Dietary α-Linolenic Acid Reduces Inflammatory and Lipid Cardiovascular Risk Factors in Hypercholesterolemic Men and Women[1]

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ABSTRACT α-Linolenic acid (ALA) reduces cardiovascular disease (CVD) risk, possibly by favorably changing vascular inflammation and endothelial dysfunction. Inflammatory markers and lipids and lipoproteins were assessed in hypercholesterolemic subjects ($n = 23$) fed 2 diets low in saturated fat and cholesterol, and high in PUFA varying in ALA (ALA Diet) and linoleic acid (LA Diet) compared with an average American diet (AAD). The ALA Diet provided 17% energy from PUFA (10.5% LA; 6.5% ALA); the LA Diet provided 16.4% energy from PUFA (12.6% LA; 3.6% ALA); and the AAD provided 8.7% energy from PUFA (7.7% LA; 0.8% ALA). The ALA Diet decreased C-reactive protein (CRP, $P < 0.01$), whereas the LA Diet tended to decrease CRP ($P = 0.08$). Although the 2 high-PUFA diets similarly decreased intercellular cell adhesion molecule-1 vs. AAD (−19.1% by the ALA Diet, $P < 0.01$; −11.0% by the LA Diet, $P < 0.01$), the ALA Diet decreased vascular cell adhesion molecule-1 (VCAM-1, −15.6% vs. −3.1%, $P < 0.01$) and E-selectin (−14.6% vs. −8.1%, $P < 0.01$) more than the LA Diet. Changes in CRP and VCAM-1 were inversely associated with changes in serum eicosapentaenoic acid (EPA) ($r = -0.496$, $P = 0.016$; $r = -0.418$, $P = 0.047$), or EPA plus docosapentaenoic acid ($r = -0.409$, $P = 0.053$; $r = -0.357$, $P = 0.091$) after subjects consumed the ALA Diet. The 2 high-PUFA diets decreased serum total cholesterol, LDL cholesterol and triglycerides similarly ($P < 0.05$); the ALA Diet decreased HDL cholesterol and apolipoprotein AI compared with the AAD ($P < 0.05$). ALA appears to decrease CVD risk by inhibiting vascular inflammation and endothelial activation beyond its lipid-lowering effects. J. Nutr. 134: 2991–2997, 2004.

KEY WORDS: • *α-linolenic acid* • *PUFA* • *C-reactive protein* • *cell adhesion molecules* • *lipids and lipoproteins*

Activation of the vascular endothelium is an early event in the development of atherosclerosis, and a chronic inflammatory response is involved in atherogenesis (1–3). Recent evidence indicated that markers of inflammation are predictive of cardiovascular disease (CVD)[3] risk (3). For example, an elevated C-reactive protein (CRP) is strongly associated with clinical manifestations of atherothrombotic disease (2,4,5). CRP also exerts a direct proinflammatory effect on the human endothelium (6). Increased expression of the proinflammatory cytokines interleukin (IL)-6, IL-1 and tumor necrosis factor-α was demonstrated in atherosclerotic lesions, and both monocyte-derived macrophages and activated endothelium produce proinflammatory cytokines (7–9). These cytokines stimulate endothelial expression of cell adhesion molecules, including vascular cell adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), and E-selectin, which mediate monocyte attachment to the endothelium and transmigration into the subendothelial space.

Studies evaluated the direct antiatherogenic and anti-inflammatory effects of dietary (n-3) PUFA in addition to their lipid-lowering effects. Fish oil supplementation suppresses proinflammatory cytokine production by human peripheral blood mononuclear cells (PBMC) (10–12) and inhibits lymphocyte proliferation (12,13). Docosahexaenoic acid (DHA) results in a dose-dependent inhibition of VCAM-1 and E-selectin, and to a lesser extent, ICAM-1 gene expression in cultured endothelial cells (14,15). Thus, (n-3) fatty acids appear to attenuate inflammatory responses that are important in the initiation of atherosclerosis.

Evidence from both epidemiologic studies and clinical trials demonstrate substantial cardioprotective effects of α-linolenic acid (ALA) (16–21), despite modest or no changes in lipids and lipoproteins (17,21). Little is known about the effects of ALA on vascular inflammation and endothelial activation.

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[3] Abbreviations used: AA, arachidonic acid; AAD, average American diet; ALA, α-linolenic acid; apo, apolipoprotein; CRP, C-reactive protein; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; en, energy; EPA, eicosapentaenoic acid; HDL-C, HDL cholesterol; ICAM-1, intercellular cell adhesion molecule-1; IL, interleukin; LA, linoleic acid; LDL-C, LDL cholesterol; MUFA, monounsaturated fatty acids; PBMC, peripheral blood mononuclear cells; TC, total cholesterol; TG, triglycerides; VCAM-1, vascular cell adhesion molecule-1.

