Effects of Linoleate-Enriched and Oleate-Enriched Diets in Combination With \( \alpha \)-Tocopherol on the Susceptibility of LDL and LDL Subfractions to Oxidative Modification in Humans

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Abstract This report describes the effects of feeding linoleate- or oleate-enriched diets to subjects who were concurrently taking 1200 mg/d of \( \alpha \)-tocopherol on the susceptibility of low-density lipoprotein (LDL) and buoyant and dense LDL subfractions to oxidation. LDL isolated from subjects who consumed linoleate-enriched diets was more susceptible to copper-mediated oxidation, as measured by formation of conjugated dienes and lipid peroxides and loss of unsaturated fatty acids, compared with LDL isolated from subjects who consumed their usual or oleate-enriched diets. In all subjects, buoyant LDL had a higher content of \( \alpha \)-tocopherol per particle and a lower 18:2 to 18:1 ratio and was considerably more resistant to oxidation than dense LDL. Although dense LDL from all groups had comparable \( \alpha \)-tocopherol levels, dense LDL from the linoleate group was most susceptible to oxidation, followed by that from the standard diet, whereas dense LDL isolated from the oleate diet group was most resistant. In summary, high dosages of \( \alpha \)-tocopherol did not prevent enhanced susceptibility to oxidation of LDL isolated from subjects fed linoleate-enriched diets. Furthermore, dense LDL was more susceptible to oxidation than was buoyant LDL, and this effect was greatly exaggerated in the dense LDL isolated from subjects fed linoleate-enriched diets. Conversely, dense LDL isolated from subjects fed oleate-enriched diets was the most protected. If oxidation of LDL is important in the pathogenesis of atherosclerosis, then these data suggest that in people with increased amounts of small, dense LDL, dietary enrichment in oleic acid may decrease the susceptibility of their LDL to oxidation. (Arterioscler Thromb. 1994;14:557-566.)

Key Words • atherosclerosis • conjugated dienes • lipid oxidation • dense LDL • LDL subclasses • vitamin E • fatty acids

There is evidence that oxidative modification of low-density lipoproteins (LDLs) occurs in vivo\(^1\)\(^-\)\(^4\) and may increase LDL atherogenicity, as recently reviewed.\(^5\)\(^-\)\(^6\) During the generation of oxidatively modified LDL, polyunsaturated fatty acids undergo peroxidative decomposition, yielding reactive aldehydes, some of which form covalent bonds with LDL apoprotein B.\(^7\)\(^-\)\(^8\) In addition, direct adduct formation between LDL lipids and protein may occur.\(^9\) These modifications generate a particle that is recognized by the scavenger receptor(s) of the macrophage\(^10\)\(^-\)\(^11\) and lead to enhanced uptake and foam cell formation. Oxidatively modified LDL or soluble LDL oxidation products have other effects that promote atherogenesis, including monocyte chemotaxis,\(^12\) cytotoxicity,\(^13\) alteration of gene expression in arterial cells,\(^14\)\(^-\)\(^15\) and alteration of vascular tone and responsiveness (reviewed in Reference 6).

A number of factors can influence the susceptibility of LDL to oxidation. An early step in the oxidation of LDL is peroxidation of polyunsaturated fatty acids. Since linoleic acid (18:2) constitutes nearly 90% of the polyunsaturated fatty acids in LDL, it is the major substrate of LDL oxidation. Diets enriched in linoleate increase the content of linoleic fatty acid in plasma LDL and increase its susceptibility to oxidation.\(^16\)\(^-\)\(^18\) In contrast, diets enriched in oleate (18:1) and depleted of linoleate generate LDL particles that are more resistant to oxidation. A second important determinant of LDL susceptibility to oxidation is the content of endogenous antioxidants. Esterbauer et al\(^20\) have suggested that after the consumption of antioxidants in LDL, the rapid propagation phase of oxidation commences.\(^20\) Vitamin E, a potent free-radical scavenger and the most abundant antioxidant in LDL, may therefore provide important antioxidant protection to LDL. In support of this concept are studies showing that enrichment with vitamin E, both in vitro and in vivo, significantly reduces LDL susceptibility to oxidation.\(^21\)\(^-\)\(^28\) In addition to compositional features, particle size and density have been shown to influence the susceptibility of LDL to oxidation.

Several investigators have demonstrated that smaller, more dense particles are more susceptible to oxidation than are larger, more buoyant LDL.\(^20\)\(^-\)\(^29\) In studies that directly compared LDL subfractions, the susceptibility to oxidation progressively increased as LDL particle density increased.\(^26\)\(^-\)\(^28\) The goal of the current study was...
to determine the effect of diet-induced changes in LDL fatty acid composition, in combination with α-tocopherol supplementation, on the susceptibility of LDL to oxidation. Of particular interest was whether the effects of these interventions were different between buoyant and dense LDL subfractions.

Methods

Participants

Eighteen healthy volunteers (9 women and 9 men) aged 22 to 61 years were recruited from the local community. Each subject was given Δ-α-tocopherol (Hoffmann-La Roche, Inc) supplementation at 1200 mg/d (approximately 100 times the recommended daily allowance) for at least 3 months before randomization to diet groups. All subjects had been consuming average American diets, and none were taking lipid-lowering medications or other antioxidants. The study was approved by the Human Studies Committee of the University of California, San Diego, and was conducted in the outpatient facilities of the University of California, San Diego, General Clinical Research Center (GCRC).

