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Decreased aortic early atherosclerosis and associated risk factors in hypercholesterolemic hamsters fed a high- or mid-oleic acid oil compared to a high-linoleic acid oil

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Abstract

Currently, diets higher in polyunsaturated fat are believed to lower blood cholesterol concentrations, and thus reduce atherosclerosis, greater than diets containing high amounts of saturated or possibly even monounsaturated fat. The present study was designed to investigate the effect of diets containing mid- or high-linoleic oil versus the typical high-linoleic sunflower oil on LDL oxidation and the development of early atherosclerosis in a hypercholesterolemic hamster model. Animals were fed a hypercholesterolemic diet containing 10% mid-oleic sunflower oil, high-oleic olive oil, or high-linoleic sunflower oil (wt/wt) plus 0.4% cholesterol (wt/wt) for 10 weeks. After 10 weeks of dietary treatment, only the animals fed the mid-oleic sunflower oil had significant reductions in plasma LDL-C levels (-17%) compared to the high-linoleic sunflower oil group. The high-oleic olive oil–fed hamsters had significantly higher plasma triglyceride levels ($+41\%$) compared to the high-linoleic sunflower oil–fed hamsters. The tocopherol levels in plasma LDL were significantly higher in hamsters fed the mid-oleic sunflower oil ($+77\%$) compared to hamsters fed either the high-linoleic sunflower or high-oleic olive oil. Measurements of LDL oxidation parameters, indicated that hamsters fed the mid-oleic sunflower oil and high-oleic olive oil diets had significantly longer lag phase ($+66\%$ and $+145\%$, respectively) and significantly lower propagation rates (-26% and -44%, respectively) and conjugated dienes formed (-17% and -25%, respectively) compared to the hamsters fed the high-linoleic sunflower oil. Relative to the high-linoleic sunflower oil, aortic cholesterol ester was reduced by -14% and -34% in the mid-oleic sunflower oil and high-oleic olive oil groups, respectively, with the latter reaching statistical significance. Although there were no significant associations between plasma lipids and lipoprotein cholesterol with aortic total cholesterol and cholesterol esters for any of the groups, the lag phase of conjugated diene formation was inversely associated with both aortic total and esterified cholesterol in the high-oleic olive oil-fed hamsters ($r = -0.69$, $P < 0.05$). The present study suggests that mid-oleic sunflower oil reduces risk factors such as lipoprotein cholesterol and oxidative stress associated with early atherosclerosis greater than the typical high-linoleic sunflower oil in hypercholesterolemic hamsters. The high-oleic olive oil not only significantly reduced oxidative stress but also reduced aortic cholesterol ester, a hallmark of early aortic atherosclerosis greater than the typical high-linoleic sunflower oil. © 2004 Elsevier Inc. All rights reserved.

Keywords: Monounsaturated fatty acids; Polyunsaturated fatty acids; Plasma lipoprotein cholesterol; LDL oxidation; Tocopherol; Early aortic atherosclerosis

1. Introduction

A review of several human studies has reported that dietary fats containing saturated fatty acids (SFA) of chain length 12:0–16:0 increase serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C), and that monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) decrease LDL-C [1].

Although these elevations of plasma TC and LDL-C as a result of increased saturated fat and cholesterol consumption are important risk factors in the development of atherosclerosis, other factors such as oxidative modification of LDL may also play a key role [2–4]. From these studies, it

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has been hypothesized that early atherosclerosis progresses when macrophages in the subendothelial space of an artery accumulate modified or oxidized LDL through a nonregulated scavenger receptor pathway and are converted to foam cells that contain excessive lipid, especially cholesterol ester. The continuing aggregation of foam cells in the subendothelial space is associated with the formation of fatty streaks, which are the earliest identifiable lesions of atherosclerosis and can be referred to as early aortic atherosclerosis.

