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Effects of Eating Walnuts beyond their Effects on Lipid Levels [Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL), and High-Density Lipoprotein Cholesterol (HDL)]

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Walnuts are unique, among the nuts, in their composition relative to alpha-linolenic acid (ALA). ALA in the body desaturates and elongates adequately to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), provided that the LA/ALA ratio in the diet is 4/1 or less, and the trans fatty acid intake is less than 2% of total energy intake [Reviewed in reference 1]. As Table 1 shows, walnuts contain the highest amount of ALA and a ratio of linoleic (LA) to ALA of 4 to 1, precisely the ratio in the Lyon Heart Study [2-4] that showed a decrease of 70% in overall mortality and a decrease in the reinfarction rate in the myocardial infarction (MI) patients by 50% within 27 months of the study.

In the Indo-Mediterranean diet study, equally significant results were obtained at a ratio of 3.8/1 of LA/ALA [5]. In the Nurses Health Study, Hu et al. [6] showed that a higher intake of ALA was associated with a relative risk (RR) of 0.55 in the lowest quintile, p for a trend of 0.01. The ALA intake ranged from 0.71 g/d in the lowest quintile to 1.36 g/d in the highest quintile [6]. In the Singh et al. study [5] the ALA intake was 1.8 g/d. In the Lyon Heart Study, about 2 g of ALA came from canola oil margarine [2]. The estimation to use about 2 g of ALA came from the data from Crete (Table 2) [7]. One ounce of walnuts contains 2.57 g of ALA. The plasma cholesteryl esters in the population of Crete were three times as high as those of Zutphen, Holland, and had the lowest coronary heart disease mortality rate than any other population in the Seven Countries Study [8]. Thus, intervention trials involving 605 patients in the Lyon Heart Study and 1000 patients in the Indo-Mediterranean diet study, as well as the Nurses Health Study, show a beneficial effect of ALA relative to coronary heart disease, both in the primary

prevention [6,9] and in the secondary prevention [2-5]. Simon et al [10] showed an increase of one standard deviation (SD) in the serum level of ALA in cholesterol esters was related to a 37% reduction of stroke risk.

Protective Effects of Alpha-Linolenic Acid (ALA) in Cardiovascular Disease

Several mechanisms have been proposed to explain the protective effect of ALA [11,12]. Dog studies have shown that acute infusion of ALA can prevent ischemia-induced ventricular fibrillation [13,14]. Other beneficial effects include an improvement in arterial compliance [15], modest blood pressure reduction [16] and antiplatelet effect [17,18]. Finally, an anti-inflammatory effect has been proposed [19], which is supported by the results of the Rallidis et al. study [20]. In their study, ALA led to a decrease in C-reactive protein (CRP) [20], an acute phase protein reactant that is an independent risk factor for coronary heart disease [21], whereas LA had no effect [20].

Specific Effects of Alpha-Linolenic Acid (ALA) in Walnuts that Decrease Coronary Heart Disease Risk

Human Studies

- Decrease in small dense LDL [22]. Small dense LDL is the most atherogenic form of LDL.
- Does not lead to increases in weight gain [23] or may even lead to weight loss [22]. Similar studies obtained from feeding ALA to animals. ALA-fed animals had less weight gain (due to higher oxidation of ALA relative to LA).
- Feeding walnuts to hypercholesterolemic men and women at a dietary ratio of 4/1 of LA/ALA of the total diet showed a significant decrease in CRP [24].

- Effects on endothelial function. Perez-Heras et al. [25] found that substituting walnuts improved endothelial function in men and women with hypercholesterolemia, as shown by decreases in vascular cell adhesion molecule (VCAM) [25].
- Zhao [24] has confirmed the results of Perez-Heras [25], showing a decrease in VCAM, and in addition, ICAM and E-selectin.
- ALA from walnuts decreased lipoprotein (a) [Lp(a)] [26]. LDL particles became enriched with ALA, but their resistance to oxidation was preserved.

Total Antioxidant Content of Walnuts

The total antioxidant content of walnuts indicates that they have the highest content (Table 3) which adds to their uniqueness [27]. Oxidative stress is involved in the pathogenesis of most chronic diseases, including coronary heart disease. So, the total antioxidant content of foods is an important factor in the prevention of chronic diseases.

Conclusion

The background diet becomes important in the control of risk factors for coronary heart disease, and walnuts are unique among nuts since they provide a ratio of LA/ALA of 4/1, which has been shown to decrease the risk for coronary heart disease in intervention trials. Walnuts not only provide the highest amount of ALA, but they also provide the highest concentration of antioxidants relative to other nuts. To group walnuts together with other nuts is unscientific, because it ignores an important body of epidemiological and clinical research on the beneficial effects of ALA at doses comparable to that obtained with less than one ounce of walnuts.