Study Design

Subjects were randomized either to receive linoleate- (n=6) or oleate- (n=6) enriched diets or to continue with their usual (standard) diets for 6 weeks (n=6). Throughout the study, subjects and investigators remained blinded to the predominant type of fatty acid in the diets. Participants picked up their fully prepared diets on Monday, Wednesday, and Friday of each week. Unused portions of the diet were returned at each visit. Participants were allowed to ingest their daily diet according to their own schedule, although the entire day’s allocation was to be ingested by bedtime. Subjects were instructed to refrigerate the perishable foods and the liquid portions of the diet at all times when not in use. Daily dietary records were reviewed weekly by a registered dietitian to ensure adherence and to monitor acceptability.

Diet Preparation

An oleate-enriched variant of sunflower oil (Trisun 80), provided by SVO Enterprises, and conventional sunflower oil (Wesson Sunlite) were incorporated into various foods and liquid formulas. Oleic acid accounts for more than 80% of the total fatty acids in Trisun 80, whereas in Wesson Sunlite, approximately 60% of the total fatty acids are linoleic acid. Total α-tocopherol levels in the Trisun 80 oil (0.54 mg/g) and Wesson Sunlite oil (0.51 mg/g) were similar, and carotene contents were undetectable. No other antioxidants were measured. Composition of the liquid formulas, which provided approximately 40% to 45% of total calories, was similar to that previously described except that milk protein was replaced with soy protein. Baked foods were prepared by the American Institute of Baking on the basis of recipes formulated by the GCRC Nutrition Unit. Additional foods (tuna, chicken, pasta, salad, fruits; granola; etc) and all liquid portions were provided by the GCRC Nutrition Unit. Food samples were analyzed for fat composition, and aliquots of liquid diets were analyzed weekly to ensure stability of fatty acid composition (see “Appendix”).

On the basis of food preferences, a 7-day cycle of meals was selected from a list of available food items and prepared individually for each subject by the GCRC Nutrition Unit. Initial daily caloric intake for each individual was calculated from estimates of energy requirements based on 3-day food records, activity levels, and estimated basal metabolic rates for each individual. The diets provided approximately 40% of calories as fat, 45% as carbohydrates, and 15% as protein. The prepared diets varied essentially only in the content of linoleic or oleic fatty acids. Total daily energy intake for each individual was adjusted weekly as needed to maintain body weight.

Preparation of LDL

Fasting blood samples were obtained from each subject at baseline and after 4 and 6 weeks of the diet period. Plasma was separated from blood that had been collected in EDTA (4.0 mmol/L) containing tubes and placed immediately on ice. A final concentration of 0.22 mmol/L gentamicin, 0.15 mmol/L chloramphenicol, 1 μmol/L D-phenylalanine-L-prolyl-L-arginine chloromethyl ketone, and 2 mmol/L benzamidine was added to all plasma samples. LDL (d=1.019 to 1.063 g/mL) was isolated by sequential ultracentrifugation as previously described and dialyzed extensively against phosphate-buffered saline containing 0.27 mmol/L EDTA (PBS-EDTA). Plasma was also divided into two equal aliquots and separated into buoyant (d=1.026 to 1.032 g/mL) and dense (d=1.040 to 1.054 g/mL) fractions by performing two sequential ultracentrifugation steps with each portion as follows. Aliquots were brought to the appropriate density by addition of NaBr and were centrifuged in 6-mL polycarbonate tubes in a Beckman 40.3 fixed-angle rotor at 40 000 rpm for 20 hours at 10°C. Buoyant and dense LDL isolated by this methodology corresponded to LDL subfractions LDL-I and LDL-III, respectively. Lipoprotein Vitamin E Content

α-Tocopherol was measured by high-performance liquid chromatography according to the method of Arnaud et al. The formation of conjugated dienes was measured at room temperature in incubations containing 100 μg of LDL protein with 5 μmol/L Cu(II) in 1 mL PBS. The absorbance at 234 nm (A234) was measured at 10-minute intervals in a Uvikon 810 spectrophotometer as described. Results are expressed as the absolute increase in absorbance or as the time required for initiation of rapid oxidation (lag time). The intra-assay coefficients of variation for these measurements are less than 1%. The formation of lipid peroxides was measured by the iodometric method in separate incubations. For these experiments, LDL was incubated at 100 μg LDL protein per milliliter in PBS at 37°C, and aliquots were removed for lipid- peroxide measurement at 0, 2, 3, and 4 hours after addition of 5 μmol/L Cu(II). Lipoprotein Vitamin E Content

α-Tocopherol was measured by high-performance liquid chromatography according to the method of Arnaud et al. α-Tocopherol acetate was used as an extraction internal standard and for standard-curve preparation. Actual concentrations of α-tocopherol standards were determined by measuring the absorbance of prepared solutions and calculating the concentrations on the basis of known spectral data. Sample values were determined from a standard curve of peak area ratios of sample to internal standard.
The calculated average energy intake (mean±SD) from the total diet was 2685 ±801 kcal/d for the linoleate diet group, which contained several lower-weight subjects, than 10% Silar SCP on a Gas Chrom Q11, 100/120 mesh. A 15:0 internal standard (pentadecanoic acid) was added to each sample before extraction, and the content of fatty acid was determined from the peak area ratios of sample to internal standard.

**Lipoprotein and Diet Fatty Acid Compositions**

Lipids from LDL and liquid-formula aliquots were extracted by a modification of the method of Folch et al. The fatty acids were transmethylated and analyzed in a Varian model 3700 gas chromatograph equipped with a column of 10% Silar SCP on a Gas Chrom Q11, 100/120 mesh. A 15:0 internal standard (pentadecanoic acid) was added to each sample before extraction, and the content of fatty acid was determined from the peak area ratios of sample to internal standard.

