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

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Abstract **Although comparative studies of the cholesterolemic properties of *trans* fatty acids relative to *cis*-unsaturates and saturates have been conducted in humans and animals, there is no recent information relating these lipid responses to susceptibility to atherosclerosis. Therefore, hamsters were fed diets containing equivalent amounts of cholesterol (0.12% wt/wt) and test fats (20% wt/wt) for 8 weeks. Each test fat contained between 50–52% of the total triacylglycerols as a single fatty acid, i.e., 8:0, 14:0, 18:0, *cis*-18:1, or *trans*-18:1 while the balance consisted of 16:0, *cis*-18:1 and 18:2 that were the same for all groups. Plasma total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) levels were not different for 8:0, *cis*-18:1, and *trans*-18:1, whereas 14:0 caused a significant rise in plasma TC, LDL-C, and HDL-C. LDL oxidation measurements showed that the lag phase of conjugated diene formation was longest for the *trans*-18:1 and *cis*-18:1 groups while rate of conjugated diene formation was lowest for the *trans*-18:1 and *cis*-18:1 groups. The *trans*-18:1- and *cis*-18:1-fed animals had significantly higher levels of LDL α-tocopherol relative to the 8:0- and 14:0-fed animals. Aortic fatty streak formation was highest for the 14:0- and 8:0-fed animals and lowest for the *trans*-18:1. In conclusion, the plasma lipid and antioxidant properties of *trans*-18:1 and *cis*-18:1 were comparable while the *trans*-18:1-fed hamsters had the least amount of early atherosclerosis. In addition, 8:0-fed animals unexpectedly had early atherosclerosis formation similar to the 14:0-fed animals.—**Nicolosi, R. J., T. A. Wilson, E. J. Rogers, and 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.* 1998.** 39: **1972–1980.**

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It has been suggested that *trans*-unsaturated fatty acids resemble their saturated counterparts with regard to metabolism and utilization. The purpose of this study was to compare a common *trans* unsaturated fatty acid (*trans*-18:1) with a medium chain fatty acid (8:0), two longer chain fatty acids (14:0 and 18:0), and its corresponding *cis* isomer (*cis*-18:1).

Several studies have evaluated the effects of *trans* fatty acids on plasma lipids and lipoproteins in humans. Most studies comparing partially hydrogenated vegetable oil (PHVO) with their non-hydrogenated native oil counterpart showed that feeding PHVO resulted in higher plasma total cholesterol (TC) levels, whereas these levels were significantly less than those observed with highly saturated fat sources (e.g., butter, palm oil, coconut oil) (1–6). In other clinical studies in which all dietary variables were held constant except the fatty acids of interest, Mensink and Katan (7) and Judd et al. (8) showed that natural food diets enriched in *trans*-18:1 resulted in higher plasma TC and low density lipoprotein cholesterol (LDL-C) levels compared to *cis*-18:1, whereas compared to 12:0–16:0 saturated fatty acids (SFA), *trans*-18:1 resulted in lower TC and LDL-C levels, despite some inconsistencies between genders (7). In addition, *trans*-18:1 produced a plasma high density lipoprotein cholesterol (HDL-C) lowering effect compared to *cis*-18:1, 18:2, and 12:0–16:0 SFA (7–9), although this effect has not been consistently observed (5, 8, 10, 11). In another study, Zock and Katan (9) showed that *trans*-18:1 raised TC and LDL-C levels compared to 18:2 but not compared to 18:0.

Similar to numerous studies showing that 18:0 does not raise TC and LDL-C levels compared to other SFA, *trans* fatty acids may have cholesterol-raising effects compared to *cis*-18:1 and 18:2 fatty acids but the effect is less than that of the 12:0–16:0 SFA and may be similar to the effects of 18:0. However, current human study designs cannot unequivocally distinguish the independent plasma lipid effects of individual

Abbreviations: AT, α-tocopherol; CE, cholesteryl ester; FC, free cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; MCT, medium chain triacylglycerol; ORO, Oil Red O; PHVO, partially hydrogenated vegetable oil; PL, phospholipid; Prot, protein; SFA, saturated fatty acid; TAG, triacylglycerol; TC, total cholesterol.