Table 1. 18:3 and 18:2 fatty acid composition of nuts (g per 100 g edible portion)

Nut	*18:3 (omega-3)*	*18:2 (omega-6)*
Walnuts	9.081	38.095
Pecans	0.986	20.628
Pistachios	0.247	12.831
Macadamia Nuts	0.206	1.296
Hazelnuts	0.087	7.833
Brazil Nuts	0.062	23.807
Chestnuts	0.053	0.440
Peanuts	0.003	15.555
Almonds	0	12.214
Coconut Meat	0	0.366

Data (except pecans and chestnuts) from Table 1 in Feldman EB. The scientific evidence for a beneficial health relationship between walnuts and coronary heart disease. LSRO Report. J Nutr 2002:132;1062S-1101S. Data for "Pecans" and "Chestnuts" from the online USDA Nutrient Database.

Table 2. Fatty acid composition of serum cholesterol esters (%)[1]

	Crete (n = 92)	Zutphen (n = 97)
	%	
16:0	11.1 ± 0.1	11.9 ± 0.1[2]
18:0	0.7 ± 0.0	1.1 ± 0.0[2]
18:1n-9	31.0 ± 0.3	21.4 ± 0.4[2]
18:2n-6	41.9 ± 0.4	53.1 ± 0.7[2]
18:3n-3	0.9 ± 0.1	0.3 ± 0.0[2]

[1] $\chi \pm$ SE. Adapted from Sandker et al. [7]
[2] Significantly different from Crete, $P < 0.001$.

Table 3. Total antioxidant concentration of nuts and seeds[1-2]

Nuts and seeds	Sample A	nmol/100g	Sample B	nmol/100g	Sample C	nmol/100g	Overall mean
Walnut	Diamond (n=3)[3]	17.89	Helios (n=3)	19.76	Helios (n=3)	25.25	20.97
Sunflower seed	Natuvit, Denmark (n=3)	5.41	Natuvit, Denmark (n=3)	4.57	Natuvit, Denmark (n=3)	6.18	5.39
Sesame seed	Natana, Denmark (n=3)	1.09	Natana, Denmark (n=3)	1.25	Natana, Denmark (n=3)	1.28	1.21
Hazelnut	Nottefabrikken (n=3)	0.48	Solbaetorvet (n=3)	0.50	Nottefabrikken (n=3)	0.49	0.49
Almond	Solbaetorvet (n=3)	0.44	ICA, Norway (n=3)	0.23	Meny, Norway (n=3)	0.23	0.30
Cashew nut	Nottefabrikken (n=3)	0.22	Nottefabrikken (n=3)	0.23	Nottefabrikken (n=3)	0.24	0.23

[1]Modified from Table 7 in Reference 27.

[2]Electron-donating antioxidants were determined by FRAP assay. Values represent mean concentration per 100 g fresh weight of edible portion if not otherwise stated. The origin and brand of each sample are indicated. If available, Samples A, B and C represent separate samples of the same dietary plant obtained from different sources such as geographical location or manufacturer.

[3]The number of items analyzed is indicated in parenthesis.

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v:/Elsevier Science/Shannon/Ath/articles/Ath8126/ATH8126.3d[x] 9 January 2003 9:16.9

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Dietary α-linolenic acid decreases C-reactive protein, serum amyloid A and interleukin-6 in dyslipidaemic patients

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Received 1 July 2002; received in revised form 18 November 2002; accepted 6 December 2002

