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Journal of Nutritional Biochemistry 13 (2002) 392–402

THE JOURNAL OF Nutritional Biochemistry

Decreased aortic early atherosclerosis in hypercholesterolemic hamsters fed oleic acid-rich TriSun oil compared to linoleic acid-rich sunflower oil

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Received 17 December 2001; received in revised form 9 April 2002; accepted 12 April 2002

Abstract

Previous studies have demonstrated that low density lipoprotein (LDL) enriched in polyunsaturated fatty acids (PUFA) are more susceptible to oxidation (ex vivo) than those containing monounsaturated fatty acids (MUFA). To test whether this observation was associated with various parameters considered to be related with the development of early aortic atherosclerosis, hamsters were fed commercial hypercholesterolemic diets (HCD) containing either the PUFA, sunflower oil (SF) or the MUFA, TriSun oil (TS) at 10% with 0.4% cholesterol (wt/wt). LDL isolated from hamsters fed TS had significantly longer lag phase (30%, $P < 0.05$), a decreased propagation phase (−62%, $P < 0.005$), and fewer conjugated dienes formed (−37%, $P < 0.007$) compared to hamsters fed SF. Aortic vasomotor function, measured as degree of aortic relaxation, was significantly greater in the TS vs SF-fed hamsters whether acetylcholine or the calcium ionophore A23187 was used as the endothelium-dependent agonist. As a group, the SF-fed hamsters had significantly more early atherosclerosis than hamsters fed TS (46%, $P < 0.006$). When animals across the two diets were pair-matched by plasma LDL-C levels, there was an 82% greater mean difference ($P < 0.002$) in early atherosclerosis in the SF versus the TS-fed hamsters. While there were no significant associations with plasma lipids and lipoprotein cholesterol, early atherosclerosis was significantly correlated with lag phase ($r = -0.67$, $p < 0.02$), rate of LDL conjugated diene formation ($r = 0.74$, $p < 0.006$) and maximum dienes formed ($r = 0.67$, $p < 0.02$). Compared to TS-fed animals, aortic sections from hamsters fed the SF-containing diet revealed that the cytoplasm of numerous foam cells in the subendothelial space reacted positively with the monoclonal anti-bodies MDA-2 and NA59 antibody, epitopes found on oxidized forms of LDL. The present study suggests that compared to TS, hamsters fed the SF-diet demonstrated enhanced LDL oxidative susceptibility, reduced aortic relaxation, greater early aortic atherosclerosis and accumulation of epitopes found on oxidized forms of LDL.

Keywords: Monounsaturated fatty acids; Polyunsaturated fatty acids; Plasma lipoprotein cholesterol; Endothelial vasodilator function; LDL oxidation; Early aortic atherosclerosis

1. Introduction

A report from the American Heart Association (AHA) reveals that up to 30% of Americans in the 45–55 year age group have blood cholesterol levels equal to or greater than 6.20 mmol/L (240 mg/dl) [1]. A review article summarizes several human studies showing that saturated fats (SFA) containing fatty acids of chain length 12:0–16:0 increase serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) and that monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) decrease LDL-C [2]. Thus, the initial therapeutic approach for hypercholesterolemic individuals at increased risk for premature coronary heart disease (CHD) is the AHA Step I and Step II

Abbreviations: TS, TriSun; SF, Sunflower; TC, total cholesterol; TAG, triacylglycerol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

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0955-2863/02/$ – see front matter
PII: S0955-2863(02)00202-4

diets, which focus on reductions in dietary SFA (< 10 and 7% of energy, respectively), and cholesterol intakes (< 300 and 200 mg/day, respectively and increases in PUFA (up to 10% of energy) and MUFA (up to 17% of energy) [3].

Although the elevations in plasma TC and LDL-C as a result of increased saturated fat and cholesterol consumption are important risk factors in the development of atherosclerosis, oxidative modification of LDL may also play a key role [4–7]. It has been hypothesized that atherosclerosis progresses when macrophages in the subendothelial space of an artery take up oxidized LDL through a non-regulated scavenger receptor and are converted to foam cells that contain excessive lipid, especially cholesterol ester. The continuing aggregation of foam cells in the subendothelial space leads to the formation of fatty streaks, which are the earliest identifiable lesions of atherosclerosis and can be referred to as early aortic atherosclerosis.

Non-human primate studies from our laboratory, as well as 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 [8–12]. However, we are hypothesizing that the significant reductions of the quantity of LDL in these experiments (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 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 oftentimes less, and thus, a significant number of these LDL particles enriched in linoleic acid are more susceptible to oxidation [13–14] and are presumably more atherogenic [15]. Thus a relative enrichment of MUFA in the diet rather than PUFA might confer additional protection by generating LDL particles relatively resistant to oxidative modification while optimizing both plasma LDL-C and HDL-C concentrations.

The principal aim of the present study was 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 atherosclerosis. This was accomplished by feeding diets rich in MUFA derived from TriSun oil (high oleate-sunflower oil) with those of a diet rich in PUFA derived from sunflower oil (high linoleate) with enough dietary cholesterol to produce elevated LDL in hamsters. The effects on plasma lipids and lipoprotein cholesterol, LDL fatty acid composition, LDL tocopherol concentrations, LDL oxidative susceptibility, aortic vasodilator function and the development of early aortic atherosclerosis as measured by fatty streak formation and accumulation of aortic oxidized LDL were evaluated.

2. Materials and methods

2.1. Animals and experimental protocol

Fifty-six, 8 week old male, Golden Syrian hamsters (*Mesocricetus auratus*) (LVG strain, Charles River Laboratory, Wilmington, MA) were housed in groups of five or six in polystyrene cages containing wood chips in a temperature controlled room (25°C) with a 12-h light:dark cycle. Hamsters were given food and water ad libitum and maintained in AAALAC (American Association for the Accreditation of Laboratory Animal Care) accredited facilities. Animals were fed commercial Purina chow 5001 (Ralston Purina, St. Louis, MO) for a period of 2 weeks prior to the start of the study to get acclimated to the facility. After 2 weeks, food-deprived hamsters were bled and plasma total cholesterol (TC) concentrations were measured. The hamsters were then divided into two groups of twenty-eight hamsters per group based on similar average plasma TC and body weights, and placed on experimental diets for 12 weeks. Each of the experimental diets were comprised of a commercial hypercholesterolemic diet (HCD) consisting of the polyunsaturated fat (10% wt/wt sunflower oil) [SF] with over 70% of the fatty acids as linoleic acid) or the monounsaturated fat (10% TriSun oil) oleic acid-enriched sunflower [TS] with over 70% of the fatty acids as oleic acid) and 0.4% cholesterol (wt/wt) added to the appropriate oil and blended in with the commercial chow. A commercial diet, rather than a semipurified diet was used because published data from our laboratory [16] and those from another [17] 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. The animals were maintained in accordance with the guidelines of the Committee on Animal Care of the University of Massachusetts Lowell Research Foundation, and the guidelines prepared by the Committee on Care in Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW publication no. 85–23, revised 1985).