**Lipoprotein Lipid and Protein Compositions**

Phospholipid phosphorus was analyzed according to the method of Bartlett. Total cholesterol and triglycerides were measured by standard enzymatic methods on a system 3500 Gilford Computer Directed Analyzer. Unesterified and esterified cholesterol contents were determined by gas-liquid chromatography on a Hewlett-Packard 5890A gas chromatograph. Protein concentrations were determined by the Lowry method that was modified to include sodium dodecyl sulfate.

**Statistical Analyses**

Comparisons between diet group means were performed by ANOVA; within diet groups were compared by Student's paired t tests. Repeated-measures ANOVA was used to compare differences in the formation of lipid peroxides between diet groups. Pearson's correlation coefficients were used to evaluate the associations between measures of oxidation and composition of LDL and LDL subfractions.

**Results**

Seventeen of 18 participants completed the 6-week diet study without significant problems. One subject from the oleate diet group removed herself from the study for personal reasons. Weekly food records and measurement of unused portions of the diets indicated that compliance was excellent, with greater than 90% of the provided diets being ingested by all participants. The calculated average energy intake (mean±SD) from the total diet was 2685 ±801 kcal/d for the linoleate group and 2513 ±597 kcal/d for the oleate group, with 56% and 58%, respectively, of energy from solid food sources. As a percent of total energy intake in the linoleate diet group, 9% was monounsaturated fatty acids, 26% was polyunsaturated, and 5% was saturated.

In contrast, in the oleate diet group, 29% of total energy intake was monounsaturated fatty acids, 5% was polyunsaturated, and 6% was saturated. The standard-diet group, which contained several lower-weight subjects, had an average total energy intake of 1804 ±503 kcal/d with 10% as monounsaturated fatty acids, 5% as polyunsaturated, and 12% as saturated.

Mean values for body weight, plasma lipids, and lipoproteins at baseline and at 6 weeks are shown in Table 1. There were no significant changes in weight or lipid values in the standard-diet group. In the linoleate and oleate diet groups, weight decreased by approximately 3%. Total cholesterol decreased significantly in both study groups; LDL cholesterol decreased by 16.1% in the linoleate group and 13.4% in the oleate group, although only the latter reduction was significant. HDL cholesterol decreased by 7.8% and 4.0% in the linoleate and oleate groups, respectively.

**α-Tocopherol and Fatty Acid Composition of LDL**

LDL α-tocopherol levels (per milligram of LDL protein) were 2 to 2.5 times higher than those reported for LDL from subjects who did not receive such supplements. However, the α-tocopherol content in LDL was similar in the three groups throughout the study (Fig 1).

Before the study subjects began the special diets, there were no significant group differences in the percent distribution of any measured long-chain fatty acids in LDL (Table 2). In the standard-diet group, LDL fatty acid content was similar to previously reported values and did not change during the 6-week period, reflecting the consistency of their diet. In contrast, after 6 weeks of linoleate (18:2) or oleate (18:1) diet enrichment, mean levels of 18:2 and 18:1 increased significantly (P<.001) in the linoleate and oleate diet groups, respectively. Within the oleate diet group, subjects had increases in oleic acid that ranged from 22% to 73% and small reductions in linoleic acid. In the linoleate diet group, all participants had marked reductions in 18:2 and increases in 18:1 that ranged from 14% to 57%

Thus, all 11 subjects who consumed the prepared diets had substantial changes in their LDL fatty acid content that reflected the changes in the composition of their LDL.
Baseline 4 wk 6 wk

**Effects of Diet on Parameters of LDL Susceptibility to Oxidation**

The rate and extent of conjugated-diene formation was measured for each individual LDL sample ($d=1.019$ to $1.063$ g/mL) at room temperature in the presence of $5 \mu$mol/L Cu$^{2+}$ (Fig 2). At baseline, there were no differences in lag times of LDL oxidation among the three study groups. However, after 4 and 6 weeks, lag time values in the linoleate diet group were less than those in the other two groups and by 6 weeks had decreased significantly compared with baseline.

On completion of the study, there was a significant correlation between lag time and preoxidation levels of LDL α-tocopherol ($r=0.59$, $P<0.05$) and the LDL 18:2 to 18:1 ratio ($r=0.58$, $P=0.05$) for all subjects.

The time course of LDL lipid-peroxide formation as measured by the iodometric method during Cu$^{2+}$-mediated oxidation is shown in Fig 3. The rate and extent of lipid-peroxide formation varied substantially between individuals and from assay to assay (note the large standard deviations and the differences in peak lipid-peroxide values). However, despite this variation, mean levels of lipid peroxides after 4 and 6 weeks of dietary supplementation were consistently higher in the linoleate diet group at each time point, with differences achieving statistical significance at the 4-week interval (panels B and C).