Animal studies, including nonhuman primates from our laboratory and others, have shown that feeding a predominantly linoleic acid–containing diet such as corn oil or safflower oil can result in dramatic decreases in plasma levels of LDL-C that are associated with reductions in atherosclerosis [5–9]. However, we are hypothesizing that these significant reductions in the quantity of plasma LDL in these experiments, i.e., 39–77%, may have reduced the possible contribution that LDL quality such as the fatty acid composition and the oxidative susceptibility of the LDL particle can make in the development of early atherosclerosis. In contrast, in human studies, the magnitude of the plasma LDL-C reduction with linoleic acid–rich diets is generally up to 20%, and often less, and thus a significant number of these LDL particles enriched in linoleic acid are more susceptible to oxidation [10,11] and are presumably more atherogenic [12]. Therefore a relative enrichment of MUFA in the diet rather than PUFA might confer additional protection against the development of early atherosclerosis by generating LDL particles relatively resistant to oxidative modification while optimizing both plasma LDL-C and HDL-C concentrations. Recently published studies in hypercholesterolemic hamsters from our laboratory that demonstrated reduced early atherosclerosis in animals fed the very high oleic acid–containing TriSun versus linoleic acid enriched sunflower oil, despite similar LDL-C levels, would appear to have supported this hypothesis [13].

The principal aims of the present study were the following: a) to compare the efficacy of a mid-oleic level sunflower oil to the more highly enriched refined oleic acid olive oil and the linoleic acid–enriched sunflower oil as it relates to plasma lipids, lipoprotein cholesterol levels, and LDL oxidative susceptibility; and b) to determine whether LDL oxidative susceptibility as a result of consuming diets with varying degrees of dietary fat saturation in a background of elevated LDL levels would be associated with more early atherosclerosis. This was accomplished by feeding diets with varying levels of MUFA derived from olive oil (high-oleic) and mid-oleic sunflower oil versus one rich in PUFA derived from sunflower oil (high-linoleic) with enough dietary cholesterol to produce elevated plasma LDL in hamsters. The effects on plasma lipids and lipoprotein cholesterol, LDL fatty acid composition, LDL tocopherol concentrations, LDL oxidative susceptibility, and development of early aortic atherosclerosis as measured by aortic cholesterol analyses were evaluated.

2. Methods and materials

2.1. Animals and experimental protocol

A total of 60 male Golden Syrian hamsters (Charles River Laboratories, Wilmington, MA), 9 weeks of age, were individually housed in stainless steel suspended rodent cages at room temperature with a 12-hour light:dark cycle. Hamsters were given food and water ad libitum and maintained in American Association for the Accreditation of Laboratory Animal Care–accredited facilities. Initially, all hamsters were fed a hypercholesterolemic nonpurified diet (PMI rodent chow 5001, PMI Feeds, Inc., St. Louis, MO) containing 10% by weight high-linoleic sunflower oil and 0.4% cholesterol for 2 weeks (Control diet). After 2 weeks, fasted hamsters (12 hours) were bled and plasma low-density lipoprotein cholesterol (LDL-C) concentrations were measured and, based on similar plasma LDL-C concentrations and body weights, distributed into the following groups: group 1 remained on the control diet; group 2 was fed 10% high-oleic olive oil in place of high-linoleic sunflower oil; and group 3 was given 10% mid-oleic sunflower oil (NuSun, donated by the National Sunflower Oil Association) in place of high-linoleic sunflower oil. Refined as opposed to extra virgin or virgin olive oil was used to eliminate >90% of the phenolic compounds (data not shown). Dietary cholesterol at 0.4% wt/wt was added to insure elevated levels of plasma LDL-C from the low SFA and highly unsaturated fatty acid vegetable oil–containing diets.

A commercial diet rather than a semipurified diet was used because published data from our laboratory [14] and from another [15] indicate that animals on the commercial diet are more responsive to various cholesterolemic interventions and the resultant lipoprotein profile (LDL-C > HDL-C) is more similar to that of humans. The diets were fed in cake form, and food disappearance and body weights were monitored on a weekly basis throughout the study.