2.2. Plasma lipid determinations

Blood samples were taken at 8 and 12 weeks from food deprived hamsters (12 hr) and collected via the retro-orbital sinus 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 x g at room temperature for 20 min and plasma total cholesterol (TC) [18] and triacylglycerol (TAG) [19] concentrations were measured enzymatically. Plasma very low- and low-density lipoprotein cholesterol which we combined and termed (LDL-C) was precipitated with phosphotungstate reagent [20] 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 procedures 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 [21]. 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 underlayed beneath 3.4 ml of 0.154 M NaCl in an Optiseal ultracentrifuge tube (Beckman Instruments, Palo Alto, CA). Optiseal tubes were placed in a pre-cooled Beckman NVT 65.2 near vertical rotor and centrifuged for 80 min at 170,000 × 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 [22] of the Lowry et al. method [23].

2.4. LDL oxidation

LDL oxidation was measured as conjugated diene production by the method of Frei and Gaziano [24]. Briefly, freshly isolated LDL was incubated at a concentration of 0.1 mg protein/ml assay volume, which included 250 μl of 20 mM HEPES buffer, 40 μl of 80 μM $CuSO_4$ and 0.154 M 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 Inc., Palo Alto, CA). Conjugated diene formation was monitored every 10 min as the change in 234 nm wavelength absorption as described by Esterbauer et al. [25]. 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 [26] 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 × g for 5 min 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/0.2M ammonium acetate [90:10 v/v]) and Solvent B (methanol/1 propanol/1M ammonium acetate [70:20:2 v/v/v]) and injected into the HPLC. The HPLC conditions used were a modification [26] of the method of Kaplan et al. [27]. Accuracy and precision of tocopherol measurements were monitored by participation in the National Institute of Standards and Technology (NIST) 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 [28]. Briefly, plasma LDL was first mixed with 5 ml methanol containing 0.2% BHT followed immediately by the addition of 10 ml chloroform and vortexed vigorously for 30 s. Following the addition of 1.0 ml of 0.09% saline and agitation in a vortex mixer, the mixture was centrifuged at 500 × g for 10 min. 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. Prior to analyses, samples were evaporated to dryness under N_2 and esterified as we have previously described [29] utilizing an Instant Methanolic HCl Kit (Alltech-Applied Science, Deerfield, IL). Fatty acid methyl esters were analyzed utilizing a Hewlett Packard model 5890 GLC, with a DB-23 (J & W Scientific) column, complete with autosampler and integrator.

2.7. Aortic fatty streak analysis

At the end of the 12 weeks, hamsters were anesthetized with an IP injection of sodium pentobarbital (62.5 mg/ml at a dosage of 0.2–0.25 ml/200 g body weight) (Henry Schein, Port Washington, NY) and aortic tissue was obtained for aortic fatty streak analysis (early aortic atherosclerosis) as we have previously described [30,31]. Briefly, hamsters were anesthetized with sodium pentobarbital (50 mg/kg body wt), cut midventrically and blood collected into a heparinized tube by puncture to the left ventricle. The right atrium was punctured allowing the circulatory system to be flushed via the left ventricle for approximately 1 min with a 4 g/L phosphate-buffered formalin solution. The right atrium was clamped and the flow rate was reduced while flushing continued for an additional 30 min. The hamster aortas were completely dissected of all surrounding tissue and the heart and descending aorta were removed and discarded. The aorta was stretched out and pinned down in 4 g/L phosphate-buffered formalin to fix for 12 h. The aorta was placed in a vial containing 4 g/L phosphate buffered saline (PBS) and stored at 4°C until morphometric analysis. Each individual en face preparation (approximately 8 mm in length) of the aortic arch was cleaned of adventitia and the inner surface was stained for 15 min with Oil Red O (ORO).

The tissue was then rinsed and opened longitudinally, and mounted on a glass slide cover slip and aqueous mounting medium. Images from the entire mounted section were captured and analyzed using a model 3000 Image Analysis system (Image Technology Corp., Deer Park, NY). The images were calibrated to square microns (μm^2) and the threshold to identify ORO was set subsequent to analysis of the total ORO-stained macrophage-derived foam cells which constituted the fatty streak or early atherosclerosis in the aortic arch. Units of measurement of fatty streak area are expressed in $\mu m^2/mm^2 \times 1000$ of aortic tissue.

2.8. Histological analyses

Aortas were fixed in 10% phosphate buffered formalin, processed into paraffin, sectioned (6 μm), and mounted on gelatin coated slides and air dried overnight at room temperature. Sections were deparaffinized with Hemo-De clearing agent (Fisher Scientific, Springfield, NJ), rehydrated through a decreasing series of ethanol concentrations and immersed for 15 min in PBS (pH 7.4).

Monoclonal antibodies, MDA-2 and NA59 (generous gift from Dr. Joseph Witztum, produced in the Immunology Core Laboratory of the La Jolla SCOR program) were used to detect malondialdehyde-lysine and 4-hydroxynonenal-lysine (4-HNE-LDL), respectively. Both of these epitopes are found on oxidized forms of LDL. Tissues were pretreated with Target Unmasking Fluid (TUF, Signet Laboratories, Inc., Dedham, MA) at 90°C for 10 min to uncover masked antigens. The sections were blocked with a 1:20 dilution of normal goat serum (Sigma, St. Louis, MO) in PBS, pH 7.4 for 20 min at room temperature. Visualization of reaction was done with the StrAviGen Supersensitive Universal Staining Kit using the Biotin-Strepavidin Amplified (B-SA) Alkaline Phosphatase Detection System (BioGenex Laboratories, San Ramon, CA) and fast red chromagen. The MDA-2 and NA59 were diluted 1:5000 and 1:4000 respectively, in PBS containing 1% sodium azide and 1% BSA. Duplicate slides of the aortic arch segments were used as negative controls by omitting the primary antibodies and substituting PBS. All sections were counterstained with Mayer's hematoxylin. For routine histopathology, duplicate sections were stained with hematoxylin and eosin. Results were examined and photographically documented.

2.9. In vitro assessment of aortic vascular function

After 12 weeks on the two diet treatments, a cohort of sixteen hamsters, eight from each diet treatment were shipped to the Boston University laboratories of Drs. Vita and Keaney for subsequent measurement of aortic vascular functions. Hamsters were sacrificed with pentobarbital 5 mg/100 g by intraperitoneal injection. The thoracic aorta was excised and immediately placed in ice-cold PBS containing 10 μM indomethacin [32]. Extraneous tissue was carefully removed and a 5 mm segment of mid aorta was placed in an organ chamber (37°C, pH 7.4) containing 20 ml of PBS and aerated with 15% O_2/80% N_2/5% CO_2. The ring was suspended between two tungsten stirrups and isometric tension was measured with a force transducer (Model FT-03, Grass, Instrument Co., Quincy, MA) and a chart recorder (Model RS 3800, Gould Instrument Co., Columbus, OH). Each ring was gradually stretched to the previously determined optimum tension of 2 g and allowed to equilibrate for 1 hr before the introduction of vasoactive drugs. The vessels were precontracted with norepinephrine (1 μM), and then endothelium-dependent relaxation was assessed by the addition of the muscarinic agonist acetylcholine (final concentration 10^{-9} to 10^{-5} M) and the calcium ionophore A23187 (10^{-9} to 10^{-6} M). Endothelium-independent relaxation was assessed by addition of nitroglycerin (10^{-9} to 10^{-5} M).