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TABLE 1

Characteristics of the study subjects at entry[1]

	Men	Women	All
n	20	3	23
Age, *y*	48.6 ± 1.6	58.3 ± 2.7	49.8 ± 1.6
Body weight, *kg*	88.5 ± 2.8	74.9 ± 8.3	86.7 ± 2.8
BMI, *kg/m*^{2}	28.0 ± 0.7	28.5 ± 2.4	28.1 ± 0.7
TC, *mmol/L*	5.74 ± 0.12	6.58 ± 0.28	5.85 ± 0.12
LDL-C, *mmol/L*	3.90 ± 0.11	4.53 ± 0.23	3.98 ± 0.11
HDL-C, *mmol/L*	1.12 ± 0.05	1.36 ± 0.11	1.16 ± 0.04
TG, *mmol/L*	1.55 ± 0.18	1.51 ± 0.29	1.54 ± 0.16

[1] Values are means ± SEM.

Several studies reported that ALA has anti-inflammatory effects; however, a recent in vitro study reported that linoleic acid (LA) and to a lesser extent ALA, stimulated the development of a proinflammatory environment within the vascular endothelium (22). Given conflicting results, we designed a controlled feeding study to evaluate the effects of ALA on multiple CVD risk factors including CRP, markers of endothelial activation, and lipids and lipoproteins. Because analysis of serum fatty acids provides an objective measure of the dietary intake of fatty acids (23–25), we incorporated serum fatty acid biomarkers as exposure variables related to the outcome variables.

SUBJECTS AND METHODS

Subjects. The study protocol was approved by the Institutional Review Board of Pennsylvania State University. Men ($n = 20$; 36–60 y) and women ($n = 3$; 55–65 y) who met the eligibility criteria [moderate hypercholesterolemia with serum total cholesterol (TC) between 5.17 and 6.21 mmol/L and LDL cholesterol (LDL-C) between the 40th and 90th percentile; overweight/obesity class I with BMI between 25 and 35 kg/m^2; not taking any lipid-lowering or anti-inflammatory medications and/or dietary supplements] participated in the study (**Table 1**). Subjects were nonsmokers, had no documented atherosclerotic disease, inflammatory disease, diabetes mellitus, uncontrolled hypertension (≥140/90 mm Hg), or other systemic diseases. The 3 women were postmenopausal and not receiving hormone replacement therapy.

Study design. A randomized, controlled, 3-diet, 3-period, crossover study design was employed. Subjects were assigned to a sequence of 3 test diets: an average American diet (AAD) that was the control; a diet high in PUFA and ALA (ALA Diet), and a diet high in PUFA and linoleic acid (LA Diet). Each diet period was 6 wk with a ≤3-wk break between diet periods to improve diet compliance. After each diet period, blood samples were taken on 2 consecutive days after a 12-h fast; 2-d means are reported.

Diets. The 3 experimental diets provided comparable amounts of total fat [35% energy (en)], carbohydrate (50% en), protein (15% en), and cholesterol (300 mg/d). Each diet was developed at 8 energy levels (ranging from 7524 to 16,302 kJ), and a 6-d cycle menu for each energy level was planned using the Nutritionist V database (First DataBank Division). All diets were nutritionally adequate; the target and assayed macronutrient composition of the diets is shown in **Table 2**. The AAD provided 13% en from SFA, 13% en monounsaturated fatty acids (MUFA) and 9% en from PUFA. In the 2 high-PUFA diets, SFA was partially replaced with PUFA resulting in diets that provided ~8% en from SFA and 16–17% en from PUFA; MUFA were held constant. The 2 high-PUFA diets provided varying amounts of LA and ALA: 12.6% en from LA and 3.6% en from ALA for the LA Diet, and 10.5% en from LA and 6.5% en from ALA for the ALA Diet. In the 2 high-PUFA diets, half of the total fat was derived from walnuts and walnut oil because they are rich sources of PUFA and, particularly, ALA (100 g of walnuts provides ~38 g of LA and 9 g of ALA; 100 g of walnut oil provides 53 g of LA and 10 g of ALA.). The daily consumption of walnuts and walnut oil was ~37 and 15 g, respectively, when the energy intake was kept at 10,032 kJ/d. In addition, flaxseed oil, which is especially high in ALA (55 g/100 g oil), was used to increase the ALA content of the ALA Diet. The ratios of LA to ALA [(n-6) to (n-3)] were ~10:1, 4:1, and 2:1, respectively, in the AAD, LA Diet, and ALA Diet.

Clinical and biochemical analyses. At end of each diet period, serum samples were taken from fasting subjects and stored at −70°C until the end of the study when all samples were analyzed at the same time.