Abstract

Background: Inflammation plays an important role in the pathogenesis of coronary artery disease. We examined whether dietary supplementation with α-linolenic acid (ALA, 18:3n-3) affects the levels of inflammatory markers in dyslipidaemic patients. *Methods*: We recruited 76 male dyslipidaemic patients (mean age = 51 ± 8 years) following a typical Greek diet. They were randomly assigned either to 15 ml of linseed oil (rich in ALA) per day ($n = 50$) or to 15 ml of safflower oil (rich in linoleic acid (LA, 18:2n-6)) per day ($n = 26$). The ratio of n-6:n-3 in linseed oil supplemented group was 1.3:1 and in safflower oil supplemented group 13.2:1. Dietary intervention lasted for 3 months. Blood lipids and C-reactive protein (CRP), serum amyloid A (SAA), and interleukin-6 (IL-6) levels were determined prior and after intervention. CRP and SAA were measured by nephelometry and IL-6 by immunoassay. *Results*: Dietary supplementation with ALA decreased significantly CRP, SAA and IL-6 levels. The median decrease of CRP was 38% (1.24 vs. 0.93 mg/l, $P = 0.0008$), of SAA 23.1% (3.24 vs. 2.39 mg/l, $P = 0.0001$) and of IL-6 10.5% (2.18 vs. 1.7 pg/ml, $P = 0.01$). The decrease of inflammatory markers was independent of lipid changes. Dietary supplementation with LA did not affect significantly CRP, SAA and IL-6 concentrations but decreased cholesterol levels. *Conclusions*: Dietary supplementation with ALA for 3 months decreases significantly CRP, SAA and IL-6 levels in dyslipidaemic patients. This anti-inflammatory effect may provide a possible additional mechanism for the beneficial effect of plant n-3 polyunsaturated fatty acids in primary and secondary prevention of coronary artery disease.

Keywords: α-Linolenic acid; C-reactive protein; Dyslipidaemia; Interleukin-6; Linoleic acid; Serum amyloid A

1. Introduction

There is substantial evidence that inflammation plays a central role in all phases of the atherosclerotic process [1]. Our understanding of atherosclerosis has evolved beyond the view that it is a bland lipid storage disease. Clinical studies show correlation of circulating acute phase reactants or cytokines with increased risk for future vascular events and raise the possibility of active contribution to their pathogenesis [2,3].

Dietary fats rich in α-linolenic acid (ALA, 18:3n-3) have been reported to modulate some of the inflammatory responses in experimental animal models [4] and clinical trials [5]. ALA is an essential fatty acid present in vegetable oils. It is the precursor for the formation of the marine long chain n-3 polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) which can displace arachidonic acid (20:4n-6) and reduce the production of proinflammatory eicosanoids prostaglandin E_2 and leukotriene B_4. Arachidonic acid is derived by desaturation and elongation of linoleic acid (LA) (18:2n-6).

Epidemiological studies indicate that there is an inverse association between dietary ALA and risk of

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0021-9150/02/$ - see front matter © 2002 Published by Elsevier Science Ireland Ltd.
PII: S0021-9150(02)00427-6

myocardial infarction [6–8]. In the Lyon Diet Heart Study a Mediterranean diet rich in ALA was beneficial in secondary prevention of myocardial infarction [9]. A number of studies have also investigated the effect of ALA on blood lipid levels and indicated that its possible antiatherogenic properties are not due to the improvement in blood lipid profile [10–14].

As there is no study to correlate dietary ALA with inflammatory markers we investigated whether dietary supplementation with ALA affects the levels of acute phase reactants C-reactive protein (CRP) and serum amyloid A (SAA) and the cytokine interleukin-6 (IL-6) in dyslipidaemic patients.

2. Materials and methods

2.1. Subjects

Subjects were recruited from the Department of Cardiology of Laiko Hospital and were then screened regarding their health status, medication usage, diet and exercise pattern. Subjects with presence of infection, endocrine, liver or vascular disease, blood pressure > 145/95 mmHg, on medication known to affect lipoprotein metabolism, habitual consumption of >30 units alcohol per week, smoking habits (≥10 cigarettes per day) or habitual undertaking of >6 h of vigorous exercise per week were excluded from the study. Intake of anti-inflammatory drugs or vitamin or other dietary supplements was forbidden during the experimental period.

2.2. Study design

Our study was an interventional study of parallel design. We followed a 2:1 process to form the ALA (subjects supplied with ALA) and LA (subjects supplied with LA) group, respectively. Experimental period lasted for 12 weeks. Subjects screened at entry to be following the average Greek diet. They were asked to maintain their dietary habits and usual lifestyle. Subjects were randomly divided into two groups and assigned to one of the two oils supplementation: ALA group 15 ml of linseed oil per day containing approximately 8 g of ALA and LA group 15 ml of safflower oil per day containing approximately 11 g of LA. The intake was designed to be such in order to achieve a n-6:n-3 ratio of 1.3:1 in the ALA group and 13.2:1 in the LA group as well as to keep the total fat intake constant in both groups. Linseed and safflower oils were provided by Savant International, UK Supplement was taken three times per day, one teaspoon of 5 ml per meal. Blood samples collection was carried out following a 12-h fast. The volunteers were asked to keep a record of what they consumed the day before the first blood collection and they were also asked to consume exactly the same meals the day before the second blood collection.