**Effects of Oxidation on LDL Fatty Acid Composition**

At the end of the study, determinations of fatty acids were performed on all LDL samples from each dietary group before and after 16 hours of Cu$^{2+}$-mediated oxidation (Fig 4). Values are presented as a percentage of the preoxidation amounts rather than absolute units because of differences in the initial amounts of fatty acids within LDL from the three groups. In response to oxidation, marked decreases in the LDL content of 18:2 and 20:4 occurred in all diet groups, but a significantly greater percent loss of unsaturated fatty acids occurred in the LDL from the linoleate diet group (8% decrease in 18:1, $P=0.07$; 75% decrease in 18:2, $P=0.06$; and a 95% decrease in 20:4, $P=0.05$) compared with the other diet groups. The absolute fatty acid loss due to oxidation was also significantly greater in the linoleate diet group.
Reaven et al. Olate-Enriched Diets, α-Tocopherol Supplementation, and LDL Oxidation Susceptibility

Figs 3 and 4. Line plots showing lipid-peroxide formation in copper-oxidized low-density lipoprotein (LDL). LDL (100 μg/mL) samples from subjects who ate either a standard diet (A) or an oleate- (B) or linoleate- (C) supplemented diet were incubated in phosphate-buffered saline with 5 μmol/L Cu²⁺, and aliquots were removed for measurement of lipid peroxides at 0, 2, 3, and 4 hours (H). Values are mean±SD for the baseline (A), 4-week (B), and 6-week (C) visit.

Composition of LDL Subfractions

To allow an overall comparison between buoyant and dense subfractions, compositional data from all diet groups were combined (Table 3). There were significantly greater amounts of dense LDL in plasma compared with buoyant LDL. Triglyceride, free cholesterol, phospholipid, and total lipid contents (as a percent of total LDL mass) were significantly less in the dense fraction, whereas cholesteryl ester and protein were higher. These values for the buoyant and dense LDL fractions are consistent with those previously reported. α-Tocopherol content per milligram of LDL protein (or per LDL particle) was nearly twofold higher in buoyant LDL compared with dense LDL. α-Tocopherol levels per milligram of total lipid also were higher in buoyant LDL (data not shown), although the α-tocopherol to polyunsaturated fatty acid content was similar in buoyant and dense LDL. The composition of dense LDL subfractions from each diet group are shown in Table 4. There were no notable differences in lipid or vitamin E content in the three diet groups. However, the total polyunsaturated fatty acid per LDL particle was higher and the α-tocopherol to polyunsaturated fatty acid ratio significantly lower in the dense LDL of the linoleate group. A similar pattern was seen in the buoyant LDL subfractions from each diet group (Table 5).

Effect of Diets on LDL Subfraction Fatty Acid Composition

The fatty acid composition of buoyant and dense LDL is shown in Fig 5. As in unfractionated LDL, 18:2 is significantly higher and 18:1 significantly lower in both subfractions from the linoleate group and 18:1 is increased in both subfractions from the oleate diet

Table 3. Characteristics and Composition of Combined Buoyant and Combined Dense Fractions

<table>
<thead>
<tr>
<th></th>
<th>Buoyant LDL</th>
<th>Dense LDL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LDL mass, mg/mL plasma</td>
<td>0.92±0.47</td>
<td>1.32±0.48t</td>
</tr>
<tr>
<td>TG content*</td>
<td>11.2±2.4</td>
<td>6.9±2.1t</td>
</tr>
<tr>
<td>Free cholesterol*</td>
<td>8.3±0.5</td>
<td>6.8±0.7t</td>
</tr>
<tr>
<td>Cholesterol ester*</td>
<td>37.5±2.6</td>
<td>41.3±2.3t</td>
</tr>
<tr>
<td>Phospholipid*</td>
<td>21.3±1.9</td>
<td>19.9±1.4t</td>
</tr>
<tr>
<td>Protein*</td>
<td>22.1±1.65</td>
<td>25.0±0.8t</td>
</tr>
<tr>
<td>α-Tocopherol/LDL protein, μg/mg</td>
<td>13.4±4.4</td>
<td>5.6±1.2t</td>
</tr>
<tr>
<td>α-Tocopherol/PUFAs, μg/mg</td>
<td>22±7</td>
<td>22±6</td>
</tr>
<tr>
<td>18:2/18:1</td>
<td>2.42±2.0</td>
<td>2.95±1.5t</td>
</tr>
<tr>
<td>Particle diameter, nm</td>
<td>27.4±0.8</td>
<td>25.5±0.7t</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; TG, triglycerides; and PUFAs, polyunsaturated fatty acids (18:2+20:4). Values from buoyant LDL fractions from all three diet groups (N=15) were combined (buoyant LDL) as were values for dense LDL fractions (dense LDL).

*Percent of total LDL mass.
†Significant compared with the buoyant-group value at P<.05.
group compared with the standard-diet group. With all diet groups combined, the ratio of 18:2 to 18:1 in dense LDL was significantly higher than in buoyant LDL (2.95 versus 2.42, P<.01, Table 3). The ratios of 18:2 to 18:1 in dense and buoyant LDL were 4.3- and 4.0-fold higher, respectively, in the linoleate diet group compared with the oleate diet group (Tables 4 and 5).

**Effects on LDL Subtraction Susceptibility to Oxidation**

Conjugated-diene formation in buoyant and dense LDL during Cu^{2+}-mediated oxidation is shown in Fig 6. In panel A, data from all three diet groups are combined to provide a better illustration of differences in oxidation between subfractions. Buoyant LDL was relatively resistant to oxidation over the 6-hour incubation period, whereas dense LDL was extensively oxidized during this time. Dense LDL fractions from the linoleate group generated greater absolute levels of conjugated dienes and exhibited a shorter lag time than did samples from both the oleate and standard-diet groups (Fig 6B). The rate and extent of conjugated-diene formation from the oleate group were markedly reduced compared with the other diet groups. The conjugated-diene lag time of dense LDL from all groups was positively correlated with LDL α-tocopherol content (r=.67, P=.05) and α-tocopherol to total polyunsaturated fatty acid ratio (r=.76, P=.01) and negatively with the 18:2 to 18:1 ratio (r=-.54, P=.08). There was no significant correlation of lag time with any LDL lipid or protein values. The extent of conjugated-diene formation was correlated positively with the percentage of 18:2 in LDL (r=.80, P<.01) and negatively with the percentage of 18:1 in LDL (r=-.85, P<.01) but not with LDL α-tocopherol content (r=-.02, not significant) or any LDL lipid or protein values.