2.10. Statistical analysis

SigmaStat software was used for all statistical evaluations (Jandel Scientific, San Rafael, CA). A one-way analysis of variance (ANOVA) was used to analyze all data with the exception of aortic vasodilator function described below. When statistical significance was found by ANOVA, the Student-Newman-Keuls separation of means was used to determine group differences. Correlations (r) between early atherosclerosis and the various parameters were performed using Pearson's product-moment correlation coefficient. Because these correlations were conducted on pooled samples, the two diet treatments were combined to provide sufficient sample size as we have described previously [33]. The vasodilator responses to acetylcholine, A23187, and nitroglycerin are reported as the percent relaxation compared to the contraction produced by 1 μM norepinephrine. The dose responses between treatment groups were compared using two way (treatment group and dose) repeated measures analysis of variance. All values were expressed as mean $\pm$ SD and statistical significance was set at the minimum $p < 0.05$ [34].

3. Results

All hamsters in each group survived the entire length of the study. No significant differences were observed between dietary treatments for body weight prior to the treatment period or at the end of the study. Also, there were no significant differences for food consumption between the treatment diets (data not shown).

LDL isolated from hamsters fed the TS diet, compared to hamsters fed the SF diet, contained significantly more oleate (32.3% vs 16.8%, $P = 0.0003$) and less linoleate (26.7% vs 42.0, $P < 0.0001$) (Table 1).

Plasma lipid and lipoprotein cholesterol concentrations between weeks 8 and 10 were not significantly different within dietary treatments and therefore the values were

Table 1
Fatty acid composition (%) of LDL isolated from hamsters after 12 weeks of dietary treatment

Fatty Acid	TriSun (TS)	Sunflower (SF)
16:0	17.92 ± 1.02	17.38 ± 1.08
16:1	0.86 ± 0.06	0.90 ± 0.03
18:0	12.85 ± 1.31	11.65 ± 0.49
18:1	32.29 ± 1.92	16.76 ± 1.26*
18:2	26.68 ± 1.00	41.97 ± 1.08**
18.3	0.13 ± 0.11	0.15 ± 0.13
20:4	4.87 ± 0.31	7.08 ± 0.79#
22:6	4.39 ± 0.46	4.16 ± 0.79

Values are mean ± SD, n = 4 pools are 5 animals each.
*$P = 0.0003$, **$P < 0.0001$, #$P < 0.02$ vs TS diet.

Table 3
LDL tocopherol concentrations (umol/L) and LDL oxidation, measured as conjugated diene formation, in hamsters after 12 weeks of dietary treatment

	TriSun (TS)	Sunflower (SF)
α-Tocopherol	5.43 ± 1.25	5.29 ± 1.00
γ-Tocopherol	1.14 ± 0.11	1.23 ± 0.06
Total Tocopherol	6.57 ± 1.34	6.52 ± 1.05
Lag Phase (min)	111 ± 8.5	85.3 ± 17.9*
Propagation Phase (nmol/min/mg LDL protein)	6.0 ± 1.63	15.7 ± 3.65**
Maximum Dienes Formed (nmol/mg LDL protein)	512 ± 36	816 ± 136**

Values are mean ± SD, n = 4 pools of 5 animals each.
$P < 0.05$; **$P < 0.01$ vs TS.

averaged (Table 2). No significant differences were observed for plasma TC, LDL-C, and HDL-C concentrations between the hamsters fed the TS and SF diets. The TS-fed hamsters had significantly greater concentrations of plasma TAG compared to the SF-fed hamsters (37%, $P = 0.0001$) (Table 2).

Although there were no significant differences in plasma total and lipoprotein cholesterol concentrations between the two dietary treatments, the hamsters fed SF had significantly greater aortic fatty streak area compared to the hamsters fed TS (46%, $P < 0.006$) (Table 2). When hamsters were paired-matched by plasma LDL-C, there was an 82% greater mean difference ($P < 0.002$) in aortic fatty streak formation in the SF-fed hamsters compared to the TS (data not shown).

Relative to the hamsters fed the SF diet, LDL from the TS-fed hamsters had a significantly longer lag phase (23%, $P < 0.05$), a decreased propagation phase (−61%, $P < 0.005$), and fewer conjugated dienes formed (−37%, $P < 0.007$). No differences were observed between the SF- and TS-fed hamsters for plasma LDL tocopherol concentrations (Table 3).

Correlations on pooled LDL samples were performed on combined diet treatments to provide adequate sample size and are presented in Table 4. Early aortic atherosclerosis was significantly correlated with the lag phase ($r = -0.666$, $p < 0.018$), rate of LDL conjugated diene formation ($r = 0.744$, $p < 0.006$), and maximum dienes formed ($r = 0.673$, $p < 0.016$).

Aortic sections for histopathology showed more areas of subendothelial foam cells in the aortas from hamsters fed the SF vs TS diet (Figs. 1A and 1B). The endothelium was intact in both groups, as was the internal elastic lamina. There was no proliferation of extracellular matrix or the presence of smooth muscle cells in the subendothelial spaces of aortas from either group. The sections reacted with MDA-2 showed a diffuse staining throughout the tunica media. The sections from SF-fed hamsters contained numerous foam cells in the subendothelial space and the cytoplasm of these cells reacted very strongly with MDA-2 as shown by the intense staining in all of the cells in this area (Fig. 1C). Aorta sections from hamsters fed the TS did not show the presence of foam cells or any difference in medial staining (Fig. 1D). Aortas reacted with NA59 did not show medial staining as was observed with MDA-2. Foam cells were again noted in the sections from the SF-fed hamsters and the cytoplasm of these subendothelial cells reacted positively with NA59 antibody (Fig. 1E). Sections

Table 2
Plasma lipid and lipoprotein cholesterol concentrations (average of week 8 and 12) (mmol/L) and aortic fatty streak area (AFSA) ($\mu m^2/mm^2 \times 1000$) in hamsters after 12 weeks of dietary treatment

	TriSun (TS)	Sunflower (SF)
TC	8.47 ± 1.74	8.87 ± 2.25
LDL-C	5.35 ± 1.91	5.40 ± 1.58
HDL-C	2.49 ± 0.41	2.50 ± 0.30
TG	6.10 ± 1.36	3.84 ± 1.47*
AFSA	7.52 ± 4.95	10.96 ± 5.16**

Values are mean ± SD, n = 20.
*$P = 0.0001$; **$P < 0.006$ vs TS diet.

Table 4
Correlations (r) between early atherosclerosis, plasma and lipoprotein cholesterol, LDL α-tocopherol (AT) and various parameters of conjugated diene formation

	Early Atherosclerosis	
Variable	r	*P*
TC	0.14	NS
LDL-C	0.17	NS
HDL-C	–0.36	NS
LDL AT	–0.10	NS
Lag phase	–0.666	0.018
Propagation phase	0.744	0.006
Maximum dienes formed	0.673	0.016

Values are derived from the two diet treatments combined, i.e., 4 pools of 5 animals each × 2 diet treatments (n = 8 pools). r is the correlation between the various parameters using Pearson's product-moment correlation coefficient. *P* is the statistical significance set at the minimum $p < 0.05$.