Serum CRP and cell adhesion molecules. Serum CRP levels were measured using a high-sensitivity ELISA assay developed in the Cytokine Core Laboratory of the Penn State General Clinical Research Center. Briefly, a 96-well plate was preincubated with a primary antibody against human CRP (Calbiochem) in a humidified container at 4°C overnight. The plate was washed and blocked with PBS containing 1% bovine serum albumin. After washing, 50 μL of serum or standards was added to each well followed immediately by 50 μL of biotinylated CRP and mixed thoroughly. The plate was incubated overnight at 4°C. The final color reaction was achieved by adding 100 μL of streptavidin conjugated with horseradish peroxidase

TABLE 2

Target and assayed macronutrient compositions of the 3 experimental diets[1,2]

	AAD		LA Diet		ALA Diet	
Nutrient	Calculated	Assayed	Calculated	Assayed	Calculated	Assayed
CHO,[3] *% en*	50	49.8 ± 0.8	50	46.8 ± 0.7	50	46.3 ± 0.6
Protein, *% en*	15	15.7 ± 0.3	15	16.1 ± 0.3	15	16.1 ± 0.3
Total fat, *% en*	35.9	34.5 ± 0.9^a	35.7	37.1 ± 0.6^b	35.2	37.6 ± 0.4^b
SFA	13.0	12.7 ± 0.3^b	8.1	8.5 ± 0.1^a	7.9	8.2 ± 0.1^a
MUFA	12.6	13.2 ± 0.2	12.6	12.2 ± 0.1	12.5	12.3 ± 0.2
PUFA	7.6	8.7 ± 0.4^a	12.3	16.4 ± 0.2^b	13.0	17.2 ± 0.2^b
LA	6.6	7.7 ± 0.4^a	9.4	12.6 ± 0.1^c	7.6	10.5 ± 0.1^b
ALA	0.8	0.8 ± 0.1^a	2.7	3.6 ± 0.04^b	5.2	6.5 ± 0.1^c
LA:ALA (n-6):(n-3)	9	9.5 ± 0.5^c	3.5	3.5 ± 0.04^b	1.5	1.6 ± 0.02^a
Cholesterol, *mg/d*	311	—	304	—	305	—

[1] Based on an intake of 10,032 kJ.
[2] For the calculated composition, values are means, $n = 6$. Means were calculated using Nutritionist V. For the assayed data, values are means ± SEM, $n = 6$; statistical analyses were performed using assayed data only. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[3] CHO, carbohydrate.

(Pierce) to each well (incubated for 30 min), followed by the addition of 100 μL of substrate solution [2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid dissolved in 0.1 mol/L monohydrate citric acid at 0.03% (wt:v) (pH 4.35), Sigma] and incubated for another 1.5 h. The absorbent units were measured at a wavelength of 405 nm.

Serum ICAM-1, VCAM-1, and E-selectin were measured using quantitative sandwich enzyme immunoassay kits (R&D Systems) following protocols provided by the manufacturer. Murine monoclonal antibodies against human ICAM-1, VCAM-1, and E-selectin were precoated onto microplates. Diluted conjugates (100 μL; antibodies against recombinant human ICAM-1, VCAM-1, and E-selectin conjugated to horseradish peroxidase) were added to each well. Then, 100 μL of standards (recombinant human ICAM-1, VCAM-1, and E-selectin), controls (lyophilized human serum containing natural and recombinant human cell adhesion molecules), or diluted serum samples were added to each well. The plates were sealed and incubated at room temperature for 1.5 h. After washing (6 times) to remove any unbound substances and/or antibody-enzyme reagent, 100 μL of a stabilized substrate solution (tetramethylbenzidine) was added to each well and the plates were sealed and incubated at room temperature for 30 min. Color development was stopped by adding 100 μL of stop solution to each well. The optical density of each well was determined at a wavelength of 450 nm with the wavelength corrected at 620 nm. The minimal detectable levels of ICAM, VCAM-1, and E-selectin were 0.35, 2, and 0.1 μg/L, respectively.

Lipids, lipoproteins and apolipoproteins. Assays for serum TC, HDL cholesterol (HDL-C) and triglycerides (TG) were conducted at the Mary Imogene Bassett Research Institute, using an enzymatic method as described by Yu-Poth et al. (26). LDL-C levels were calculated by Friedewald's equation: LDL-C = TC − (HDL-C + TG/5) (27). Apolipoprotein (apo) AI and apo B were determined by rate immunonephelometry on a Beckman Array (Beckman Instruments).