**Discussion**

Our study was designed to answer three questions: (1) Does the enrichment of LDL in both α-tocopherol and

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**Table 4. Comparison of Composition of Dense LDL Fractions (d=1.040 to 1.054 g/mL) After 6 Weeks of Dietary Modification**

<table>
<thead>
<tr>
<th></th>
<th>Standard Diet (n=6)</th>
<th>Olate Diet (n=4)</th>
<th>Linoleate Diet (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG content*</td>
<td>7.6±2.7</td>
<td>7.3±0.6</td>
<td>5.8±1.8</td>
</tr>
<tr>
<td>Free cholesterol*</td>
<td>6.7±0.8</td>
<td>7.3±0.8</td>
<td>6.7±0.7</td>
</tr>
<tr>
<td>Cholesteryl ester*</td>
<td>42.1±2.4</td>
<td>39.9±1.5</td>
<td>41.6±2.5</td>
</tr>
<tr>
<td>Phospholipid*</td>
<td>19.3±0.6</td>
<td>19.8±1.8</td>
<td>20.8±2.5</td>
</tr>
<tr>
<td>Protein*</td>
<td>24.4±0.8</td>
<td>25.8±0.2</td>
<td>25.1±0.6</td>
</tr>
<tr>
<td>a-Tocopherol/LDL protein, µg/mg</td>
<td>6.2±1.1</td>
<td>4.8±0.8</td>
<td>5.2±0.6</td>
</tr>
<tr>
<td>Total PUFAs/LDL protein, µg/mg</td>
<td>244.9±16.5</td>
<td>215.0±19.2</td>
<td>322.3±7.3†</td>
</tr>
<tr>
<td>a-Tocopherol/PUFAs, µg/mg</td>
<td>25±4</td>
<td>22±3</td>
<td>18±5†</td>
</tr>
<tr>
<td>18:2/18:1</td>
<td>2.0±0.2</td>
<td>1.2±0.1</td>
<td>5.2±1.8†</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; TG, triglycerides; and PUFAs, polyunsaturated fatty acids (18:2+20:4). Total LDL mass in milligrams per milliliter of plasma values are as follows: standard diet, 1.39±0.23; oleate diet, 1.33±0.90; and linoleate diet, 1.24±0.30.

*Percent of total LDL mass.

†Significant differences compared with the standard-group final value at P<.05.

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**Table 5. Comparison of Composition of Buoyant LDL Fractions (d=1.026 to 1.032 g/mL) After 6 Weeks of Dietary Modification**

<table>
<thead>
<tr>
<th></th>
<th>Standard Diet (n=6)</th>
<th>Olate Diet (n=4)</th>
<th>Linoleate Diet (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG content*</td>
<td>11.4±3.2</td>
<td>10.7±2.2</td>
<td>11.3±1.9</td>
</tr>
<tr>
<td>Free cholesterol*</td>
<td>8.1±0.4</td>
<td>8.4±0.5</td>
<td>8.3±0.7</td>
</tr>
<tr>
<td>Cholesteryl ester*</td>
<td>38.0±2.7</td>
<td>36.5±1.9</td>
<td>37.6±3.1</td>
</tr>
<tr>
<td>Phospholipid*</td>
<td>21.3±0.8</td>
<td>20.7±0.5</td>
<td>21.0±1.4</td>
</tr>
<tr>
<td>Protein*</td>
<td>21.2±1.0</td>
<td>23.7±1.1</td>
<td>21.8±1.9</td>
</tr>
<tr>
<td>a-Tocopherol/LDL protein, µg/mg</td>
<td>13.1±1.3</td>
<td>11.0±0.3</td>
<td>12.3±2.5</td>
</tr>
<tr>
<td>Total PUFAs/LDL protein, µg/mg</td>
<td>568±4.4</td>
<td>491±22</td>
<td>740±116†</td>
</tr>
<tr>
<td>a-Tocopherol/PUFAs, µg/mg</td>
<td>22±3</td>
<td>22±3</td>
<td>17±5</td>
</tr>
<tr>
<td>18:2/18:1</td>
<td>1.7±0.2</td>
<td>1.02±0.1</td>
<td>4.1±1.11</td>
</tr>
</tbody>
</table>

LDL indicates low-density lipoprotein; PUFAs, polyunsaturated fatty acids (18:2+20:4). Total LDL mass in milligrams per milliliter of plasma values are as follows: standard diet, 1.14±0.49; oleate diet, 0.73±0.43; and linoleate diet, 0.81±0.45.

*Percent of total LDL mass.

†Significant differences compared with the standard-group final value at P<.05.
oleate lead to a greater degree of protection against in vitro oxidation than does \( \alpha \)-tocopherol supplementation alone? (2) Does enrichment of LDL in linoleate increase LDL susceptibility to oxidation despite concurrent supplementation with \( \alpha \)-tocopherol? (3) Do the effects of diet and \( \alpha \)-tocopherol supplementation differ among LDL density subtractions?

Considering first the effect of these interventions on unfractionated LDL, several points are demonstrated. LDL enriched in both \( \alpha \)-tocopherol and oleic acid was not more resistant to oxidation than LDL enriched in \( \alpha \)-tocopherol alone. This suggests that for \( \alpha \)-tocopherol-enriched LDL, there is no additional benefit (as measured by our in vitro assays of oxidation) of enrichment with oleic acid. However, it is possible that in subjects who take lower doses of \( \alpha \)-tocopherol, diets enriched in oleic acids may still further reduce the susceptibility of LDL to oxidation.