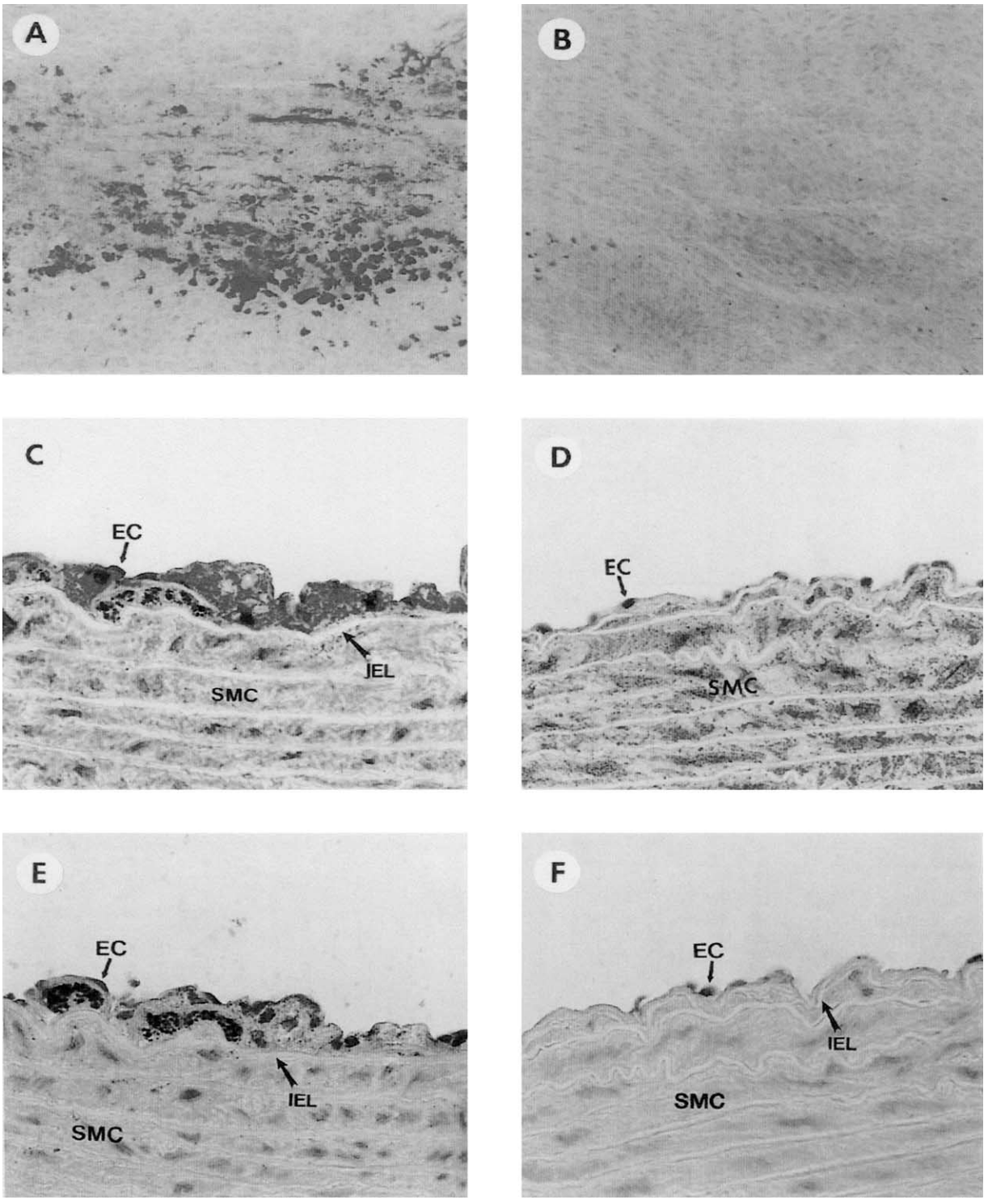

Fig. 1. Hamster aortic arches. (A) Oil red O stained en face preparation of the inner curvature of the aortic arch from a SF-fed hamster (photographed at 40×). (B) Oil red O en face preparation of the inner curvature of the aortic arch from a TS-fed hamster (photographed at 40×). (C) A cross section of an aorta from a SF-fed hamster reacted with MDA-S antibody showing subendothelial foam cells with intense positive staining for reaction with MDA-2 in the cytoplasm. (D) Aortic cross section from a TS-fed hamster reacted with MDA-2 antibody that does not contain foam cells but has the same diffuse reaction staining in the medial SMC. (E) Aortic cross section from a SF-fed hamster reacted with NA59 antibody showing subendothelial foam cells and the presence of NA59 in the cytoplasm of these cells. (F) An aortic cross section from a TS-fed hamster reacted with NA59 antibody showing the absence of foam cells and NA59 reaction product. All cross sections were photographed at 400×; EC = endothelial cell, SMC = smooth muscle cell, IEL = internal elastic lamina.

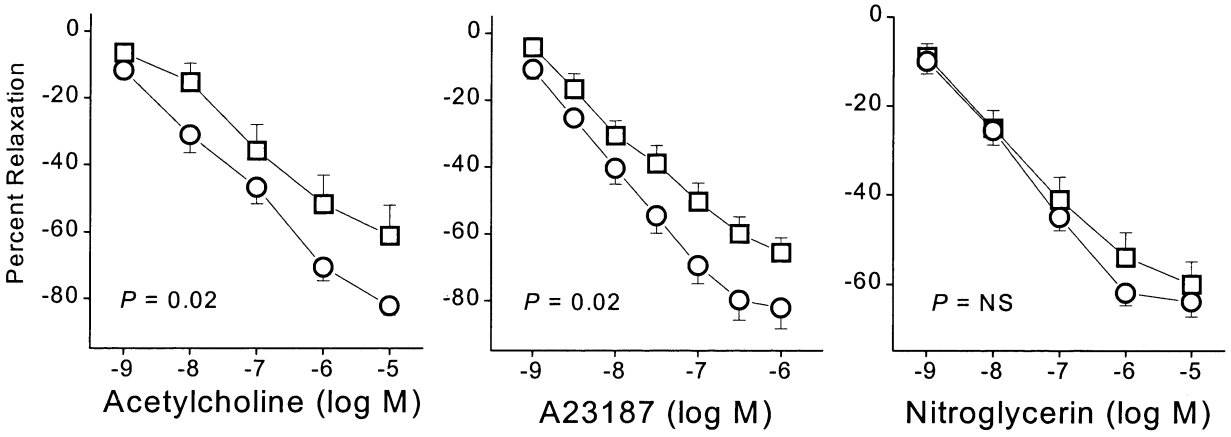

Fig. 2. Effect of dietary treatment on vascular function. Endothelium-dependent relaxation to acetylcholine and A23187 and endothelium-independent relaxation to nitroglycerin was examined in segments of mid-thoracic aorta harvested from hamsters after 12 weeks of dietary treatment as described in Methods. Relaxation to acetylcholine ($P = 0.02$) and A23187 ($P = 0.02$) was less in the Sunflower oil group (□) compared to the Trisun oil group (○) by repeated measures ANOVA. Data represent 8 animals per treatment group and are expressed as mean ± SEM.

from TS-fed hamsters did not show foam cell presence and there was no NA59 reaction product observed (Fig. 1F).