Table 1
Oils composition per 100 g

	linseed oil	safflower oil
Palmitic acid (g)	5.9	5.7
Stearic acid (g)	3.6	2.4
Arachidonic acid (g)	–	0.5
Oleic acid (g)	18.2	11.5
Eicosapentaenoic acid (g)	–	0.5
Linoleic acid (g)	13.9	74.4
α-Linolenic acid (g)	54.2	0.5
Total sterols (g)	0.4	0.4
Total tocopherols (mg)	54.27	48.21

The composition of the two oils is given in Table 1. The study design was approved by the Ethical Committee of the Harokopio University and the volunteers gave their informed consent. The subjects visited the Department of Cardiology at Laiko Hospital once a month and the oils were provided to them. The subjects were weighed at each visit and were asked for their smoking and physical activity habits.

2.3. Diets

Dietary assessment based on a food frequency questionnaire was used to define the background diet of the subjects. Frequency of milk, bread, fruits, vegetables, legume, olive oil, fish and meat consumption was recorded together with a dietary 24 h recall. The subjects' dietary intake during the intervention period was checked by one 3-day dietary record per month. The recorded days comprised of 2 weekdays and 1 weekend day. The diet diaries were analyzed by the NUTRITIONIST v program (Version 2.1 First Data Bank Inc. USA). Supervision for smoking habit, body mass index, physical activity and dietary habits was checked by phone calls once a week and by monthly visits to the hospital.

2.4. Blood sampling and laboratory methods

The volunteers attended the Department of Cardiology at 'Laiko' Hospital twice for blood collection, at the beginning and the end of the experimental period. At each visit, and after a 12-h overnight fast, blood samples were collected at 08:00 h. Subjects laid supine for 10 min prior to the blood collection. Blood was collected into a glass tube without preservative (Vacutainer tube, Becton Dickinson) for serum lipids and inflammatory indices determination. All samples were collected without

occlusion. The Vacutainer tubes were kept on ice and were then centrifuged at 3000 rpm for 10 min at 4 °C within 2 h from blood collection. Serum samples were stored at −80 °C for further analysis.

Plasma total cholesterol, triglycerides, and high density lipoprotein (HDL) cholesterol levels were determined using enzymatic colorimetric assays on an ACE analyzer (Sciapparelli Biosystems Inc., USA). Total cholesterol and triglycerides determination included reactions of hydrolyzation and oxidation. Analysis of serum HDL cholesterol concentrations was carried out by a liquid stable reagent-immunoinhibition method. Low density lipoprotein (LDL) cholesterol was calculated by the Friedewald equation [15].

CRP was assayed by high sensitivity particle-enhanced immunonephelometry (N Latex, Date-Behring Marburg GmbH, Marburg, Germany) with a range from 0.175 to 1100 mg/l. SAA was also measured by particle-enhanced immunonephelometry (N Latex, Date-Behring Marburg GmbH) with a range from and 0.75 to 1000 mg/l. IL-6 was assayed by enzyme linked immunoassay (R & D Systems Europe Ltd, Abingdon, UK) with a range from 0.156 to 10 pg/ml. The intra-assay and inter-assay coefficient of variation for CRP and SAA was $<5\%$ and for IL-6 $<12\%$. All samples were assayed by a single operator blinded to the timing of sampling and type of dietary intervention. The measurements were performed at the Biochemistry Laboratory of General Hospital of Nikea.

2.5. Statistical analysis

The data on CRP, SAA and IL-6 which were not normally distributed were expressed as medians. Differences of inflammatory markers within and between groups were analysed by Wilcoxon signed rank test and Mann-Whitney *U*-test. Differences for the normally distributed variables were analysed with the Student *t*-test. Pearson's correlation coefficients were used to assess relationships between variables. Discontinuous variables were tested by a contingency x^2-test. A *P* value <0.05 was considered significant. The Stat View™ II statistical package was used.

3. Results

3.1. Baseline characteristics

A total number of 76 subjects completed the study. ALA and LA group were matched for age (50.4 ± 7.3 vs. 52 ± 7.7, $P = 0.33$) and body mass index (28.42 ± 3.44 vs. 28 ± 3.19, $P = 0.63$). The percentage of light smokers (< 10 cigarettes per day) was similar in the two groups (24 vs. 23%, $P = 0.85$). None of the participating subjects changed his smoking habits during the intervention period. The n-6:n-3 ratios were comparable in the background diets of the two groups (7.5:1 and 7:1) while after the supplementation the ratios changed to 1.3:1 and 13.2:1 in the ALA and LA groups, respectively. Despite supplementation the calories intake from fat in both groups was similar. In addition, no differences in nutrient intake between ALA and LA groups were observed, during the intervention period (Table 2).