2.2. Plasma lipid and lipoprotein cholesterol determinations

Blood samples were taken at 6 and 10 weeks from fasted hamsters (12 hours) via the retro-orbital sinus and collected into heparinized capillary tubes under ultrapure CO_2/O_2 (50/50) gas (Northeast Airgas, Salem, NH) anesthesia. Plasma was harvested after centrifugation at $1500 \times g$ at room temperature for 20 minutes and plasma total cholesterol (TC) [16] and triacylglycerol (TAG) [17] concentrations were measured enzymatically. Plasma very–low-density and low-density lipoprotein cholesterol, which we combined and termed LDL-C, was precipitated with phosphotungstate reagent [18], and high-density lipoprotein cholesterol (HDL-C) was measured in the supernatant. The concentration of LDL-C was calculated as the difference between plasma TC and HDL-C. The accuracy of the pro-

cedures used for the measurement of plasma TC, HDL-C, and TAG concentrations are maintained by participation in the Lipid Standardization Program of the Center for Disease Control and the National Heart, Blood, and Lung Institute.

2.3. LDL isolation

Plasma LDL was isolated by single near-vertical spin, discontinuous density gradient ultracentrifugation as we have previously described [19]. Briefly plasma was adjusted to a density of 1.21 g/mL by addition of 0.4898 g solid KBr to 1.5 mL plasma. These treated plasma samples were then underlayered beneath 3.4 mL of 0.154 mol/L NaCl in an Optiseal ultracentrifuge tube (Beckman Instruments, Palo Alto, CA). Optiseal tubes were placed in a precooled Beckman NVT 65.2 near vertical rotor and centrifuged for 80 minutes at $170,000 \times g$ and 7°C in a Beckman L8-70 ultracentrifuge. LDL (0.7–0.9 mL) was removed from centrifuge tubes by aspiration through the side of the tube using a 1-mL syringe with a 25-gauge needle. The LDL fraction obtained was then filtered through an Acrodisc 0.2 μm sterile syringe filter (Gelman Sciences, Ann Arbor, MI). Protein concentration of the isolated LDL was determined by a modification [20] of the method of Lowry et al. [21].

2.4. LDL oxidation

LDL oxidation was measured as conjugated diene production by the method of Frei and Gaziano [22]. Briefly, freshly isolated plasma LDL was incubated at a concentration of 0.1 mg protein/mL assay volume, which included 250 μL of 20 mmol/L HEPES buffer, 40 μL of 80 μmol/L $CuSO_4$, and 0.154 mol/L NaCl (volume = 710 μL − volume of LDL). Incubations were conducted at 37°C in a thermostatted 12-cell holder in a Cary 1E spectrophotometer (Varian Associates, Palo Alto, CA). Conjugated diene formation was monitored every 10 minutes as the change in 234 nm wavelength absorption as described by Esterbauer et al. [23]. Parameters of the conjugated diene assay measured included lag phase (resistance to oxidation), propagation phase (rate of oxidation), and maximum dienes formed.

2.5. Plasma LDL tocopherol analyses

Plasma LDL tocopherol levels were determined as we have previously described [24] by treating 200 μL of each LDL sample (approximately 50 μg LDL protein) with 2.0 mL of acetone containing butylated hydroxytoluene (15 mg/L) and 2.0 mL petroleum ether followed by vortex mixing. The samples were centrifuged at $500 \times g$ for 5 minutes and the organic layer transferred to a 7.0-mL brown borosilicate screw-top vial. The sample residues were re-extracted with 2.0 mL of petroleum ether and the organic layers were combined. Samples were evaporated under N_2 and reconstituted with mobile phase consisting of a 50:50 solution of solvent A (methanol/0.2 mol/L ammonium ac-

etate [90:10 v/v]) and solvent B (methanol/1 propanol/1 mol/L ammonium acetate [70:20:2 v/v/v]) and injected into the high-performance liquid chromatograph. The HPLC conditions used were a modification of the method of Kaplan et al. [25]. Accuracy and precision of tocopherol measurements were monitored by participation in the National Institute of Standards and Technology Lipid Soluble Vitamin Quality Assurance Program.