3.1. Effect of dietary treatment on aortic vascular function

The contractile response to 1 μM norepinephrine was similar in the TS group (0.6 ± 0.1 g) and the SF group (0.5 ± 0.2 g, $P = 0.44$). The effects of 12 weeks of dietary treatment on vascular function are presented in Fig. 2. Acetylcholine produced dose-dependent relaxation with a maximal response of 81 ± 7% ($P < 0.001$) in the TS group and 64 ± 21% ($P < 0.001$) in the SF group. The dose responses to acetylcholine were different between treatment groups by repeated measures ANOVA ($P = 0.02$). A23187 produced dose-dependent relaxation with a maximal response of 82 ± 5% ($P < 0.001$) in the TS group and 66 ± 5% ($P < 0.001$) in the SF group. The dose responses to A23187 were different between treatment groups by repeated measures ANOVA ($P = 0.02$). Nitroglycerin produced dose-dependent relaxation with a maximal response of 80 ± 3% ($P < 0.001$) in the TS group and 79 ± 5% ($P < 0.001$) in the SF group. The between group responses were not different by repeated measures ANOVA.

Vasomotor function was also assessed in a group of 7 hamsters consuming normal chow without cholesterol or oil additions. Acetylcholine produced dose-dependent relaxation in these animals with a maximal response of 79 ± 3% ($P < 0.001$). This response was not significantly different than the response to acetylcholine in the TS group. There was a strong trend for a reduced response to acetylcholine in the SF group compared to the animals consuming normal chow ($P = 0.058$). A23187 produced dose-dependent relaxation in the normal chow group with a maximal response of 81 ± 4% ($P < 0.001$). This response was not significantly different than the response to A23187 in hamsters in the TS group. The response to A23187 was lower in the group of animals consuming SF compared to control ($P = 0.05$). Nitroglycerin produced dose-dependent vasodilator in the normal chow group with a maximal response of 88 ± 6% ($P < 0.001$). This response was not significantly different than the response to nitroglycerin in the SF and Trisun oil groups.

4. Discussion

The aim of the present study was to test whether short-term feeding of MUFA or PUFA-enriched diets would alter the distribution of these fatty acids in plasma LDL and whether this would affect the susceptibility of LDL to oxidative modification and subsequently decrease aortic vasodilator function, increase early aortic atherosclerosis and accumulation of oxidized LDL. There were no differences in plasma TC, LDL-C, or HDL-C concentrations observed between hamsters fed the enriched MUFA or PUFA diets in the present study. This result is in agreement with some studies [35–40] but not others [41–42].

Twelve weeks of consuming diets enriched in oleate or linoleate resulted in increased LDL content with the corresponding fatty acid, in agreement with other studies [13–14]. Ex vivo oxidation of LDL from SF-fed hamsters generated more conjugated dienes and, at a faster rate, than did oxidation of LDL from TS-fed hamsters. There was also a greater lag time in conjugated diene formation in the TS-fed hamsters compared to the hamsters fed SF. The increased oxidative susceptibility of LDL enriched in PUFA compared to MUFA is in agreement with many studies [13,15, 43–47]. However, as suggested by others [14], 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

the Lee et al. study [45] suggest that enriching lipoproteins with oleate may reduce oxidation by (a) direct "antioxidant"-like effect (b) reducing the amount of linoleate available for oxidation and (c) reducing the generation of bioactive particles that occur during mild oxidation. Since in the present study, LDL tocopherol levels were similar between the SF and TS diet treatments, it seems doubtful that LDL antioxidant levels were a factor in the observed differences in LDL oxidation between diets, suggesting that LDL fatty acid composition may have been the predominant factor influencing LDL oxidative susceptibility. Another interesting finding was the apparent fatty acid effects on aortic vasodlilator function. In the present study, the vasodilator responses to endothelium-dependent agonists, acetylcholine and A23187 were impaired in animals consuming the hypercholesterolemic SF compared to the hypercholesterolemic TS diet. There also was a strong trend for an impaired response in the SF group compared to normal control animals. Vasodilator responses to nitroglycerin were preserved in all groups. These findings suggest that dietary intake of oil rich in MUFA preserves endothelial vasomotor function in the setting of cholesterol feeding. Alternatively, dietary consumption of oil rich in PUFA may worsen endothelial vasomotor function in this setting.

These findings are consistent with an earlier study of the effects of dietary fatty acids on endothelial function [48]. In that study, consumption of a single meal enriched with sunflower oil was associated with the acute impairment of endothelium-dependent flow-mediated dilation in healthy human subjects. However, no prior human or animal study has examined the effects of chronic consumption of different fatty acids on endothelium-dependent vasodilation. Fatty acids have complex effects on other aspects of endothelial function. For example, Carluccio et al. [49] demonstrated that oleic acid inhibits cytokine-induced expression of vascular cell adhesion molecule-1 (VCAM-1) and other indices of endothelial activation in cultured cells, a finding also shared by the n-3 fatty acid docosahexaenoic acid (DHA) [50]. Studies by Toborek et al. [51] demonstrated that exposure of human endothelial cells to linoleic 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, vascular adhesion molecule 1 and intracellular adhesion molecule 1. On the other hand, oleic acid exposure either had no effect or reduced the transcriptional activation and inflammatory gene mRNA levels in these endothelial cells. Although not relevant to the present study because all vessels were pretreated with indomethacin, there is evidence that PUFAs but not MUFAs impair prostacyclin production in cultured endothelial cells [52]. The precise mechanisms for preserved endothelial vasodilator function in the animals treated with TS are unclear. The findings of the present study of increased LDL oxidative susceptibility coupled with the accumulation of epitopes found on oxidized LDL in foam cells found in the subendothelial space of aortas from SF-fed hamsters suggests a role for increased formation of oxidized LDL in vascular wall pathology associated with early atherosclerosis. Accumulation of oxidized LDL might influence the bioactivity of endothelium-derived nitric oxide by several mechanisms [53]. For example, oxidized LDL is cytotoxic to endothelial cells [54] and inactivates nitric oxide directly [55]. Oxidized LDL may also decrease expression of endothelial nitric oxide synthase (eNOS) [56]. Oxidized LDL stimulates recruitment of inflammatory cells to the vascular wall [57], which may increase vascular production of reactive oxygen species and destruction of nitric oxide. Oxidized LDL has also been shown to inhibit receptor-dependent NO release from endothelial cells, an effect that is attributable to formation of lysophosphatidylcholine and altered cell membrane signal transduction [58]. This latter mechanism may be less important in the present study because the vasodilator responses were also altered in response to A22187, which stimulates nitric oxide by non-receptor-dependent mechanisms.