Serum fatty acid profile. Serum fatty acid composition was determined using GC. Serum total lipids were extracted using a chloroform:methanol mixture (1:1, v:v) containing BHT (Sigma) and heptadecanoic acid (used as an internal standard, Nu-Chek-Prep). FAME were separated on a SP-2330 capillary column (30 m × 0.25 mm with 0.2 μm film, Supelco) on a Hewlett-Packard 5890 II gas chromatograph equipped with a flame-ionization detector. The column was programmed to set the initial temperature at 150°C for 8 min, then raise the temperature from 150 to 190°C at 2°C/min with the final temperature held at 190°C for 20 min. Helium was used as a carrier and make-up gas at a flow rate of 40 mL/min and a split ratio of 1:100. The temperatures for injector and detector were set at 250 and 265°C, respectively. Peak areas were integrated as relative weight using Hewlett-Packard ChemStation software. The percentage of individual fatty acids was calculated according to the peak areas relative to the total area (total fatty acid was set at 100%).

Statistical analyses. Statistical analyses were performed using SAS v8.2 (SAS Institute). ANOVA was used to test for difference among the 3 experimental diets. For all other outcome variables, data from men and women were pooled because the women were postmenopausal. Results are expressed as means ± SEM unless otherwise noted. For variables with nonnormal distributions (i.e., CRP), medians and geometric means ± SEM are reported, and statistical analyses were conducted after a logarithmic (base 10) transformation. The mixed procedure (PROC MIXED) was used to test for effects of diet, order (the sequence of the 3 diets given to each subject), and their interactive effects on outcome variables. Significant diet effects were examined with Tukey's least significant difference test. Pearson correlation analyses were conducted to test associations between lipid/lipoprotein variables and novel CVD risk factors. Probability values ≤ 0.05 were considered significantly different; a P-value ≤ 0.1 denoted a trend. For all outcome variables reported in the Results, none of the order effects or the interactions of diets with orders were significant; therefore, only diet effects are presented.

RESULTS

Fatty acid composition of serum lipids. Over the course of the dietary interventions, changes in serum fatty acid profiles reflected the fatty acid composition of the 3 test diets **(Fig. 1)**, indicating the subjects' compliance with the test diets. As expected, relative to the AAD, serum total (n-6) PUFA (and LA) was higher after subjects consumed the LA Diet ($P < 0.05$), and serum total (n-3) PUFA [i.e., ALA, eicosapentaenoic acid (EPA), and docosapentaenoic acid (DPA), but not DHA], was higher after subjects consumed both the LA and ALA Diets ($P < 0.05$, **Fig. 2**). Serum total (n-6) PUFA was lower [both in LA and arachidonic acid (AA)] and serum total (n-3) PUFA was higher (in ALA, EPA, and DPA) when subjects consumed the ALA Diet compared with the LA Diet (Fig. 2). Serum ratios of LA:ALA and (n-6):(n-3) were lower when subjects consumed the LA and ALA Diets vs. the AAD ($P < 0.05$ for both, Fig. 2).

Serum CRP concentrations. One subject had an abnormally high CRP level (12.4 mg/L) on the AAD because of a severe cold. Therefore, this data point was eliminated for the data analyses. The medians of serum CRP were 1.51, 0.83, and 0.37 mg/L, respectively, after subjects consumed the AAD, LA Diet, and ALA Diet. CRP levels decreased ~75% when subjects consumed the ALA Diet ($P < 0.01$, **Fig. 3**), and 45% when subjects consumed the LA Diet ($P = 0.08$) compared

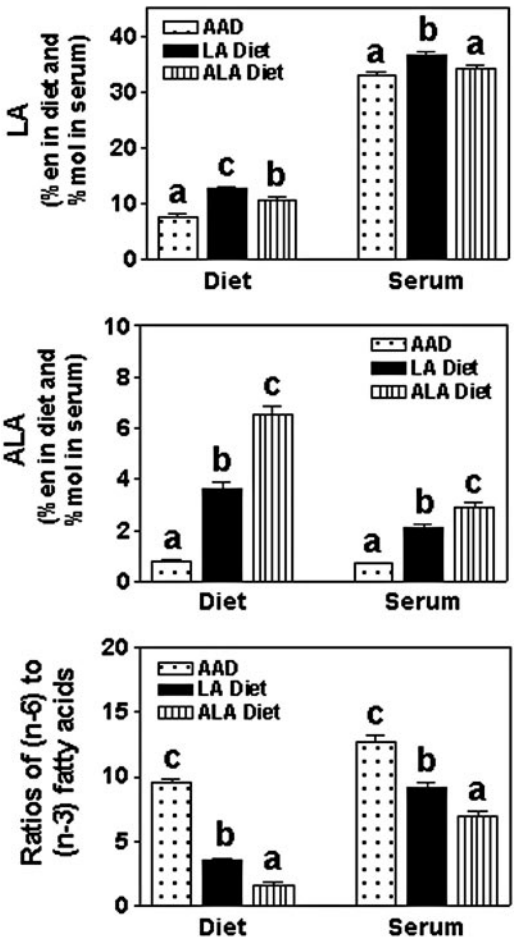

FIGURE 1 Increases in LA and ALA levels and decreases in (n-6):(n-3) ratios in the LA and ALA Diets and in sera of the subjects when they consumed the LA and ALA Diets vs. the AAD. LA and ALA are expressed as % en from total fat in the diets, and are expressed as % mol from serum total lipids (set as 100%). Values are means ± SEM, $n = 6$ in the diets and $n = 23$ in the sera. Means for a variable without a common letter differ, $P < 0.05$.

