acid exchange. Recently suggestive evidence has been provided that phytosterols in nuts and VOO in the PREDIMED study relate to the LDL-cholesterol response of the experimental diets [20].

The Mediterranean diets enriched with VOO or mixed nuts were associated with HDL-cholesterol raises in the PREDIMED study [8]. This effect has also been reported after consumption of high-phenolic content VOO [9], but was not detected in a recent pooled analysis of nut feeding trials [5] or a meta-analysis of walnut studies [16]. There were no among diet changes in HDL-cholesterol in our study. Likewise, we could not find any significant differences among diets in the response of several oxidation and inflammation markers. However, the lack of significant changes in some of the biomarkers was probably due to insufficient statistical power (Table 4). As reviewed [6], prior studies with almonds or walnuts failed too find changes in CRP, the main circulating inflammatory biomarker. Two recent studies reported conflicting results on this topic [21,22]. Casas-Agustench et al. [21] fed one serving of mixed nuts to patients with metabolic syndrome and failed to show CRP changes, while Rajaram et al. [22] reported significant CRP decreases in healthy subjects who were given diets enriched with almonds at two doses (10% and 20% of energy) compared to a control diet. Again, in the much more powered PREDIMED study reduced circulating biomarkers of inflammation and oxidation were reported after both VOO and nut-supplemented diets [8,23], although CRP was decreased only after the VOO diet [8]. A further substudy of the PREDIMED trial found reduced monocyte expression of pro-inflammatory ligands after the two intervention diets [24]. At any rate, there is increasing evidence of the antioxidant and anti-inflammatory properties of both high-phenolic VOO [4] and nuts [6].

Our study has limitations, such as the small number of participants who completed the study, which reduced statistical power and blunted the significance of dietary associations with various cardiovascular risk markers. Another limitation is that we studied patients with hypercholesterolemia and the magnitude of cholesterol reduction observed with the nut diets may not be extrapolated to persons with normal blood cholesterol levels, who would have lesser responses [5]. Strengths of the study are that it reproduced real-life conditions with home-prepared foods for the background diet, used supplemental foods that are commonly available and consumed by the public, and verified compliance with objective markers of intake.

In conclusion, our results confirm the well known cholesterol lowering properties of nut-enriched diets. They also suggest that replacing common, mostly refined olive oil by phenolic-rich VOO has a cholesterol lowering effect that is independent of the oils' fatty acid content. This effect, which has been formerly shown in a single study, more powered but less tightly controlled than the present one [8], is intriguing and clearly deserves further study. Nonetheless, it is valid to suggest that VOO, walnuts and almonds, which are foods rich in unsaturated fatty acids and other bioactive nutrients, should be included in healthy diets, especially those aimed at reducing blood cholesterol and cardiovascular risk.

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Author's contributions

JS-S and ER designed the study and obtained funding. AP-H and MS delivered the intervention and estimated food and nutrient intake. MC and AS-V performed laboratory determinations. NRTD, MC, and ER analyzed and interpreted the data. NRTD and ER drafted the article. All authors critically

reviewed the article for important intellectual content and approved the final version.

Conflict of interest

JS-S has received research funding from and is a non paid member of the International Nut and Dried Fruit Foundation, Reus, Spain. ER has received research funding from the California Walnut Commission, Sacramento, CA and is a non paid member of its Scientific Advisory Committee. All other authors have no other conflicts of interest to declare.

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