Lipid profile and inflammatory markers at baseline in the two groups are shown in Table 3.

3.2. Serum lipids and inflammatory markers after dietary intervention

Regarding plasma lipid levels, in ALA group cholesterol levels remained unaltered whereas in LA group they were decreased ($P = 0.04$) (Table 3). On the other hand, in ALA group, HDL cholesterol levels were decreased ($P = 0.005$).

CRP, SAA and IL-6 levels showed a statistically significant reduction after administration of ALA (Table 3). The median decrease of CRP was 38%, of SAA 23.1% and of IL-6 10.5%. Inflammatory markers did not show any statistical difference after the administration of diet rich in LA (Table 3). In addition, we compared changes (Δ = levels prior-levels after intervention) in CRP, SAA and IL-6 in ALA group with changes in CRP, SAA and IL-6 in LA group. ΔCRP, ΔSAA and ΔIL-6 in the ALA group differed significantly from corresponding changes in the LA group (0.525 (−0.13–2.46) vs. 0.04 (−0.41–0.4), $P = 0.01$, 0.75 (−0.03–1.91) vs. 0.04 (−0.6–0.59), $P = 0.03$ and 0.19 (−0.04–1.46) vs. 0.04 (−1.05–0.48), $P = 0.04$, respectively).

The reduction of inflammatory markers in the ALA group was independent of lipid changes since there was no significant correlation between changes in inflammatory markers with those in lipids (Table 4). Changes in one inflammatory marker in the ALA group were correlated significantly with changes of each other inflammatory marker (ΔCRP vs. ΔSAA, $r = 0.62$, $P = 0.001$, ΔCRP vs. ΔIL-6, $r = 0.53$, $P = 0.0001$ and ΔSAA vs. ΔIL-6, $r = 0.62$, $P = 0.001$).

4. Discussion

Our study indicates that dyslipidaemic patients demonstrate a significant reduction in inflammatory indices when supplied with ALA but not with LA diet for 3 months.

We did not use exactly the same amounts of ALA and LA supplements in the ALA and LA groups, respectively. This occurred because we were aiming to lower the n-6:n-3 ratio at physiologically realistic levels. Our target was to decrease the n-6:n-3 ratio in the ALA group and to increase the n-6:n-3 ratio in the LA group.

Table 2
Mean daily intakes of energy and nutrients for dyslipidaemic subjects on ALA and LA enriched diets

Diet	ALA group (mean ± S.D.)	LA group (mean ± S.D.)	P value
Proteins %	15.1 ± 2.4	14.9 ± 2.2	0.564
Carbohydrates %	47.7 ± 3.8	47.8 ± 4.2	0.613
Fats %	35.9 ± 4.1	35.9 ± 4.4	0.477
Alcohol %	1.3 ± 0.4	1.4 ± 0.4	0.524
Energy (kcal)	2181.8 ± 243.1	2183.2 ± 237.6	0.638
Proteins (g)	82.4 ± 10.3	81.3 ± 11.5	0.356
Carbohydrates (g)	260.2 ± 20.6	260.9 ± 21.8	0.672
Fats (g)	87.0 ± 11.1	87.1 ± 10.7	0.521
Alcohol (g)	4.1 ± 1.1	4.4 ± 1.3	0.395
Cholesterol (mg)	216.4 ± 27.0	218.3 ± 30.6	0.449
Saturated fats (g)	22.8 ± 2.6	23.2 ± 2.8	0.518
Monounsaturated fats (g)	46.5 ± 4.3	46.6 ± 4.8	0.753
Polyunsaturated fats (g)	11.9 ± 1.2	12.0 ± 1.4	0.687
α-Linolenic acid (g)	0.9 ± 0.2	1.0 ± 0.2	0.524
Eicosapentaenoic acid (g)	0.3 ± 0.06	0.3 ± 0.08	0.373
Docosahexaenoic acid (g)	0.2 ± 0.05	0.2 ± 0.04	0.465
Vitamin C (mg)	120.1 ± 14.6	120.7 ± 15.3	0.316
Dietary fiber (g)	25.4 ± 3.0	25.2 ± 3.6	0.759

Therefore, with the amounts of ALA and LA that we chose we achieved a n-6:n-3 ratio in the ALA group of 1.3:1 and 13.2:1 in the LA group. In addition, these dietary supplementations that we used provided a similar intake of calories from fat in both groups. This is important since differences in total fat intake can modulate the inflammatory responses [16].