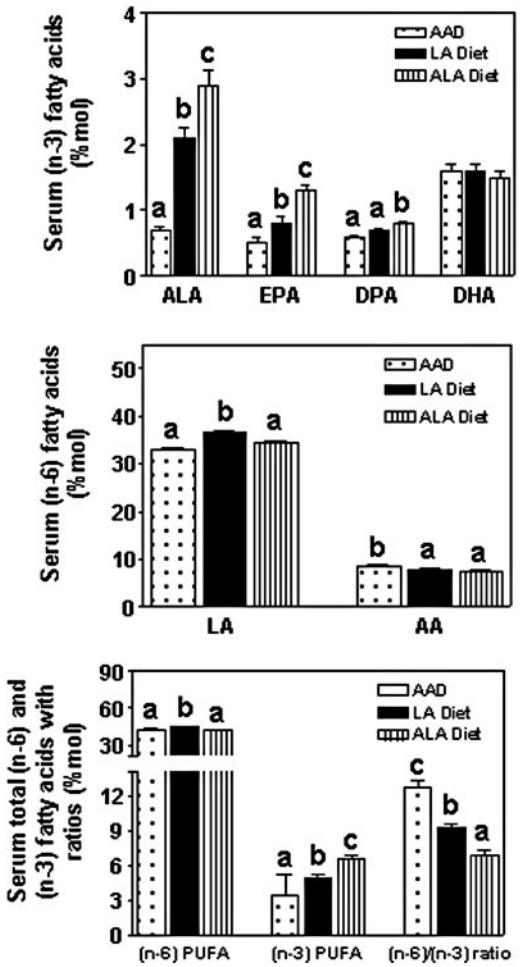

FIGURE 2 Effects of the 3 experimental diets on serum (n-3) and (n-6) fatty acid profiles, serum total (n-6) PUFA, (n-3) PUFA, and (n-6):(n-3) ratios in subjects with hypercholesterolemia. Values are means ± SEM, $n = 23$. Means for a variable without a common letter differ, $P < 0.05$.

with the AAD. However, CRP levels did not differ after subjects consumed the LA and ALA Diets.

When subjects consumed the ALA Diet, significant positive correlations between CRP and TG ($r = 0.504$, $P < 0.05$) and between CRP and TC:HDL-C ($r = 0.482$, $P < 0.05$) were observed, suggesting that lower CRP levels were associated with lower TG levels and TC:HDL-C ratios when subjects consumed the ALA Diet; these relations were not observed when subjects consumed the other 2 diets. In contrast, CRP and HDL-C were inversely correlated when subjects consumed the LA Diet and AAD ($r = -0.360$ and $r = -0.344$, $P < 0.05$ for both). In addition, when subjects were divided into low and high CRP groups based on their CRP levels when they consumed the AAD (i.e., CRP < 2 mg/L vs. CRP ≥ 2 mg/L), subjects with lower CRP levels had a greater reduction in LDL-C levels after they consumed the 2 high-PUFA diets (vs. AAD) than those with higher CRP levels (−0.51 ± 0.06 vs. −0.36 ± 0.06 mmol/L, $P = 0.068$). However, other lipid variables as well as the serum fatty acid profile did not differ between the 2 groups, i.e., those with high vs. low CRP levels.

Serum cell adhesion molecule concentrations. For cell adhesion molecules, individual responses to the 3 experimental diets appear in **Figure 4**. The 2 high-PUFA Diets significantly decreased serum ICAM-1 and E-selectin compared with the AAD ($P < 0.01$; **Fig. 5**). The ALA Diet also reduced VCAM-1 levels compared with the AAD ($P < 0.01$); however, VCAM-1 levels did not differ after subjects consumed the LA Diet and the AAD. In addition, the ALA Diet resulted in greater decreases in both VCAM-1 and E-selectin levels compared with the LA Diet [decreased by 12.9% ($P < 0.01$) and 7.2% ($P < 0.01$), respectively].

When subjects consumed the ALA Diet, significant positive correlations were observed between ICAM-1 and TC:HDL-C ($r = 0.444$, $P < 0.05$), and between ICAM-1 and LDL-C:HDL-C ($r = 0.464$, $P < 0.05$). This suggests that dietary ALA decreases serum ICAM-1and lipid ratios in a parallel manner.