Finally, the present study revealed that hamsters fed a MUFA compared to a PUFA-enriched diet developed less early aortic atherosclerosis. This protective finding for a MUFA-enriched diet is not supported by one study in monkeys [10], a study in LDL receptor-null, human apoB-overexpressing transgenic mice [59] and one study in LDL receptor-deficient mice [60]. On the other hand, the anti-atherosclerotic properties of MUFA-enriched diets have been demonstrated in rabbits [61–64], swine [65,66] and monkeys [67,68]. There are several possible mechanisms derived from both in vitro and in vivo studies which might explain the beneficial effects of MUFA-containing diets as they relate to early atherosclerosis. For example, in one of the rabbit studies cited above by Li [61], the reduced atherosclerosis observed in aortas from rabbits fed peanut oil versus corn oil, was associated with less VCAM-1 expression in aortic intimal cells in the peanut oil group despite high 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) [69]. Similar findings of reduced monocyte adhesion and also chemotaxis with oleate versus linoleate rich diets were reported by Tsimikas et al. [47].Our finding of decreased accumulation of oxidized LDL in the MUFA group coupled with a proposed role of oxidized LDL in stimulating VCAM-1 expression [70] suggests another possible mechanism for the anti-atherosclerotic 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. [71]. In these studies, individuals fed a Mediterranean-type MUFA compared to either a low fat NCEP –I or a high saturated fat diet, had reduced levels of endothelial products, von Willebrand factor (v WF), Thrombomodulin and Tissue Factor Pathway Inhibi-

tor (TFBI) and Plasminogen Activator Inhibitor (PAI-1). Our finding that the degree of early aortic atherosclerosis was significantly correlated with measurements of LDL oxidative susceptibility and not lipoprotein cholesterol levels was unexpected but receives some support from the studies of Hennig et al. [72] 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. In conclusion, these studies in hypercholesterolemic hamsters have demonstrated that MUFA-enriched diets reduce early aortic atherosclerosis. Moreover, to our knowledge, this communication represents the first demonstration that diets enriched in MUFA reduce the aortic accumulation of oxidized LDL. In addition, our finding that MUFA-enriched diets increased aortic vasodilator function suggests an additional mechanism for its anti-atherosclerotic properties. 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

Drs. Keaney and Vita are recipients of Established Investigator Awards from the American Heart Association.

References

[1] A. Chait, J.D. Brunzell, M.A. Denke, D. Eisenberg, N.D. Ernst, F.A. Franklin, H. Ginsberg, T.A. Kotchen, L. Kuller, R.M. Mullis, M.Z. Nichaman, R.J. Nicolosi, E.J. Schaefer, N.J. Stone, W.H. Weidman, Rational of the Diet-Heart Statement of the American Heart Association: Report of the Nutrition Committee, Circulation 88 (1993) 3008–3029.

[2] P.M. Kris-Etherton, Y. Shaomei, Individual fatty acid effects on plasma lipids and lipoproteins: human studies, Am J Clin Nutr 65 (suppl) (1997) 1628S–1644S.

[3] NCEP Expert Panel, Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults: (Adult Treatment Panel II), J Am Med Assoc 269 (1993) 3015–3023.

[4] D. Steinberg, S. Parthasarathy, T.W. Carew, J.C. Khoo, J.L. Witztum, Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity, N Engl J Med 320 (1989) 915–924.

[5] J.L. Witztum, D. Steinberg, Role of oxidized low-density lipoprotein in atherosclerosis, J Clin Invest 88 (1991) 1785–1792.

[6] I. Jialal, S. Devaraj, Low-density lipoprotein oxidation, antioxidants, and atherosclerosis: a clinical biochemistry perspective, Clin Chem 42 (1996) 498–506.

[7] H. Esterbauer, J. Gebicki, H. Puhl, G. Jurgens, The role of lipid peroxidation and antioxidants in oxidative modification of LDL, Free Rad Biol Med 13 (1992) 341–390.

[8] M.S. Wolfe, J.K. Sawyer, T.M. Morgan, B.C. Bullock, L.L. Rudel, Dietary polyunsaturated fat decreases coronary artery atherosclerosis in a pediatric-aged population of African green monkeys, Arterioscler Thromb 14 (1994) 587–597.

[9] L.L. Rudel, F.L. Johnson, J.K. Sawyer, M.S. Wilson, J.S. Parks, Dietary polyunsaturated fat modifies low density lipoproteins and reduces atherosclerosis of nonhuman primates with high and low diet responsiveness, Am J Clin Nutr 62 (suppl) (1995) 463S–470S.

[10] L.L. Rudel, J. Parks, J.K. Sawyer, Compared with dietary monounsaturated and saturated fat, polyunsaturated fat protects African green monkeys from coronary artery atherosclerosis, Arterioscler Thromb Vasc Biol 15 (1995) 2101–2110.

[11] J.K. Sawyer, F. Johnson, L.L. Rudel, Atherosclerosis is less in Cynomolgus monkeys fed polyunsaturated (ϖ6) fat in spite of lower HDL concentrations and large LDL size, Arteriosclerosis 9 (1996) 722a.

[12] R.J. Nicolosi, J.L. Hojnacki, N. Llansa, K.C. Hayes, Diet and lipoprotein influence on primate atherosclerosis, Proc Soc Exp Biol Med 156 (1977) 1–7.

[13] P. Reaven, S. Parthasarathy, B.J. Grasse, E. Miller, D. Steinberg, J.L. Witztum, Effects of oleate-rich and linoleate-rich diets on the susceptibility of low density lipoprotein to oxidative modification in mildly hypercholesterolemic subjects, J Clin Invest 91 (1993) 668–676.

[14] P. Reaven, S. Parthasarathy, B.J. Grasse, E. Miller, F. Almazan, F.H. Mattson, J.C. Khoo, D. Steinberg, J.L. Witztum, Feasibility of using an oleate-rich diet to reduce the susceptibility of low-density lipoprotein to oxidative modification in humans, Am J Clin Nutr 54 (1991) 701–706.

[15] S. Parthasarathy, J.C. Khoo, E.Miller, J. Barnett, J.L. Witztum, D. Steinberg, Low density lipoprotein rich in oleic acid is protected against oxidative modification: implications for dietary prevention of atherosclerosis, Proc Natl Acad Sci USA 87 (1990) 3894–3898.

[16] A.H.M. Terpstra, J.C. Holmes, R.J. Nicolosi, The hypocholesterolemic effect of dietary soy protein vs. casein in hamsters fed cholesterol-free or cholesterol-enriched semi-purified diets, J Nutr 121 (1991) 944–947.

[17] B.R. Krause, R.F. Bousley, K.A. Kieft, R.L. Stanfield, Effect of the ACAT inhibitor CI-976 on plasma cholesterol concentrations and distribution in hamsters fed zero- and no-cholesterol diets, Clin Biochem 25 (1992) 371–377.

[18] C.C. Allain, L.S. Poon, C.S.G. Chen, W. Richmond, P.C. Fu, Enzymatic determination of total serum cholesterol, Clin Chem 20 (1974) 470–475.