The protective effect of foods rich in ALA has been reported both in primary and secondary prevention of coronary artery disease. Ascherio et al. [7] found an inverse association between intake of ALA and risk of coronary artery disease. In the study of Hu et al. [8], the dietary intake of ALA was examined in relation to the risk of fatal ischaemic heart disease among participants in the Nurses' Health Study. According to the results of this study a higher intake of ALA was associated with a lower relative risk of fatal ischaemic heart disease. In the Lyon Diet Heart Study when a Mediterranean ALA rich diet was given in survivors of the first myocardial infarction there was significant reduction of recurrence of cardiac events and overall mortality [9].

Several mechanisms have been proposed to explain the protective effect of ALA [17,18]. Dog studies have shown that acute infusion of ALA can prevent ischaemia-induced ventricular fibrillation [19,20]. Other beneficial effects include an improvement in arterial compliance [21], modest blood pressure reduction [22] and antiplatelet effect [23]. Finally an anti-inflammatory effect has been proposed [24] which is supported by the results of our study.

Atherosclerosis is considered a chronic disorder characterized by low-grade vascular inflammation [1,2]. Inflammatory markers are found elevated in patients with chronic stable angina and acute coronary syndromes and their prognostic value for subsequent coronary events has also been reported [25–27].

In our study CRP, SAA and IL-6 showed a significant reduction after the 3-month dietary intervention with ALA. To our knowledge this is the first study to report these findings. The reduction was observed independently of lipid changes. The exact mechanism of suppression of inflammatory markers is unknown. Caughey et al. [28] reported that diet enriched in ALA inhibited the production of tumor necrosis factor-α and IL-1β in healthy volunteers. More data exist from diets enriched with marine n-3 PUFAs EPA and DHA. However, it has to be specified that ALA may not fully reproduce the effects of fish oils [17,29]. Dietary supplementation with fish oil in healthy volunteers for 6 weeks suppressed IL-2 production from peripheral mononuclear cells [30]. Kremer et al. [31] reported a reduction in macrophage IL-1 and neutrophil leukotriene B4 production when dietary fish oil was provided in patients with active rheumatoid arthritis. In another study [32], dietary supplementation with fish oil in healthy volunteers suppressed the capacity of mononuclear cells to synthesize IL-1β, IL-1α and tumor necrosis factor-α in vitro.

A possible mechanism for the decreased production of these cytokines is the alteration in the type of arachidonic acid metabolites. n-3 PUFAs induce changes in both cycloxygenase and lipoxygenase products such as reduction in production of prostaglandin E_2 and leukotriene B_4 [4]. Both metabolites enhance the release of IL-

Table 3
Lipid profile, inflammatory markers and body mass index (BMI) before and after the dietary intervention in the ALA and LA groups

Variables	Before	After	P value
ALA group (n = 50)			
Total cholesterol (mg/dl)	232 ± 34	226 ± 36	0.27
Triglycerides (mg/dl)	155 ± 84	163 ± 87	0.16
HDL cholesterol (mg/dl)	42.7 ± 10.4	41 ± 10.2	0.005
LDL cholesterol (mg/dl)	154 ± 37	152 ± 36	0.59
CRP (mg/l)	1.24 (0.93–3.7)	(0.56–1.8)	0.0008
SAA (mg/l)	3.24 (2.3–4.9)	1.7–3.9)	0.0001
IL-6 (pg/ml)	2.18 (1.35–3.9)	(1.3–2.8)	0.01
BMI (kg/m^2)	28.42 ± 3.44	28.40 ± 3.44	0.25
LA group (n = 26)			
Total cholesterol (mg/dl)	234 ± 46*	218 ± 46	0.04
Triglycerides (mg/dl)	173 ± 113*	156 ± 86	0.13
HDL cholesterol (mg/dl)	39.2 ± 7.4*	38.3 ± 8	0.25
LDL cholesterol (mg/dl)	154 ± 43*	149 ± 44	0.36
CRP (mg/l)	1.54 (0.625–3.1)*	(0.64–1.7)	0.35
SAA (mg/l)	3.52 (2.34–4.9)*	(2.15–4.4)	0.58
IL-6 (pg/ml)	1.77 (1.3–2.7)*	(1.1–2.2)	0.69
BMI (kg/m^2)	28 ± 3.19*	28 ± 3.22	0.8

Values of inflammatory markers are expressed as median and 25th and 75th percentile. CRP, C-reactive protein; HDL, high density lipoprotein; IL-6, interleukin-6; LDL, low density lipoprotein; SAA, serum amyloid A.