Changes in serum CRP and VCAM-1 predicted by changes in serum (n-3) fatty acids. After subjects consumed the ALA Diet, changes in serum CRP and VCAM-1 were inversely associated with changes in serum EPA (vs. AAD, $r = -0.496$, $P = 0.016$ and $r = -0.418$, $P = 0.047$, respectively, **Table 3**). A trend for inverse correlations also existed between changes in CRP and VCAM-1 and changes in serum EPA+DPA, and between changes in CRP and changes in serum ALA+EPA+DPA ($P < 0.1$ for all, Table 3). In addition, when subjects consumed the LA Diet, similar inverse correlations were observed between changes in CRP and changes in serum EPA ($r = -0.353$, $P = 0.099$) and EPA+DPA ($r = -0.386$, $P = 0.069$). However, changes in ICAM-1 and E-selectin were not significantly associated with changes in any of the serum (n-3) fatty acids. Taken together, these data suggest that serum EPA appears to play a role in regulating CRP and VCAM-1 levels.

Serum lipid and lipoprotein concentrations. Compared with the AAD, the lipid-lowering effects of the 2 high-PUFA diets were comparable. Serum TC, LDL-C, TG, and apo B levels were 10.9, 12.3, 18.4, and 9.4% lower, respectively, when subjects consumed the LA Diet, and were 10.8, 11.0, 18.4, and 9.7% lower, respectively, when they consumed the ALA Diet compared with the AAD ($P < 0.05$ for all, **Table 4**). Although the ALA Diet significantly decreased HDL-C (P

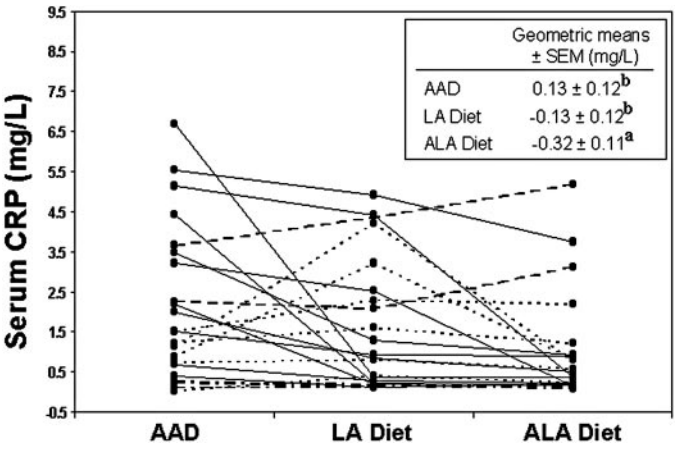

FIGURE 3 Effects of the 3 experimental diets on serum CRP levels in subjects with hypercholesterolemia. Solid lines represent subjects who responded with decreases in CRP relative to AAD; dashed lines for those subjects responding with decreases then increases in CRP; dotted lines for those subjects responding with increases then decreases in CRP; dash-dotted lines for those subjects who did not respond to the changes in diets. Values are geometric means ± SEM, $n = 22$ when subjects consumed the AAD, and $n = 23$ when subjects consumed the LA and ALA Diets. Means for a variable without a common letter differ, $P < 0.01$.

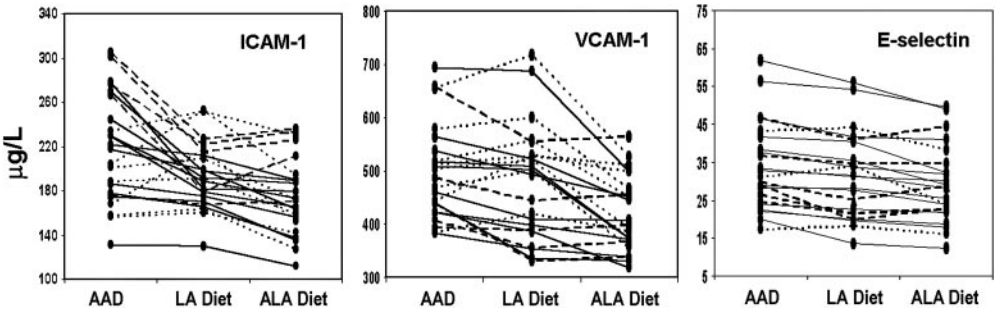

FIGURE 4 Effects of the 3 experimental diets on serum cell adhesion molecules in subjects with hypercholesterolemia. Solid lines represent subjects who responded with decreases in ICAM-1, VCAM-1, and E-selectin relative to AAD; dashed lines for those subjects responding with decreases then increases in ICAM-1, VCAM-1, and E-selectin; dotted lines for those subjects responding with increases then decreases in ICAM-1, VCAM-1, and E-selectin.