2.6. Plasma LDL fatty acid

For plasma LDL fatty acid analysis, a 300-μL aliquot of isolated plasma LDL was extracted using a modified Folch extraction technique [26]. Briefly, plasma LDL was first mixed with 5 mL methanol containing 0.2% butylated hydroxytoluene followed immediately by the addition of 10 mL chloroform and vortexed vigorously for 30 seconds. After the addition of 1.0 mL of 0.09% saline and agitation in a vortex mixer, the mixture was centrifuged at $500 \times g$ for 10 minutes. The top aqueous layer was aspirated and the bottom organic layer was transferred to a glass tube with a Teflon-lined cap and stored at −70°C under N_2. Before analyses, samples were evaporated to dryness under N_2 and esterified, as we have previously described [27], using an Instant Methanolic HCl Kit (Alltech-Applied Science, Deerfield, IL). Fatty acid methyl esters were analyzed using a Hewlett Packard model 5890 GLC, with a DB-23 (J & W Scientific) column, complete with autosampler and integrator.

2.7. Aortic cholesterol measurements

At the end of the exposure period (week 10), hamsters were anesthetized with an i.p. injection of sodium pentobarbital and aortic tissue was obtained for determination of aortic cholesterol concentration as we have previously described [28]. The heart and thoracic aorta were removed and stored in phosphate-buffered saline at 4°C for subsequent analysis. To measure cholesterol concentrations in the aortic arch, a piece of aortic tissue extending from as close to the heart as possible to the branch of the left subclavian artery was used (approximately 20–40 mg). The tissue was cleaned, weighed, and placed in a vial containing 4 mL of methanol and 10 mL of chloroform and treated. The sample was mixed vigorously and left at room temperature for 48 hours before extraction. The solution was then placed in a 37°C water bath, under N_2. When one half of the solution was evaporated, 1 mL of chloroform with 1% Triton-100 was added, mixed, and evaporated to dryness at 37°C under N_2. A 250-μL quantity of distilled water was added to the samples, vortexed, and placed in a shaking water bath at 37°C for 20 minutes to solubilize the lipid. After incubation, aortic total and free cholesterol concentrations were determined in triplicate using 25 μL of sample enzymatically (Wako Chemicals, Richmond, VA) using an ELISA assay. Aortic cholesterol ester concentration was determined as the

Table 1
Fatty acid composition of oil samples

Fatty Acid	Mid-Oleic Sunflower Oil	High-Linoleic Sunflower Oil	High-Oleic Olive Oil
C16:0	4.5	6.2	10.7
C16:1	0.1	0.1	0.2
C18:0	3.7	4.4	2.6
C18:1	62.9	18.6	75.1
C18:2	26.2	68.7	9.2
C18:3	0.3	0.2	—
C20:0	0.3	0.3	0.4
>C20:0	2.0	1.5	1.2
Total	100.0	100.0	100.0

difference between the total and the free cholesterol concentrations.

2.8. Statistical analysis

SigmaStat software was used for all statistical evaluations (Jandel Scientific, San Rafael, CA). One-way analysis of variance (ANOVA) was used to analyze all data. When statistical significance was found by ANOVA, the Student-Newman-Keuls separation of means was used to determine group differences. Correlations (r) between aortic cholesterol and the various parameters were performed using the Pearson product–moment correlation coefficient. All values are expressed as mean $\pm$ SD and statistical significance was set at the minimum $P < 0.05$ [29].

3. Results

3.1. Vegetable oil analyses

The fatty acid composition of the oils (Table 1) revealed the expected enrichment of high-oleic olive oil (75%) with oleate compared to high-linoleic sunflower oil that was enriched in linoleate (69%). Mid-oleic sunflower oil contained relatively higher levels of oleate (63%) with lower levels of linoleate at 26% than the high-linoleic sunflower oil.

The analyses of tocopherol levels of the oils (Table 2) revealed that the mid-oleic sunflower oil contained 85% and

Table 2
Vitamin E composition of vegetable oils (mg/100 g oil)

	High-Lioleic Sunflower Oil	Mid-Oleic Sunflower Oil	High-Oleic Olive Oil
AT	32.59 ± 2.39[a]	34.76 ± 3.67[a]	19.72 ± 2.18[b]
GT	11.08 ± 0.68[a]	20.36 ± 1.78[b]	5.02 ± 0.39[c]
TT	43.67 ± 4.02[a]	55.12 ± 5.87[a]	24.74 ± 2.50[b]

Data are mean ± SD, $n = 3$. Values in a row not sharing a superscript letter are significantly different at $P < 0.05$.