* P = NS (lipid, inflammatory markers and BMI of LA group vs. corresponding levels of lipid, inflammatory markers and BMI of ALA group).

Table 4
Correlation between changes in inflammatory markers and those in lipids in 50 subjects with dietary supplementation with ALA

		r	P value
ΔCRP vs.	Δcholesterol	−0.07	0.63
	Δtriglycerides	−0.25	0.09
	ΔLDL cholesterol	0.015	0.92
	ΔHDL cholesterol	−0.093	0.52
ΔSAA vs.	Δcholesterol	−0.068	0.64
	Δtriglycerides	−0.18	0.21
	ΔLDL cholesterol	0.014	0.93
	ΔHDL cholesterol	0.03	0.83
ΔIL-6 vs.	Δcholesterol	−0.28	0.06
	Δtriglycerides	−0.30	0.07
	ΔLDL cholesterol	−0.16	0.27
	ΔHDL cholesterol	−0.14	0.31

Δ values are values at baseline—values after dietary intervention. Abbreviations as in Table 3.

6 in vitro [33]. Conversely, a decrease in these eicosanoids could explain the reduction of IL-6. IL-6 is a pleiotropic cytokine [34], which controls CRP and SAA hepatic production [35,36]. Therefore, it is plausible to speculate that dietary supplementation with ALA primarily suppresses IL-6 release which secondary suppresses CRP and SAA production. The atherogenic potential of the inflammatory markers, such as CRP [37], favors our hypothesis that the anti-inflammatory effect of ALA may reflect a beneficial anti-atherogenic action.

Supplementation with LA decreased significantly total cholesterol levels. This finding is well documented and is known to be due to an increased synthesis of LDL receptors. However, supplementation with ALA did not change total cholesterol levels but it decreased HDL cholesterol levels. Other studies also suggest that there is no hypolipidaemic effect of ALA. In particular, in the MARGARIN Study, which recruited subjects with multiple cardiovascular risk factors, HDL cholesterol levels were lower in the ALA group than in the LA group [10]. This comes in accordance with another study which showed that a flaxseed oil/low fat diet providing 20 g of ALA daily for 1 month, resulted in the lowest HDL cholesterol levels, in comparison with the control diet (rich in saturated fatty acids) and a diet supplemented with canola oil (rich in oleic acid) [21]. However, in another study, the effects of an ALA-rich diet on LDL and HDL cholesterol levels did not differ from the effects of a LA-rich diet, in normolipidaemic men [14]. Therefore, the evidence regarding the cardioprotective properties of ALA, as far as the improvement of lipid profile is concerned, is not conclusive.

Few limitations of this study have to be addressed. First, we did not apply a cross-over design which is inherently much stronger than a parallel interventional study. We did it because a cross-over design has the problem of carryover effect as the wash-out period cannot be specified in lipid interventional trials. Counting an intermediate wash-out period of at least 2 months, the total study period should be prolonged to 8 months. This design could raise compliance problems, taking into consideration that compliance in Greek populations is relatively low. Second, the doses of n-3 and n-6 that we provided by the supplements in our study are difficult to be achieved by the usual dietary intake. However, products like spreads fortified with the appropriate fatty acids can be produced by relevant industries. These spreads can substitute other sources of fat in the diet of hyperlipidaemic patients.

In conclusion, dietary supplementation with ALA decreases inflammatory markers in dyslipidaemic patients. This anti-inflammatory effect may provide a possible mechanism for the beneficial effect of ALA in primary and secondary prevention of coronary artery disease. The attractiveness of this assumption is en-

forced by the growing evidence that inflammatory markers are pathophysiologically involved in atherogenesis.

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Abstract for Reference 23

Impact of walnut consumption on body composition

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Nuts are considered fattening by many people. Therefore, the aim of present study is to determine the long-term effect of walnut consumption on weight and body composition. Ninety one subjects (51 women, 40 men) with mean age 54.3 ± 10.6 (SD), mean weight 75.9 ± 14.3 kg and mean BMI 28.4 ± 3.4 kg/m^2 were randomly assigned to control and treatment groups. The treatment group received 12% of the total energy as walnuts (28-56 g/d) for six months. No additional dietary advice was given to either group. The changes in body composition were analyzed by bio-impedance analysis (Tanita ® TBF-300A). Body weight, BMI, percent fat and fat free mass were measured at months 0, 2, 4 and 6. Repeated-Measures Analysis of Variance was conducted to evaluate changes in body composition over time. The results for ANOVA indicated no significant diet-by-time interaction effect for body composition (weight Wilks' Lambda = 0.95, p=0.18; BMI Wilks' Lambda = 0.93, p = 0.09; percent fat Wilks' Lambda = 0.92, p=0.052; fat free mass Wilks' Lambda = 0.96, p=0.34). In conclusion, we did not observe a significant change (decrease/increase) in weight and body composition between two groups.
Support: California Walnut Commission