In contrast, LDL isolated from the linoleate diet group was oxidized more rapidly and had more extensive fatty acid decomposition despite a similarly high content of \( \alpha \)-tocopherol. Thus, by providing LDL with more substrate for oxidation, a diet sufficiently enriched in linoleic fatty acids can overcome the antioxidant effects of high dosages of \( \alpha \)-tocopherol. It also follows that at lower levels of \( \alpha \)-tocopherol supplementation, a diet enriched in polyunsaturated fatty acids may increase the susceptibility of LDL to oxidation even more.

Buoyant LDLs (from all diet groups) were markedly resistant to oxidation, and there was no evidence of an accelerated oxidation phase (propagation) during the 6 hours of monitored conjugated-diene formation. This did not appear to be due to prior oxidation, because the baseline optical density values were similar to those of dense LDL. Furthermore, exposure of buoyant LDL to more harsh oxidizing conditions did lead to oxidation (data not shown). In contrast, oxidation of dense LDL occurred within the 6-hour measurement period for samples from all diet groups. Although the basis of these differences in rates of oxidation between buoyant and dense LDL is not clear, there are several striking differences in composition between the two fractions. The average content of \( \alpha \)-tocopherol per dense LDL particle was less than half that in buoyant LDL, and the 18:2 to 18:1 ratio was significantly higher. Both factors correlated well with the rate and extent of dense LDL oxidation.

**Fig 5.** Bar graphs showing fatty acid content of low-density lipoprotein (LDL) subtractions. LDL lipids were extracted, and amounts of 18:1, 18:2, and 20:4 were measured as described in "Methods." Data represent the mean±SD values from all buoyant (A) and dense (B) LDL samples as a percent of total LDL fatty acid content for the standard (○), oleate (□), and linoleate (●) diet groups. *Significant difference compared with values for the standard-diet group at \( P < .05 \).

**Fig 6.** Line plots showing measurement of conjugated-diene formation in low-density lipoprotein (LDL) subtractions during copper-mediated oxidation. LDL (100 \( \mu \)g/mL) was incubated in phosphate-buffered saline medium containing 5 \( \mu \)mol/L Cu\(^{2+}\), and absorbance at 234 nm was measured at 10-minute (min) intervals in a UVikon 810 spectrophotometer. In A, buoyant (○) and dense (□) fractions from all three diet groups are combined. In B, dense LDL fractions from the standard (○), oleate (□), and linoleate (●) diet groups are shown separately. Values are mean±SD.
conjugated-diene formation. Although differences between LDL subfractions in the content of vitamin E per particle have been noted before, the presence of similar vitamin E to lipid ratios in these LDL fractions has led some investigators to minimize the importance of the reduced absolute content of vitamin E. However, the absolute content of vitamin E per LDL particle may be the determinant of the predominant LDL subclass susceptibility to oxidation, because there may be a threshold level of vitamin E particles necessary to substantially retard LDL oxidation. It is also possible that there were differences between LDL subfractions that we did not measure, such as the content of carbohydrate side chains or other antioxidants that may account for differences in the susceptibility of buoyant and dense LDL to oxidation. In particular, because the standard group maintained their usual diets, their LDL subfractions may have varied from those of the linoleate and oleate diet groups in constituents other than fatty acids. Variations in oxidation rates of LDL subfractions have also been previously attributed to differences in protein or free cholesterol content; although this was not evident in our study, possibly as a result of our manipulations of the participants’ diets and α-tocopherol intake.

Particularly striking were the differences in the rate and extent of oxidation of dense LDL among the three diet groups. Most susceptible to oxidation was linoleate-enriched dense LDL, followed by dense LDL from the standard-diet group, whereas dense LDL from the oleate diet group was least susceptible. Although dense LDL from all diet groups had comparable α-tocopherol levels, the content of 18:1 and 18:2 differed markedly, as did the α-tocopherol to 18:2 and α-tocopherol to 18:1 ratios. The α-tocopherol to 18:2 ratio was lowest in the linoleate diet group and highest in the oleate diet group, and this ratio accounted for nearly 65% of the variance ($r^2 = 0.64$) in maximal conjugated-diene formation that occurred in all dense LDL samples. Although other factors not measured in this study may contribute to LDL susceptibility to oxidation, these results indicate that the contents of linoleic and oleic acids in dense LDL are important determinants of its oxidation.

Several results from this study deserve further comment. In subjects who take a high dose of α-tocopherol, diet enrichment with linoleic or oleic fatty acid only modestly influences oxidation of unfractionated LDL ($d = 1.019$ to $1.063$ g/mL). In contrast, diet enrichment with these fatty acids reduces the susceptibility of dense LDL to oxidation. This suggests that buoyant LDL particles and intermediate-density LDL particles ($d = 1.032$ to $1.040$ g/mL, which were not isolated in this study) in the incubation mixture may influence measurement of the rate and extent of formation of conjugated dienes in smaller, more dense LDL. Our findings may simply reflect the fact that in our subjects a large portion of the plasma LDL was relatively more buoyant, and hence the oxidation of dense LDL particles was overshadowed. This result is consistent with that of a previous study, in which we found that the rate of oxidation of unfractionated LDL was correlated with the diameter of the predominant LDL subclass.