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fatty acids or their atherogenic potential because of the limitations imposed by replacing one fatty acid for the other.

Mechanistic studies in hamsters by Woollett, Spady, and Dietschy (12) have shown that the 12:0, 14:0, and 16:0 SFA raise plasma LDL-C, down-regulate the hepatic LDL receptor system, and increase hepatic LDL production rate, whereas, 18:1 and possibly 18:2 have the opposite effect by up-regulating hepatic LDL receptors (13). On the other hand, SFA with chain lengths 6:0, 8:0, 10:0, and 18:0 did not perturb LDL receptor activity in either direction and did not alter plasma LDL-C levels (12). Woollett, Daumere, and Dietschy (14) recently reported that PHVO did not alter LDL receptor activity or plasma LDL-C levels and behaved in a fashion similar to 18:0 and suggested that these fatty acids have a neutral effect on LDL metabolism.

While the plasma lipid and lipoprotein effects of *trans* fatty acids have been evaluated to a great degree, very few well-controlled systematic studies have been conducted to establish whether they play a role in the atherogenic process or influence other end-points associated with the development of atherosclerosis such as the oxidative susceptibility of LDL. Early studies with rabbits (15–17), swine (18, 19) and vervet monkeys (20) showed no effect of *trans* fatty acids on atherosclerosis. However, a lack of systematic control over the fatty acid composition of the diet, as well as inconsistent demonstration of elevated plasma cholesterol, which is an essential component for inducing experimental atherosclerosis, prevented drawing meaningful conclusions from these studies. Therefore, the aim of the present hamster study was to compare the effects of triacylglycerols (TAG) enriched in *trans*-18:1 from PHVO, *cis*-18:1, 8:0 14:0, and 18:0 on plasma lipid and lipoprotein cholesterol levels, as well as on direct and indirect parameters related to atherosclerosis.

METHODS AND MATERIALS

Animals

One hundred male Golden Syrian hamsters (LVG strain, Charles River Labs, Wilmington, MA) approximately 10 weeks old and weighing 115 ± 11 g, were housed individually in hanging cages for 2 weeks before the initiation of the experimental diets. After approximately 2 weeks of acclimatization to a chow diet, the animals were placed on the specified experimental diets (20 animals per group) for an 8-week period. Experimental protocols were approved by the Institutional Animal Care and Use Committee. Animals were maintained in accordance with the guidelines of the Animal Care Committee at the University of Massachusetts-Lowell Research Foundation and the guidelines prepared by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW publication no. 85-23, revised 1985). Animals were housed in environmentally controlled conditions with an alternating 12-h light/dark cycle and given free access to food and water at all times except when food was withheld for experimental protocols described below.

Materials

The test fats that were incorporated into the diets were designed to contain a single predominant fatty acid, which differed for each experimental diet. The fatty acids: octanoic acid (8:0), myristic acid (14:0), and stearic acid (18:0) were provided by Henkel Corp., Emery Group (Cincinnati, OH). Purified TAG containing a common fatty acid were prepared from the above fatty acids by Abitech Corp. (Columbus, OH). Kraft Foods Technology Center (Glenview, IL) supplied two test fat blends: one was developed with high oleic sunflower oil containing high levels of *cis*-octadecenoic fatty acids (*cis*-18:1; n–9) and the other was made from a partially hydrogenated version of high oleic sunflower oil containing high levels of *trans*-octadecenoic fatty acids (*trans*-18:1; n–10, 16%; n–9, 19%; n–8, 26%; n–7, 22%; and n–6, 16%).