Abstract for Reference 24

Lipid-lowering Effects of Walnuts/Walnut Oil in Moderately Hypercholesterolemic Subjects

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Walnuts/walnut oil are rich sources of polyunsaturated fatty acids (PUFA) and n-3 fatty acid (n-3FA), primarily α-linolenic acid. The present study used Walnuts/walnut oil in experimental diets high in PUFA/n-3FA to assess their lipid-lowering effects in humans. Twelve hypercholesterolemic men (45-65 y) were fed 3 different diets in a random order: an average American diet (AAD, 35% fat, 13% SFA, 7% PUFA, n-6/n-3 ≈ 10:1), a diet high in n-6 PUFA (N-6 diet, 35% fat, 8% SFA, 12% PUFA, n-6/n-3 ≈ 9:1) and a diet high in n-3 PUFA (N-3 diet, 35% fat, 8% SFA, 12% PUFA, n-6/n-3 ≈ 4:1). In the N-3 and N-6 diets, half of total fat was replaced with walnuts/walnut oil (34 g walnuts and 5 teaspoons walnut oil based on 2000 kcal/d). Other dietary components were kept constant across the 3 diets. The N-3 and N-6 diets decreased serum TC by 11.2% and 10.6%, LDL-C by 13.3% and 12.2%, triglycerides by 15.0% and 15.1%, and apo B by 9.1% and 8.7%, respectively, compared with AAD ($P<0.05$ for all). HDL-C did not change significantly. In summary, both the N-3 and N-6 diets elicited comparable effects on serum lipids, suggesting that dietary PUFA, regardless of their n-3 or n-6 series, exert significant lipid-lowering effects (Funded by California Walnut Commission).

Abstract for Reference 25

A DIET ENRICHED WITH WALNUTS IMPROVES POSTPRANDIAL ENDOTHELIAL DYSFUNCTION IN MEN AND WOMEN WITH POLYGENIC HYPERCHOLESTEROLEMIA

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Objective. Regular nut intake appears to lower coronary heart disease risk by approximately 50% in epidemiological studies, an extent that is only partly explained by the cholesterol-lowering effect (≈ 10%) observed in feeding studies. We assessed the effects of a diet enriched with walnuts on endothelial function and serum lipids in men and women with polygenic hypercholesterolemia (HC).

Methodos. In a randomized crossover study, 10 men and 10 women with HC consumed during 4 weeks a healthy, Mediterranean-type diet and a diet of similar energy and fat content in which walnuts replaced ≈ 35% of the energy from monounsaturated fat. In the last week of each dietary period, endothelial function was evaluated in the brachial artery by ultrasound 4 h after a test meal containing olive oil or walnuts. Serum lipoproteins, LDL resistance to an in vitro oxidative stress, oxidized LDL, vitamin E, homocystein, folic acid, and soluble cellular adhesion and inflammation molecules were also measured.

Results. In comparison with the Mediterranean diet, the walnut diet improved flow-mediated dilatation in the brachial artery from 3.2% to 5.8% (P=0.043); reduced the serum VCAM level by 27 (95% CI, 5 to 50,) percent, P=0.045, and produced mean changes in total cholesterol and LDL cholesterol of -4.4% and -6.9 %, respectively. The mean differences (95% CI) in the changes in serum lipids between the two diets were: total cholesterol, -11.5 (-20.7 to - 2.3) mg/dL, P=0.017, and LDL cholesterol, -12.8 (-21.7 to -3.9) mg/dL, P=0.007. Lipid changes were similar in men and women. The resistance of LDL to oxidation and the serum levels of oxidized LDL, vitamin E, homocystein, folic acid, ICAM, and C-reactive protein were similar with the two dietary treatments.

Conclusions. Substituting walnuts for part of the monounsaturated fat in a cholesterol-lowering Mediterranean diet improves endothelial function in men and women with hypercholesterolemia. The beneficial vascular effect of walnuts may help explain the cardioprotective effect of habitual nut consumption beyond cholesterol-lowering