The results of this study have important implications. Several epidemiological studies have shown LDL of greater density to be associated with an increased risk for coronary artery disease. Austin et al demonstrated that for men and women there was overall a threefold higher risk for coronary artery disease in those with increased amounts of dense LDL. In a study of Framingham subjects, individuals with coronary artery disease had a predominance of dense LDL in their plasma compared with control subjects. Similar findings were reported in a study by Crouse et al. Greater uptake by artery wall macrophages of dense LDL as a result of enhanced susceptibility to oxidation is one potential explanation for these findings, although both in vitro and animal studies have suggested additional mechanisms. Stender and Zilversmit and Nordestgaard and Zilversmit have demonstrated that entry of lipoproteins into the artery wall is size dependent, with smaller particles, such as dense LDL, gaining entry more readily. Additionally, the sialic acid content of lipoproteins may influence their interactions with artery wall proteoglycans, with reduced sialic acid content being associated with increased avidity of proteoglycan binding. Small, dense LDL has less total carbohydrate and a lower sialic acid content than larger LDL particles. These properties are probably important, because oxidation of dense LDL is unlikely to occur in plasma because of abundant plasma antioxidants. On the other hand, if dense LDL enters the arterial wall more readily and/or is retained preferentially within the intima, the ease of oxidation of dense LDL may be critically relevant. For these reasons, therapies directed toward reducing LDL oxidation should focus on dense LDL in particular. The current results suggest that diets enriched in monounsaturated fatty acids alone or in combination with antioxidants such as vitamin E may be a particularly safe and effective method of achieving this goal.

Previous diet studies have in general used specially prepared liquid diets to alter the LDL fatty acid composition. While these studies have helped demonstrate the feasibility of altering LDL susceptibility to oxidation through dietary manipulations, the specific diets are not practical for general use. The current study demonstrates that standard baked goods and other food items can be sufficiently enriched in oleate to create a more practical, palatable diet that will effectively increase the 18:1 content of LDL. The amount of total calories per day that must be replaced by monounsaturated fatty acids to reduce LDL susceptibility to oxidation may be less than previously thought. Abbey et al have recently shown that diets with less than 20% of total daily calories enriched in monounsaturated fatty acids or polyunsaturated fatty acids may influence LDL susceptibility to oxidation. With the marked effect that linoleate- or oleate-enriched diets appear to have on the oxidation of dense LDL, it is conceivable that even more modest changes in dietary fatty acids will influence atherogenesis.

In summary, whereas diet-induced differences in susceptibility of unfractionated LDL (representing a mixture of buoyant, intermediate, and dense LDL) to oxidation were small when large supplements of vitamin E were given concurrently, the effect on the susceptibility of dense LDL to oxidation was substantial. Interventions designed to reduce lipoprotein oxidation should also assess differences in efficacy between LDL subfractions. α-Tocopherol supplementation alone, even at
high doses, did not prevent dense LDL from undergoing oxidation. In contrast, oleate-enriched diets decreased the extent of oxidation of dense LDL and therefore may be an effective method of reducing oxidation of this fraction. This may be particularly relevant for individuals with familial combined hyperlipidemia, hypertriglyceridemia, or diabetes, who have an increased amount of dense LDL. Individuals with this LDL pattern, also called the type B phenotype, appear to be at greater risk for coronary artery disease and may benefit more from therapy that can specifically reduce the oxidation of dense LDL.

Acknowledgments

This work was supported by grant HL-14197 from the National Heart, Lung, and Blood Institute (SCOR); GCRC Program MO1 RR00827 of the National Center for Research Resources, National Institutes of Health; and the American Philosophical Society. Part of this work was conducted at the Lawrence Berkeley Laboratory through the US Department of Energy under contract No. DE-AC03-76SF00098. We thank Dr Joseph Witztum for his advice and comments regarding the manuscript; Haven Webb, Joellen Barnett, Elizabeth Miller, Pat Blanche, and Laura Holl for important assistance in the conduct of these studies; and Annie Durning-Canty, Eva Brzezinski, Carol Walsh, and Ann Lake from the GCRC Nutrition Unit for excellent assistance in diet preparation and nutrient analysis. We also thank SVO Enterprises for supplying Trisun 80 oil and for additional financial support; Protein Technologies International for providing soy protein; and Hoffmann-La Roche, Inc, for supply of tocopherol capsules.