Experimental diets

An analysis of the *trans*-18:1 showed approximately 44% of the total fatty acids contribution was *trans*-18:1, while the balance was made up of: 16:0, 5%; 18:0, 7%; *cis*-18:1, 29%; 18:2, 11%; and 4% as other fatty acids. Based on the upper levels achievable for *trans*-18:1, all other test fats used in this study were designed to contain approximately 50% of the test fatty acid being evaluated and the same balance of the other fatty acids described for the *trans*-18:1 diet. The test fats were developed by blending amounts of purified TAG containing a common fatty acid (e.g., 8:0, 14:0, and 18:0) as well as high oleic sunflower oil and regular safflower oil in order to achieve targeted levels of fatty acids. **Table 1** shows the fatty acid composition of the TAG added to the five experimental diets. Analysis of α- and γ-tocopherol levels from aliquots of each diet preparation revealed no significant differences between diets with a mean ± SD of 5.6 ± 1.3 mg/100 g.

Dietary TAG blends were supplied to Research Diets, Inc., (New Brunswick, NJ) for blending and pelleting into a chow-based diet that consisted of 75% chow (Teklad, No. 8604w), 20% TAG blend, 5% maltodextrin, and 0.12% cholesterol and were stored at −20°C. Chow-based instead of semi-purified diets were chosen for study because previous work in our laboratory (21) and others (22) has demonstrated that feeding the former results in lipoprotein profiles (nonHDL > HDL) more similar to those of humans. An 8-week diet duration was chosen because previous studies from our laboratory (23–25) and others (26) have shown that diet-induced atherogenesis is observed in hamsters after 4–10 weeks of receiving a hypercholesterolemic diet.

TABLE 1. Fatty acid composition of the triacylglycerols (TAG) added to the various experimental diets

	Diet				
	8:0[a]	14:0[a]	18:0[a]	*cis*-18:1[a]	*trans*-18:1[a]
	% of total fatty acids				
8:0	47.4	0.2	0.2	0.2	0
10:0	0.05	0.03	0.02	0.04	0
12:0	0.04	0.45	0.14	0	0
14:0	0.11	44.5	0.28	0.13	0.13
16:0	5.5	5.7	7.4	5.25	5.5
16:1	0.13	0.14	0.27	0	0
17:0	0	0	0.07	0.02	0.03
18:1	5.7	5.5	48.0	5.6	7.0
cis-18:1	28.0	29.6	29.1	74.0	29.0
trans-18:1	0	0	0	0	44.0
18:2	11.6	12.7	12.0	12.8	11.3
18:3	0.67	0.7	1.3	0.85	0.6
20:0	0.17	0.15	0.44	0.29	0.35
22:0	0.33	0.33	0.73	0.8	0.93
Other	0.3	0.18	0.23	0.2	1.16
Total	100.0	100.0	100.0	100.0	100.0

[a] These fatty acids are the major acids in the respective triacylglycerols added to the diet.

Plasma lipid determinations

After an overnight fast (12 h), hamsters were anesthetized with a mixture of 50% oxygen and 50% carbon dioxide. Blood samples were collected from the retro-orbital sinus into heparinized capillary tubes. Whole blood samples were kept on ice until centrifugation. Plasma was prepared from whole blood by centrifugation at 1500 *g* at 4°C for 15 min. Plasma very low, low, and high density lipoprotein cholesterol (VLDL-C, LDL-C, and HDL-C) levels were measured enzymatically after ultracentrifugation (27) as previously reported (28). Plasma total cholesterol (TC) was measured by adding plasma VLDL-C, LDL-C, and HDL-C levels. Plasma TAG was measured enzymatically using previously reported methods (28). Plasma lipid determinations in our laboratory are standardized by participation in the Center for Disease Control–National Heart, Lung and Blood Institute Lipid Standardization Program.

Hepatic fatty acid and cholesterol analysis

For liver fatty acid analysis, liver tissue was extracted using a standard Folch (29) extraction technique as described below. One hundred mg (wet wt) of homogenized liver 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 sec. After 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 (30) 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.

Hepatic cholesterol levels were measured by a previously described method (31) with modification (32).