AT = α-tocopherol; GT = γ-tocopherol; TT = total tocopherol.

Table 3
Plasma LDL fatty acid composition (%) in hamsters

	High-Linoleic Sunflower Oil	Mid-Oleic Sunflower Oil	High-Oleic Olive Oil
16:0	15.1 ± 0.53	14.5 ± 0.56	16.2 ± 1.12
16:1	0.75 ± 0.09	0.77 ± 0.07	0.96 ± 0.04
18:0	9.57 ± 0.23	9.61 ± 0.49	9.54 ± 0.62
18:1	20.6 ± 1.74[a]	32.3 ± 2.29[b]	35.2 ± 2.25[b]
18:2	46.9 ± 0.60[a]	35.1 ± 0.67[b]	29.3 ± 0.91[c]
18:3	0.21 ± 0.09	0.28 ± 0.09	0.23 ± 0.08
20:4	4.65 ± 0.32[a]	3.23 ± 0.22[b]	2.91 ± 0.43[b]
20:5	0.12 ± 0.04[a]	0.44 ± 0.05[b]	0.60 ± 0.08[b]
22:6	2.43 ± 0.21	2.23 ± 0.16	2.00 ± 0.21

Values represent mean ± SD, $n = 10$ pools of two animals with similar plasma LDL-C concentrations/group. Values in a column not sharing a superscript letter are significantly different at $P < 0.05$.

306% more γ-tocopherol (the major form of tocopherol in foods) than high-linoleic sunflower oil and high-oleic olive oil, respectively. Although the mid-oleic sunflower oil and high-linoleic sunflower oil had similar levels of α-tocopherol (the major form of tocopherol in blood), both oils had approximately 75% more α-tocopherol than high-oleic olive oil.

3.2. Animal studies

All hamsters in each group survived the entire length of the study. No significant differences were observed between dietary treatments for body weight before the treatment period or at the end of the study (data not shown).

Plasma LDL isolated from hamsters fed the mid-oleic sunflower oil diet, compared to those fed the high-linoleic sunflower oil diet, contained significantly more oleate (32.3% vs 20.6%, $P < 0.05$) and less linoleate (−35.1% vs −46.9, $P < 0.05$) (Table 3). Although the enrichment of LDL with oleate was not significantly different between the mid-oleic sunflower oil and high-oleic olive oil–treated groups, linoleate content of LDL from mid-oleic sunflower oil–fed animals was significantly greater than high-oleic olive oil–fed animals (35.1% vs 29.3%, $P < 0.05$). Compared to high-linoleic sunflower oil, plasma LDL from both mid-oleic sunflower oil and high-oleic olive oil–fed hamsters had significantly less arachidonate (4.65% versus 3.23% and 2.91%, respectively, $P < 0.05$).

The appearance of the ω-3 fatty acids C20:5 and C22:6 in the LDL could probably be attributed to the fish meal component of the commercial diet, which was a basic ingredient in all diets.

Plasma lipid and lipoprotein cholesterol concentrations between weeks 6 and 10 were not significantly different within dietary treatments (data not shown) and therefore the values were averaged (Table 4). Plasma LDL-C levels were significantly lower only in the mid-oleic sunflower oil group compared to the high-linoleic sunflower oil group (−17%, $P < 0.05$).

Table 4
Plasma lipids and lipoprotein cholesterol concentrations (mean of weeks 6 and 10) (mmol/L)

Diet	TC	LDL-C	HDL-C	TC/HDL-C	TAG
High-linoleic sunflower oil	9.2 ± 2.3	4.6 ± 1.9[a]	4.7 ± 0.6	2.0 ± 0.35	1.7 ± 0.4[a]
Mid-oleic sunflower oil	8.1 ± 2.1	3.8 ± 1.9[b]	4.3 ± 0.5	1.9 ± 0.44	2.0 ± 0.4[a,b]
	(−12%)	(−17%)	(−9%)	(−5%)	(18%)
High-oleic olive oil	8.7 ± 2.1	4.4 ± 1.9[a,b]	4.3 ± 0.5	2.0 ± 0.38	2.4 ± 0.8[b]
	(−5%)	(−4%)	(−9%)	(0%)	(41%)

Data are mean ± SD, $n = 20$. Values in parentheses are percent differences from the high-linoleic sunflower oil group. Values in a column not sharing a superscript letter are significantly different at $P < 0.05$.