< 0.05) and apo AI ($P < 0.05$) compared with the AAD, there were no differences in HDL-C and Apo AI levels when subjects consumed the LA and ALA Diets, and the 2 diets reduced TC:HDL-C ratios similarly.

DISCUSSION

Our results demonstrate that a diet high in ALA beneficially affects multiple CVD risk factors, an important finding that was associated with significantly higher serum levels of ALA, EPA and DPA when subjects consumed the LA and ALA diets compared with the AAD. That these fatty acids were higher when subjects consumed the ALA Diet compared with the LA Diet is suggestive of a dose-response relation between dietary (n-3) fatty acids and their beneficial effects on CVD risk factors. Because ALA is a precursor for long-chain fatty acid synthesis, the increase in serum EPA and DPA may result in part from a conversion of ALA to these fatty acids. However, ALA enrichment may not suffice for DHA because of the very limited conversion rate of ALA to DHA (28,29). We propose that changes in serum ALA, EPA, and DPA are important for the biological effects observed.

Walnut supplementation, as a way of enriching serum lipids with LA and ALA, was shown to have a favorable effect on plasma lipids and lipoproteins (30–34). Our results are similar and demonstrate that a diet low in saturated fat and cholesterol, and high in PUFA, regardless of (n-3) or (n-6) series, significantly decreases serum lipid and lipoprotein levels. Higher levels of (n-3) fatty acids did not elicit more favorable effects on lipid risk factors. In addition, the diet high in PUFA and ALA decreased serum HDL-C and apo AI levels, an effect that was reported in some studies (17,33,35,36), but not in others (31,32,34). Nevertheless, the 2 high-PUFA diets in the present study significantly decreased TC:HDL-C ratios.

CRP and proinflammatory cytokines play an important role in atherogenesis (2,5). Increased CRP levels exhibit synergy with concurrent hypercholesterolemia to increase CVD risk in both men and women (37,38). Several studies reported that CRP is inversely associated with EPA and DHA in both healthy subjects and in patients with stable coronary artery disease (39,40). Consistent with this finding, our results demonstrate that a diet high in PUFA and ALA significantly decreased CRP levels, and the changes in serum EPA and EPA+DPA were inversely associated with changes in CRP. However, the magnitude of this response was variable among subjects; it will be important to determine whether there is a genetic basis for different CRP responses to diet because recent studies showed that CRP gene polymorphism influences CRP levels (41,42). Erlinger et al. (43) reported that increased CRP levels are associated with smaller decreases in TC and LDL-C in response to a reduced-fat/low-cholesterol diet. The present study also demonstrated that subjects with higher CRP levels had a diminished cholesterol-lowering response to the 2 high-PUFA diets that was reduced by 29% compared with subjects with lower CRP levels. Therefore, decreasing CRP levels by dietary and/or other interventions can improve the lipid responses, thereby reducing overall CVD risk.

Endothelial activation and enhanced expression of cell adhesion molecules are early events in atherogenesis (44). Several studies showed that DHA or oleic acid decreases the expression of cell adhesion molecules in the endothelium (14,15,45) and in PBMC (46), thereby reducing adhesion of monocytoid cells to the endothelium. Consistent with these findings, we found that ICAM-1 and E-selectin decreased after subjects consumed the 2 high-PUFA diets. Moreover, markedly favorable effects on VCAM-1 and E-selectin were elicited only by a diet high in ALA. These results suggest that diets

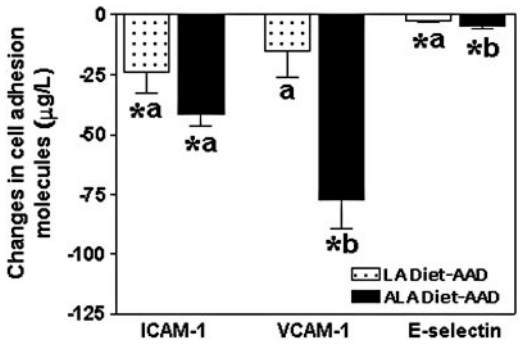

FIGURE 5 Decreases in serum cell adhesion molecules in subjects when they consumed the LA and ALA Diets vs. the AAD. Values are means ± SEM, $n = 23$. *Changes for a variable significantly differ from zero, $P < 0.01$. Means for a variable without a common letter differ, $P < 0.01$.