Plasma LDL analyses

Low density lipoprotein (LDL) (d 1.020–1.050 kg/L) was isolated by density gradient ultracentrifugation (27) from plasma pooled from 4 animals each (n = 5 pools/treatment group). LDL isolated by this method was free of other lipoproteins as judged by agarose electrophoresis (33). LDL protein concentrations were determined by the method of Markwell et al. (34) using bovine serum albumin as a standard. LDL lipid composition and fatty acid distribution, α-tocopherol levels, and LDL oxidation assays were also performed on these pools.

The composition of purified LDL was characterized by measurement of the total cholesterol and free cholesterol, TAG, phospholipid, and total protein content as we have previously described (35). Cholesteryl ester mass represents the difference between the total and free cholesterol after correction for fatty acid content.

For plasma LDL fatty acid analysis, a 300-μL aliquot of isolated plasma LDL was extracted using the same procedure as stated above for liver fatty acid analysis (29).

Plasma LDL α-tocopherol (AT) levels were determined 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 was 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 and injected into the HPLC. The HPLC conditions used were a modification (36) of the method of Kaplan et al. (37). 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.

LDL oxidation was measured as conjugated diene production by the method of Frei and Gaziano (38). 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 80 m $CuSO_4$, and 0.154 m NaCl. 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. (39). Parameters of the conjugated diene assay measured included lag phase (resistance to oxidation), propagation phase (rate of oxidation), and maximum dienes formed.

Dietary lipid absorption

Net lipid absorption was quantified by determining the amounts of dietary lipid consumed and the amounts excreted in the feces for each diet group. The amount of food consumed by each experimental diet group was measured over a 1-week period. During this period, three 24-h fecal collections were obtained. Aliquots of the diet and feces were oven-dried overnight and ground to a fine powder. Total lipid extraction with chloroform: methanol 2:1 was performed on the dried powder. After evaporation of the solvent, the amount of lipid in the diet and feces was determined gravimetrically. Apparent lipid absorption was estimated as the difference between dietary intake and fecal excretion and as percent absorption based on the calculation:

$$\text{Apparent lipid absorption (\%)} = \frac{[\text{g lipid consumed}] - [\text{g lipid excreted}]}{[\text{g lipid consumed}]} \times 100$$

Fecal neutral sterols were also measured in the 18:0 and 14:0 groups from fecal samples collected during the lipid absorption study.

Fecal neutral sterol measurements

Dry feces (200 mg) were extracted with 4 mL of methanol–water 80:20 for 1 h at 100°C in a 5-mL Reacti-vial fitted with a Mini-nert cap. Samples were then allowed to come to room temperature and centrifuged at 500 *g* at room temperature for 10 min. The supernatant was transferred to a clean screw-top glass vial. The fecal pellet was re-extracted using 4 mL of methanol–chloroform 50:50 followed by a third extraction with 4 mL of 1 m ammonium carbonate–methanol 20:80 under the same conditions as the first extraction. The three supernatants were subsequently pooled and evaporated to dryness under N_2.

Four mL of 0.1 N NaOH–ethanol 10:90 (by volume) was added to each sample and overlayered with N_2, and capped and heated at 100°C for 30 min. The samples were allowed to cool to room temperature followed by the removal of the solvent and transferred to 16 × 150 borosilicate tubes. Five mL of water and 3 mL of hexane were added to the solvent followed by vortexing and centrifugation at 500 *g* for 2 min. The hexane extraction was repeated twice more and pooled. To the hexane extract, 1 mL of 5-α-cholestane (240 μg/mL) was added as an internal standard and the solution was brought up to 10 mL with hexane in a volumetric flask. Exactly 2 mL was removed and evaporated to dryness at 100°C under N_2. One hundred μL of Tri-Sil reagent was added and the samples were capped. Samples were then heated at 85°C for 20 min, followed by evaporation under N_2 and reconstituted in 100 μL of methylene chloride. One μL was then in-

jected and analyzed using a Shimadzu GC-14A gas chromatograph with a flame ionization detector (Kyoto, Japan) using a 50 m × 0.2 mm HP-1 capillary column (Hewlett-Packard, Andover, MA). The injector and detector temperatures were set at 300°C. The initial column temperature was 220°C and was increased to 300°C at a rate of 2°C/min. The final temperature was held for 10 min. Column flow rate was 1.5 mL/min. Peak areas were quantitated using a Shimadzu CR501 integrator.