LDL-C = low-density lipoprotein cholesterol; HDL = high-density lipoprotein cholesterol; TAG = triacylglycerol; TC = total cholesterol.

The high-oleic olive oil–fed hamsters had significantly greater concentrations of plasma TAG compared to the high-linoleic sunflower oil–fed hamsters (41%, $P < 0.05$) (Table 4).

Relative to the hamsters fed the high-linoleic sunflower oil diet, LDL from the mid-oleic sunflower oil–fed hamsters had a significantly longer lag phase (66%, $P < 0.05$), and a decreased propagation phase (−26%, $P < 0.005$) (Table 5). LDL from the high-oleic olive oil–fed hamsters compared to the high-linoleic sunflower oil–fed hamsters also had significantly longer lag phase (145%, $P < 0.05$) and decreased propagation phase (−44%, $P < 0.05$) and conjugated dienes formed (−25%, $P < 0.05$). There were no statistically significant differences in any of the parameters of oxidative stress measured between the mid-oleic sunflower oil– and the high-oleic olive oil–fed hamsters, although LDL from the latter tended to demonstrate greater resistance to in vitro–induced oxidation.

Plasma LDL α-tocopherol levels in the mid-oleic sunflower oil–fed hamsters were significantly higher than either the hamsters fed high-linoleic sunflower oil (77%, $P < 0.05$) or high-oleic olive oil (60%, $P < 0.05$), which not surprisingly resulted in significantly higher total tocopherol levels in the mid-oleic sunflower oil group relative to the other two diet groups (Table 5).

Compared to high-linoleic sunflower oil, aortic cholesterol ester was reduced −14% and −34% in the mid-oleic sunflower oil and high-oleic olive oil groups, respectively, with the latter reaching statistical significance ($P < 0.05$) (Table 6). Although there were no significant associations between lipoprotein cholesterol values and early atherosclerosis for any of the groups, the lag phase of conjugated diene formation was inversely associated with both aortic total and esterified cholesterol ($r = -0.69$, $P < 0.05$) in the high-oleic olive oil–fed hamsters.

4. Discussion

The aims of the present study were to determine the following: 1) whether hamsters fed mid-oleic sunflower oil, higher in linoleate content than typical olive oil, would have lower circulating levels of plasma lipids and lipoprotein cholesterol; 2) to what degree of LDL oxidative susceptibility would exist in animals fed an mid-oleic sunflower oil with more oleate than typical sunflower oil but less than typical olive oil; and 3) to what extent any of these parameters determined would be associated with different degrees of early atherosclerosis as measured by the accumulation of aortic cholesterol. The finding that hamsters fed mid-oleic

Table 5
LDL oxidative susceptibility and LDL vitamin E concentrations

	High-Linoleic Sunflower Oil	Mid-Oleic Sunflower Oil	High-Oleic Olive Oil
Lag phase (min)	63.1 ± 32.0[a]	104.5 ± 38.4[b]	154.5 ± 63.1[b]
		(66%)	(145%)
Propagation phase (nmol/min/mg LDL protein)	10.54 ± 3.17[a]	7.77 ± 1.78[b]	5.88 ± 2.22[b]
		(−26%)	(−44%)
Maximum dienes formed (nmol/mg LDL protein)	592.8 ± 133.4[a]	494.6 ± 109.1[a,b]	443.7 ± 89.8[b]
		(−17%)	(−25%)
LDL α-tocopherol (ng/μg LDL protein)	2.46 ± 1.29[a]	4.36 ± 1.78[b]	2.73 ± 1.33[a]
		(77%)	(11%)
LDL γ-Tocopherol (ng/μg LDL protein)	0.35 ± 0.26	0.49 ± 0.25	0.32 ± 0.28
		(40%)	(−9%)
LDL total tocopherol (ng/μg LDL protein)	2.82 ± 1.53[a]	4.85 ± 1.97[b]	3.05 ± 1.56[a]
		(72%)	(8%)

Data are mean ± SD, $n = 10$ pools of two animals each. Values in parentheses are percent differences from the high-linoleic sunflower oil group. Values in a row not sharing a superscript letter are significantly different from each other at $P < 0.05$.