[19] G. Bucolo, H. David, Quantitative determination of serum triglycerides by the use of enzymes, Clin Chem 36 (1973) 476–482.

[20] K.W. Weingand, B.P. Daggy, Quantitation of high-density lipoprotein cholesterol in plasma from hamsters by differential precipitation, Clin Chem 36 (1990) 575.

[21] A.H.M. Terpstra, C.J.H. Woodward, F.J. Sanchez-Muniz, Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation: visualization by prestaining and rapid isolation of serum lipoproteins from small volumes of serum, Anal Biochem 111 (1981) 149–157.

[22] M.A.K. Markwell, S.M. Hass, L.L. Bieber, N.E. Tolbert, A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples, Anal Biochem 8 (1978) 206–212.

[23] O.H. Lowry, O.H., N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the Folin phenol reagent, J Biol Chem 193 (1951) 265–275.

[24] B. Frei, J.M. Gaziano, Content of antioxidants, preformed lipid hydroperoxides and cholesterol as predictors of the susceptibility of human LDL to metal ion dependent and independent oxidation, J Lipid Res 34 (1993) 2135–2145.

[25] H. Esterbauer, G. Striegl, H. Puhl, M. Rotheneder, The continuous monitoring of in vitro oxidation of human low density lipoprotein, Free Rad Res Commun 6 (1989) 67–75.

[26] E.J. Rogers, S.M. Rice, R.J. Nicolosi, D.R. Carpenter, C.A. McClelland, L.J. Romanczyk Jr., Identification and quantitation of γ-oryzanol components and simultaneous assessment of tocols in rice bran oil, J Amer Oil Chem 70 (1993) 301–307.

[27] L.A. Kaplan, E.A. Stein, W.C. Willett, M.J. Stampfer, W.S. Stryker, Reference ranges for retinol, tocopherols, lycopene and alpha- and beta-carotene in plasma by simultaneous high-performance liquid chromatographic analysis, Clin Physiol Biochem 5 (1987) 297–304.
[28] J. Folch, M. Lees, G.H. Sloane-Stanley, A simple method for the isolation and purification of total lipids from animal tissue, J Biol Chem 226 (1957) 497–509.
[29] K.S. Chong, R.J. Nicolosi, R.F. Rodger, D.A. Arrigo, R.W. Juan, J.J. Mackey, S. Georas, P.N. Hebert, Effect of dietary fat saturation on plasma lipoproteins and high density lipoprotein metabolism of the rhesus monkey, J Clin Invest 79 (1987) 675–683.
[30] T.L. Foxall, G.T. Shwaery, A.F. Stucchi, S.S. Wang, R.J. Nicolosi, Dose-response effects of doxazosin on plasma lipids, lipoprotein cholesterol, and aortic fatty streak formation in hypercholesterolemic hamsters, Am J Pathol 140 (1990) 1357–1363.
[31] M.C. Kowala, J.J. Nunnari, S.K. Durham, R.J. Nicolosi, Doxazosin and cholestyramine similarly decrease fatty streak formation in the aortic arch of hyperlipidemic hamsters, Atherosclerosis 91 (1991) 35–49.
[32] J.F.J. Keaney, J.M. Gaziano, A. Xu, B. Frei, J. Curran-Celentano, G.T. Shwaery, J. Loscalzo, J.A. Vita, Low-dose alpha-tocopherol improves and high-dose alpha-tocopherol worsens endothelial vasodilator function in cholesterol-fed rabbits, J Clin Invest 93 (1994) 844–851.
[33] R.J. Nicolosi, T.A. Wilson, E.J. Rogers, D. Kritchevsky, Effects of specific fatty acids (8:0, 14:0, cis-18:1, trans-18:1) on plasma lipoproteins, early atherogenic potential, and LDL oxidative properties in the hamster, J Lipid Res 39 (1998) 1972–1980.
[34] G.W. Snedecor, W.G. Cochran, Statistical Methods. 6th ed. Iowa State University Press, Ames, IA, 1967.
[35] I.B. Gustafsson, B. Vessby, M. Ohvrall, M. Nydahl, A diet rich in monounsaturated rapeseed oil reduces the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemic subjects, Am J Clin Nutr 59 (1994) 667–674.
[36] R. Mensink, M. Katan, Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low density and high density lipoprotein cholesterol in healthy women and men, N Engl J Med 321 (1989) 436–441.
[37] P. Mata, J. Garrido, J.M. Ordovas, E. Blazquel, L.A. Alvarez-Sala, M.J. Rubio, R. Alonso, M. de Oya, Effect of dietary monounsaturated fatty acids on plasma lipoproteins and apolipoproteins in women, Am J Clin Nutr 56 (1992) 77–83.
[38] E. Berry, S. Eisenberg, D. Haratz, Y. Friedlander, Y. Norman, N.A. Kaufman, Y. Stein, Effects of diets rich in monounsaturated fatty acids on plasma lipoproteins—the Jerusalem Nutrition Study: high MUFAs vs high PUFAs, Am J Clin Nutr 53 (1991) 899–907.
[39] G. Wardlaw, J. Snook, Effects of diets high in butter, corn oil, or high-oleic sunflower oil on serum lipids and apolipoproteins in men, Am J Clin Nutr 51 (1990) 815–821.
[40] M.C. Nydahl, I.G. Gustaffson, B. Vessby, Lipid-lowering diets enriched with monounsaturated or polyunsaturated fatty acids but low in saturated fatty acids have similar effects on serum lipid concentrations in hyperlipidemic patients, Am J Clin Nutr 59 (1994) 115–122.
[41] R.P. Mensink, M.B. Katan, Effect of dietary fatty acids on serum lipids and lipoproteins, Arterioscler Thromb 12 (1992) 911–919.
[42] B.V. Howard, J.S. Hannah, C.C. Heiser, K.A. Jablonski, M.C. Paidi, L. Alarif, D.C. Robbins, W.J. Howard, Polyunsaturated fatty acids result in greater cholesterol lowering and less triacylglycerol elevation than do monounsaturated fatty acids in a dose-response comparison in a multiracial study group, Am J Clin Nutr 62 (1995) 392–402.
[43] P. Mata, O. Varela, R. Alonso, C. Lahoz, M. de Oya, L. Badimon, Monounsaturated and polyunsaturated n-6 fatty acid-enriched diets modify LDL oxidation and decrease human coronary smooth muscle cell DNA synthesis, Arterioscler Thromb Vasc Biol 17 (1997) 2088–2095.
[44] P.D. Reaven, D. Tribble, Effects of vitamin E and oleic acid-rich diets on the susceptibility of LDL subfractions to oxidation, Circulation 88 (suppl II) (1993) 3033.
[45] C. Lee, J. Barnett, P.D. Reaven, Liposomes enriched in oleic acid are less susceptible to oxidation and have less proinflammatory activity when exposed to oxidizing conditions, J Lipid Res 39 (1998) 1239–1247.
[46] A. Bonanome, A. Pagnan, S. Biffanti, A. Opportuno, F. Sorgoto, M. Dorella, M. Maiorino, F. Ursini, Effect of dietary monounsaturated and polyunsaturated fatty acids on the susceptibility of plasma low density lipoproteins to oxidative modification, Arterioscler Thromb 12 (1992) 529–533.
[47] S. Tsimikas, A.P. Tsimikas, S. Alexopoulos, F. Sigari, C. Lee, P.D. Reaven, LDL isolated from Greek subjects on a typical diet or from American subjects on an oleate-supplemented diet induces less monocyte chemotaxis and adhesion when exposed to oxidative stress, Arterioscler Thomb Vasc Biol 19 (1999) 122–130.
[48] P.J. Ong, T.S. Dean, Hayward, P.L. Della Monica, T.A. Sanders, P. Collins, Effect of fat and carbohydrate consumption on endothelial function [letter], Lancet 354 (9196) (1999) 2134.
[49] M.A. Carluccio, M. Massaro, C. Bonfrate, L. Siculella, M. Maffia, G. Nicolardi, A. Distante, C. Storelli, C. De, Oleic acid inhibits endothelial activation: a direct vascular antiatherogenic mechanism of a nutritional component in the Mediterranean diet, Arterioscler Thromb Vasc Biol 19 (2) (1999) 220–228.
[50] M. Massaro, M.A. Carluccio, R. De Caterina, Direct vascular antiatherogenic effects of oleic acid: a clue to the cardioprotective effects of the Mediterranean diet, Cardiologia 44 (6) (1999) 507–513.
[51] M. Toborek, Y.W. Lee, R. Garrido, S. Kaiser, B. Hennig, Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cells, Am J Clin Nutr 75 (2002) 119–125.
[52] F. Achard, M. Gilbert, C. Benistant, S. Ben Slama, D.L. DeWitt, W.L. Smith, M. Lagarde, Eicosapentaenoic and docosahexaenoic acids reduce PGH synthase 1 expression in bovine aortic endothelial cells, Biochem Biophys Res Commun 241 (2) (1997) 513–518.
[53] M.N. Diaz, B. Frei, J.A. Vita, J.F.J. Keaney, Antioxidants and atherosclerotic heart disease, N Engl J Med 337 (1997) 408–417.
[54] A. Nègre-Salvayre, M.T. Pieraggi, L. Mabile, R. Salvayre, Protective effect of 17-beta-estradiol against the cytotoxicity of minimally oxidized LDL to cultured bovine aortic endothelial cells, Atherosclerosis 99 (1992) 207–217.
[55] J.H. Chin, S. Azhar, B.B. Hoffman, Inactivation of endothelium-derived relaxing factor by oxidized lipoproteins, J Clin Invest 89 (1992) 10–18.
[56] J.K. Liao, W.S. Shin, W.Y. Lee, S.L. Clark, Oxidized low-density lipoprotein decreases the expression of endothelial nitric oxide synthase, J Biol Chem 270 (1995) 319–324.
[57] M.T. Quinn, S. Parthasarathy, L.G. Fong, D. Steinberg, Oxidatively modified low density lipoproteins: a potential role in recruitment and retention of monocyte/macrophages during atherogenesis, Proc Natl Acad Sci USA 84 (1987) 2995–2998.
[58] K. Kugiyama, S.A. Kerns, J.D. Morrisett, R. Roberts, P.D. Henry, Impairment of endothelium-dependent arterial relaxation by lysolecithin in modified low-density lipoproteins, Nature 344 (1990) 160–162.
[59] L.L. Rudel, K. Kelley, J.K. Sawyer, R. Shah, M.D. Wilson, Dietary monounsaturated fatty acids promote aortic atherosclerosis in LDL receptor-null, human apoB 100-overexpressing transgenic mice, Arterioscler Thromb Vasc Biol 18 (1998) 1818–1827.
[60] J. George, M. Mulkins, S. Casey, R. Schatzman, E. Sigal, D. Harats, The effects of n-6 polyunsaturated fatty acid supplementation on the lipid composition and atherogenesis in mouse models of atherosclerosis, Atherosclerosis 140 (2000) 285–293.
[61] H. Li, Dietary regulation of arterial wall functions related to atherogenesis, Ph.D thesis submitted to Tufts University, 1993.
[62] A. Mortensen, P.L. Espensen, B.F. Hansen, P. Ibsen, The influence of dietary olive oil and margarine on aortic cholesterol accumulation in