TABLE 3

Correlation coefficients among the changes in serum (n-3) fatty acids and the changes in serum CRP and VCAM-1 when subjects consumed the ALA Diet compared with the AAD

	ΔCRP		ΔVCAM-1	
	r	*P*-value	*r*	*P*-value
ΔALA	−0.320	0.136	−0.348	0.104
ΔEPA	−0.496	0.016	−0.418	0.047
ΔDPA	−0.145	0.510	−0.099	0.654
ΔDHA	−0.069	0.762	−0.202	0.366
ΔEPA + DPA	−0.409	0.053	−0.357	0.091
ΔALA + EPA + DPA	−0.386	0.069	−0.311	0.149

TABLE 4

Serum lipid, lipoprotein, and apolipoprotein concentrations in subjects when they consumed the AAD, LA Diet, and ALA Diet for 6 wk[1]

	AAD	LA Diet	ALA Diet
TC, *mmol/L*	5.59 ± 0.16[b]	4.98 ± 0.13[a]	4.99 ± 0.14[a]
LDL-C, *mmol/L*	3.74 ± 0.14[b]	3.28 ± 0.12[a]	3.33 ± 0.11[a]
HDL-C, *mmol/L*	1.18 ± 0.06[b]	1.15 ± 0.06[ab]	1.11 ± 0.05[a]
TG, *mmol/L*	1.47 ± 0.13[b]	1.20 ± 0.11[a]	1.20 ± 0.11[a]
TC:HDL-C	4.90 ± 0.18[b]	4.52 ± 0.18[a]	4.65 ± 0.19[a]
Apo AI, *g/L*	1.51 ± 0.04[b]	1.45 ± 0.05[ab]	1.43 ± 0.04[a]
Apo B, *g/L*	1.11 ± 0.03[b]	1.01 ± 0.03[a]	1.01 ± 0.03[a]

[1] Values are means ± SEM, $n = 23$. Means in a row with superscripts without a common letter differ, $P < 0.05$.

high in PUFA and (n-3) fatty acids exert their cardioprotective effects in part via effects on endothelial function, and that a higher dose of ALA may have the largest beneficial effects on the markers of endothelial activation. Interestingly, we found that the changes in VCAM-1 could be predicted by the changes in serum EPA and DPA, suggesting that these are bioactive components that inhibit vascular endothelial activation. Although in vitro cell culture studies demonstrated that DHA decreases cell adhesion molecule expression in the endothelium (14,15), the present study did not replicate this effect, perhaps because we observed no significant changes in serum DHA after subjects consumed the 3 experimental diets. This may be explained by the low efficiency of ALA conversion to DHA (28,29).

Ridker et al. (37,38) showed that CRP elicits an additive value to lipid testing in assessing risk for future CVD events. Other studies reported that changes in CRP were inversely correlated with changes in HDL-C and apo AI (47), and positively correlated with serum TG (48). In addition, ICAM-1, but not VCAM-1 or E-selectin, was significantly associated with serum TC (49). In general, arterial wall lipids are susceptible to oxidation, and oxidized LDL can activate leukocytes (including monocytes and macrophages) and endothelial cells, inducing a chronic inflammatory response (50,51). On the other hand, an increase in cholesterol reverse transport by HDL (i.e., increased HDL-C) would be expected to decrease lipid accumulation in the artery wall, thereby decreasing the vascular inflammatory response. This may explain the associations between lipid risk factors and novel inflammatory markers. In the present study, we did observe an inverse association between CRP and HDL-C when subjects consumed the LA Diet and AAD. In contrast, we found both CRP and HDL-C levels decreased when subjects consumed the ALA Diet. The underlying mechanisms require further study. Nevertheless, the findings that CRP and ICAM-1 are positively correlated with TC:HDL-C ratios in the present study are of great importance given that the TC:HDL-C ratio is a significant predictor of CVD risk (37,38). Because the diet high in ALA beneficially affected both lipids/lipoproteins and CRP/cell adhesion molecules, this suggests that ALA acts via multiple mechanisms to reduce CVD risk.

On the basis of in vitro data, there has been some concern that (n-6) fatty acids, primarily LA, stimulate a proinflammatory environment within the vascular endothelium (22), promoting endothelial dysfunction. However, the present study demonstrated that a diet high in LA decreased ICAM-1 and E-selectin levels, and tended to decrease CRP. Of note is that ALA also increased in this diet, resulting in lower LA:ALA or (n-6):(n-3) ratios. Given that (n-3) fatty acids exert anti-inflammatory responses, the addition of (n-3) fatty acids to a diet rich in (n-6) fatty acids might counteract the potentially adverse effect of LA on endothelial function.

In conclusion, a diet high in PUFA, especially ALA, elicits cardioprotective effects by decreasing lipid and lipoprotein levels and by eliciting vascular anti-inflammatory effects. The fact that ALA has marked, beneficial effects on multiple CVD risk factors further underscores its potentially important role in CVD risk reduction. These findings provide additional support for the importance of ensuring adequate PUFA and ALA intake as a strategy to markedly lower CVD risk.

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