LDL = low-density lipoprotein.

Table 6
Aortic cholesterol concentrations (μg/mg of tissue)

	High-Linoleic Sunflower Oil	Mid-Oleic Sunflower Oil	High-Oleic Olive Oil
CE	1.37 ± 0.23^a	$1.18 \pm 0.08^{a,b}$ (-14%)	0.90 ± 0.08^b (-34%)
FC	0.66 ± 0.10	0.57 ± 0.09 (-14%)	0.52 ± 0.06 (-21%)
TC	2.03 ± 0.27^a	$1.75 \pm 0.10^{a,b}$ (-14%)	1.43 ± 0.07^b (-30%)

Data are mean $\pm$ SD, $n = 17$. Values in parentheses are percent differences from the high-linoleic sunflower oil. Values in a row not sharing a superscript letter are significantly different at $P < 0.05$ by analysis of variance.

CE = cholesterol ester; FC = free cholesterol; TC = total cholesterol.

sunflower oil with more linoleate had lower plasma LDL-C than those given high-oleic olive oil is in agreement with some studies [30,31] but not others [32–37]. However, the significantly lower plasma LDL-C levels in hamsters fed the mid-oleic sunflower oil versus the high-linoleic sunflower oil is difficult to explain based on linoleate levels, because high-linoleic sunflower oil has greater levels of linoleate than the mid-oleic sunflower oil. It is possible, although unlikely, that the relative enrichment of the cholesterol-raising saturated fatty acid, palmitic acid, of high-linoleic sunflower oil (38%) and high-oleic olive oil (137%) compared to mid-oleic sunflower oil may have been a contributing factor, although the absolute levels of palmitic acid would seem too low to result in significant effects on LDL-C levels.

The effects of 10 weeks of consuming diets enriched in oleate or linoleate resulted in increased LDL content with the corresponding fatty acid is in agreement with other studies [10,11]. Ex vivo oxidation of LDL from high-linoleic sunflower oil–fed hamsters generated more conjugated dienes and did so at a faster rate than oxidation of LDL from either mid-oleic sunflower oil or high-oleic olive oil-fed hamsters. There was also a greater lag time in conjugated diene formation in the mid-oleic sunflower oil– and the high-oleic olive oil–fed hamsters compared to those fed high-linoleic sunflower oil. The increased oxidative susceptibility of LDL enriched in PUFA-containing sunflower oil compared to MUFA-containing sunflower oil and olive oils is in agreement with many studies [10,12, 38–42]. However, as suggested by others [11], it is difficult to determine whether this reduction in oxidative modification of LDL is due to the increased content of oleate in the LDL, the reduced content of linoleate, or both. Results from a study by Lee et al. [40] suggest that enriching lipoproteins with oleate may reduce oxidation by a) direct "antioxidant-like" effect, b) reduction in the amount of linoleate available for oxidation, and c) reduction in the generation of bioactive particles that occur during mild oxidation. Because, in the present study, LDL tocopherol levels were similar between the high-linoleic sunflower and high-oleic olive oil diet

treatments, it seems doubtful that LDL antioxidant levels were a factor in the observed differences in LDL oxidation between these two diets, suggesting that LDL fatty acid composition may have been the predominant factor influencing LDL oxidative susceptibility. On the other hand, the greater LDL tocopherol levels in the hamsters fed mid-oleic sunflower oil versus high-linoleic sunflower oil diets may have also contributed to the reduced LDL oxidative susceptibility of the former diet treatment. Interestingly, the cross-breeding to produce this nontransgenic mid-oleic sunflower oil resulted in 84% higher levels of γ-tocopherol compared to high-linoleic sunflower oil. This may have contributed to the enrichment of plasma LDL tocopherol levels in hamsters fed mid-oleic sunflower oil versus high-linoleic sunflower oil.