cholesterol-fed rabbits maintained at similar plasma cholesterol level, Atherosclerosis 96 (1992) 159–170.

[63] P. Espensen-Leth, S. Stender, H. Ravn, K. Kjeldsen, Antiatherogenic effect of olive and corn oils in cholesterol-fed rabbits with the same cholesterol levels, Arteriosclerosis 8 (1988) 281–287.

[64] D. Kritchevsky, S.A. Tepper, D.M. Klurfeld, D. Vesselinovich, R.W. Wissler, Experimental atherosclerosis in rabbits fed cholesterol-free diets. Part 12. Comparison of peanut and olive oils, Atherosclerosis 50 (1984) 253–259.

[65] S.M. Royce, R.P. Holmes, T. Takagi, F. A. Kummerow, The influence of dietary isomeric and saturated fatty acids on atherosclerosis and eicosanoid synthesis in swine, Am J Clin Nutr 39 (1984) 215–222.

[66] T.Y. Toda, V.K. Yamamoto, F.A. Kummerow, Comparative study of the atherogenicity of dietary trans, saturated and unsaturated fatty acids on swine coronary arteries, J Nutr Sci Vitaminol 31 (1985) 233–241.

[67] D. Kritchevsky, L.M. Davidson, M. Weight, N.P.J. Kriek, J.P. duPlessis, Effect of trans unsaturated fats on experimental atherosclerosis in vervet monkeys, Atherosclerosis 51 (1984) 123–133.

[68] L.M. Alderson, L.M., K.C. Hayes, R.J. Nicolosi, Peanut oil reduces diet-induced atherosclerosis in cynomolgus monkeys, Arteriosclerosis 6 (1986) 465–474.

[69] P. Mata, R. Alonso, A. Lopez-Farre, J.M. Ordovas, C. Lahoz, C. Garces, C. Caramelo, R. Codoceo, E. Blazquez, M. de Oya, Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro, Arterioscler Thromb Vasc Biol 16 (1996) 1347–1355.

[70] R. De Caterina, M. Spiecker, G. Solaini, G. Basta., F., Bosetti, P. Libby, J. Liao, The inhibition of endothelial activation by unsaturated fatty acids, Lipids 34 (1999) 191S–193S.

[71] F. Perez-Jimenez, P. Castro, J.L. Miranda, E.P. Rojas, A. Blanco, F. L. Segura, F. Velasco, C. Marin, F. Fuentes, J.M. Ordovas, Circulating levels of endothelial function are modulated by dietary monounsaturated fat, Atherosclerosis 145 (1999) 351–358.

[72] B. Hennig, M. Toborek, G.A. Boissonneault, G.A. Shantha, N.C., E.A. Decker, P.R. Oeltgen, Animal and plant fats selectively modulate oxidizability of rabbit LDL and LDL-mediated disruption of endothelial barrier function, J Nutr 125 (1995) 2045–2054.