References


### Table 6. Fatty Acid Composition of Fat Extracted From Food Products and Liquid Diet Formulas

<table>
<thead>
<tr>
<th>Food Item</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2</th>
<th>18:3</th>
<th>20:0</th>
<th>20:1</th>
<th>22:0</th>
<th>24:0</th>
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<tbody>
<tr>
<td>Biscuit (L)</td>
<td>6.7</td>
<td>4.3</td>
<td>21.7</td>
<td>64.9</td>
<td>0.38</td>
<td>0.34</td>
<td>0.35</td>
<td>0.84</td>
<td>0.26</td>
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<td>Biscuit (O)</td>
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<td>2.7</td>
<td>60.6</td>
<td>27.3</td>
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<td>0.41</td>
<td>0.38</td>
<td>1.06</td>
<td>0.32</td>
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<td>Chicken salad (L)</td>
<td>7.4</td>
<td>4.8</td>
<td>20.3</td>
<td>62.9</td>
<td>0.66</td>
<td>0.31</td>
<td>0.30</td>
<td>0.69</td>
<td>0.24</td>
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<tr>
<td>Chicken salad (O)</td>
<td>4.9</td>
<td>4.4</td>
<td>74.2</td>
<td>12.5</td>
<td>0.30</td>
<td>0.41</td>
<td>0.37</td>
<td>0.98</td>
<td>0.38</td>
</tr>
<tr>
<td>Cookie (L)</td>
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<td>4.2</td>
<td>22.5</td>
<td>63.9</td>
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<td>0.35</td>
<td>0.38</td>
<td>0.76</td>
<td>0.27</td>
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<tr>
<td>Cookie (O)</td>
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<td>75.4</td>
<td>13.1</td>
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<td>0.34</td>
<td>1.11</td>
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<tr>
<td>Corn muffin (L)</td>
<td>6.6</td>
<td>4.2</td>
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<td>64.8</td>
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<td>0.37</td>
<td>0.35</td>
<td>0.83</td>
<td>0.28</td>
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<tr>
<td>Corn muffin (O)</td>
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<td>5.0</td>
<td>77.2</td>
<td>11.3</td>
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<td>0.36</td>
<td>0.32</td>
<td>1.29</td>
<td>0.36</td>
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<tr>
<td>Cranberry bread (L)</td>
<td>6.7</td>
<td>3.8</td>
<td>26.1</td>
<td>59.7</td>
<td>2.0</td>
<td>0.28</td>
<td>0.23</td>
<td>0.65</td>
<td>0.23</td>
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<tr>
<td>Cranberry bread (O)</td>
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<td>0.38</td>
<td>0.92</td>
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<td>Granola (L)</td>
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<td>4.1</td>
<td>20.4</td>
<td>64.5</td>
<td>1.70</td>
<td>0.30</td>
<td>0.28</td>
<td>0.76</td>
<td>0.23</td>
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<td>Granola (O)</td>
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<td>68.0</td>
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<td>Mayonnaise (L)</td>
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<td>66.0</td>
<td>0.50</td>
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<td>0.23</td>
<td>0.83</td>
<td>0.29</td>
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<tr>
<td>Mayonnaise (O)</td>
<td>4.7</td>
<td>4.1</td>
<td>75.3</td>
<td>12.8</td>
<td>0.31</td>
<td>0.40</td>
<td>0.33</td>
<td>1.17</td>
<td>0.41</td>
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<td>Ranch dressing (L)</td>
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<td>0.23</td>
<td>0.83</td>
<td>0.27</td>
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<tr>
<td>Ranch dressing (O)</td>
<td>4.8</td>
<td>4.4</td>
<td>75.1</td>
<td>12.5</td>
<td>0.29</td>
<td>0.41</td>
<td>0.35</td>
<td>1.13</td>
<td>0.39</td>
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<td>Tuna salad (L)</td>
<td>7.0</td>
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<td>0.34</td>
<td>0.27</td>
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<td>Banana bread (L)</td>
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<td>16.0</td>
<td>1.7</td>
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<td>1.1</td>
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<td>Carrot cake (O)</td>
<td>5.5</td>
<td>4.2</td>
<td>76.2</td>
<td>10.9</td>
<td>1.16</td>
<td>0.44</td>
<td>0.45</td>
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<td>0.37</td>
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<td>Muffin (L)</td>
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<td>3.9</td>
<td>77.6</td>
<td>10.9</td>
<td>0.30</td>
<td>0.46</td>
<td>0.41</td>
<td>1.12</td>
<td>0.46</td>
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<td>Pasta salad (L)</td>
<td>4.4</td>
<td>4.2</td>
<td>77.9</td>
<td>10.7</td>
<td>0.25</td>
<td>0.49</td>
<td>0.44</td>
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<tr>
<td>Formula (L)</td>
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<td>24.0</td>
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<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tr>
<tr>
<td>Formula (O)</td>
<td>4.5</td>
<td>4.5</td>
<td>78.0</td>
<td>13.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</table>

L indicates food item included in linoleate-enriched diet; O, food item included in oleate-enriched diet. Values are percent of total fatty acids.
29. Chait A, Brazg RL, Tribble DL, Krauss RM. Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. 

30. Boothby WM, Sandiford RB. Nomographic charts for the calculation of the metabolic rate by the gasometer method. 


32. Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoprotein in normal humans. 


35. Arnaud J, Fontis I, Blacher S, Kia D, Favier A. Simultaneous determination of retinal a-tocopherol and y-carotene in serum by gas-liquid chromatography compared with two other methods. 


38. Bartlett GR. Phosphorus assay in column chromatography. 

39. Hendriks MA, Wolthers BC, Groen A. The determination of total cholesterol in serum by gas-liquid chromatography compared with two other methods. 

40. Kleinveld HA, Naber AHJ, Stalenhoef AFH, Demacker PNM. Oxidation resistance, oxidation rate, and extent of oxidation of human low-density lipoprotein depend on the ratio of oleic acid content to linoleic acid content; studies in vitamin E deficient subjects. 


43. Reaven PD, Parthasarathy S, Grassie B, Miller E, Almazan F, Martin T, Koo JC, Steinberg D, Wittum J. Feasibility of using an oleate-enriched diet to reduce the susceptibility of low density lipoprotein to oxidative modification in humans. 

44. Reaven PD, Parthasarathy S, Grassie B, Miller E, Almazan F, Martin T, Koo JC, Steinberg D, Wittum J. Feasibility of using an oleate-enriched diet to reduce the susceptibility of low density lipoprotein to oxidative modification in humans. 


Effects of linoleate-enriched and oleate-enriched diets in combination with alpha-tocopherol on the susceptibility of LDL and LDL subfractions to oxidative modification in humans.

P D Reaven, B J Grasse and D L Tribble

Arterioscler Thromb Vasc Biol. 1994;14:557-566
doi: 10.1161/01.ATV.14.4.557

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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