Finally, the present study revealed that hamsters fed the MUFA-containing sunflower oil and, in particular, olive oil, compared to a PUFA-enriched sunflower oil-containing diet developed less early aortic atherosclerosis as measured by the accumulation of aortic total and esterified cholesterol. This protective finding for a MUFA-enriched diet is not supported by one study in monkeys [7], a study in LDL receptor-null, human apolipoproteinB–overexpressing transgenic mice [43] and one study in LDL receptor–deficient mice [44]. On the other hand, the antiatherosclerotic properties of MUFA-enriched diets have been demonstrated in rabbits [45–48] and monkeys [49,50], our own previous studies in hamsters [13], and in swine [51,52]. There are several possible mechanisms derived from both in vitro and in vivo studies that might explain the beneficial effects of MUFA-containing diets as they relate to early atherosclerosis. For example, in one of the above-cited rabbit studies by Li [45], the reduced atherosclerosis observed in aortas from rabbits fed peanut oil versus corn oil was associated with less vascular cell adhesion molecule–1 (VCAM-1) expression in aortic intimal cells in the peanut oil group despite higher plasma cholesterol levels. Similarly, the rate of adhesion of blood monocytes, isolated from individuals consuming different fatty acid–enriched diets to a common pool of LDL-treated human umbilical vein endothelial cells occurred in a pattern of PUFA (n-3) > PUFA (n-6) > MUFA (n-9) [53]. Although not measured in this study, our previous finding of decreased aortic accumulation of oxidized LDL in the MUFA group [13] coupled with a proposed role of oxidized LDL in stimulating VCAM-1 expression [45] suggests another possible mechanism for the antiatherosclerotic properties of MUFA-enriched diets. A beneficial effect of monounsaturated fat on endothelial function is also supported by the studies of Perez-Jiminez et al. [54]. In these studies, individuals fed a Mediterranean-type MUFA compared to either a low-fat National Cholesterol Education Program (NCEP)-I or a high–saturated fat diet had reduced levels of endothelial products, von Willebrand factor, thrombomodulin and tissue factor pathway inhibitor, and plasminogen activator inhibitor–1. Fatty acids can have complex effects on other aspects of endothelial

function. For example, Carlucci et al. [55] demonstrated that oleic acid inhibits cytokine induced expression of VCAM-1 and other indices of endothelial activation in cultured cells, a finding also shared by the n-3 fatty acid docosahexaenoic acid [56]. Studies by Toborek et al. [57] demonstrated that exposure of human endothelial cells to linoleic acid but not oleic acid markedly induced nuclear factor–κB and activator protein 1 transcriptional activation and enhanced messenger RNA levels of tumor necrosis factor–α, monocyte chemoattractant protein 1, VCAM-1, and intracellular adhesion molecule–1 (ICAM-1).

Our finding that the degree of early aortic atherosclerosis was significantly correlated with the lag phase of conjugated diene formation in the high-oleic olive oil group and not lipoprotein cholesterol levels was consistent with our previous study in hamsters [13] but also receives additional support from the studies of Hennig et al. [58] in which endothelial cell dysfunction, mediated by exposure to lipoproteins derived from animals fed different fats, was correlated with lipoprotein oxidative susceptibility and not lipoprotein levels. The finding that mid-oleic sunflower oil–fed animals had reduced risk factors for early atherosclerosis such as decreased LDL cholesterol and LDL oxidative susceptibility, but not early atherosclerosis itself (as defined by a significant reduction in aortic cholesterol ester) was unexpected, but may suggest that a certain level of oleate in the diet and/or a greater reduction in oxidative stress is necessary before one can demonstrate effects at the level of the blood vessel wall. In conclusion, these studies in hypercholesterolemic hamsters have demonstrated that MUFA-enriched diets reduce early aortic atherosclerosis. These findings, coupled with the observations of others that MUFA feeding is associated with improvement in various endothelial functions, supports the recommendations that MUFA-enriched diets be implemented in cardiovascular disease prevention strategies.

Acknowledgments

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