Aortic morphometric analysis

The quantitation of aortic fatty streak area in the hamster has been previously described (23, 24, 32).

Statistical analyses

Power calculations (80%) indicated that 20 animals were needed in each of the five diet groups to detect a significant ($P < 0.05$) difference in all the parameters measured. Analysis of variance (ANOVA) was utilized to analyze all data using the main effect of dietary fat type to determine whether there were differences in plasma lipids and other parameters that were measured. When significant main effects were detected ($P < 0.05$), a multiple means separation test was utilized to detect significant differences between diet groups. Homogeneity of variance was determined for all data and, in order to normalize the data and to stabilize the variance, all raw data were transformed to Log_{10} values before statistical analyses were performed. All data are presented as mean ± SEM. Correlations between early atherosclerosis and the various parameters were performed using Pearson's product–moment correlation coefficient. Because these correlations were conducted on pooled samples, all diet treatments were combined to provide sufficient sample size (40).

RESULTS

The data relating to 8-week food intake, final body weight, 8-week weight gain, feed efficiency, and dietary lipid absorption are presented in **Table 2**. Also, the various dietary treatments did not have a significant effect on food consumption (8:0, 9.3 ± 0.4; 14:0, 9.3 ± 0.2; 18:0, 9.8 ± 0.3; *cis*-18:1, 10.0 ± 0.4; and *trans*-18:1, 8.7 ± 0.3 g/d per animal) (mean ± SEM). Although there was a trend towards less food consumption in *trans*-18:1-fed animals especially compared to *cis*-18:1-fed animals, this difference was not significant. Final body weights, which ranged from 140 to 157 g, and weight gain, which ranged from 25 to 42 g, were generally lower in the 18:0-fed animals compared to all other treatments. Feed efficiency was reduced up to 40% in 18:0-fed animals compared to the other diet treatments. Similarly, the total amount of lipid absorbed ranged from 95 to 97% for all experimental diets except 18:0-fed animals in which only 85% absorption was observed. Because of the reduction in dietary lipid absorption in the 18:0-fed animals, fecal neutral sterols were examined in the 18:0-fed relative to the 14:0-fed animals (n = 5/group) to determine whether there was an accompanying increase in fecal neutral sterols, especially cholesterol excretion. Cholesterol excretion was increased 3-fold in the 18:0-fed animals (3.0 ± 0.15 mg/g feces) compared to 14:0-fed animals (1.0 ± 0.25 mg/g feces). Because of these significant differences in body weight, feed efficiency, % lipid absorption, and fecal cholesterol excretion of 18:0-fed animals relative to all other groups, the 18:0 group was excluded from all subsequent comparisons.

TABLE 2. Effect of test fats enriched in individual fatty acids on 8-week food intake, final body weight, 8-week weight gain, feed efficiency, and dietary lipid absorption

Diet	8-Week Food Intake	Body Weight[a]	8-Week Weight Gain	Feed Efficiency[b]	Lipid Absorption[c]
	g	*g*	*g*	*×10²*	*%*
8:0	521 ± 8[d]	153 ± 5[d]	38 ± 3[d,e]	7.29 ± 0.6[d]	95.8 ± 0.2[d]
14:0	522 ± 10[d]	155 ± 4[d]	40 ± 2[d]	7.70 ± 0.4[d]	97.3 ± 0.1[d]
18:0	549 ± 12[d]	140 ± 4[d]	25 ± 4[e]	4.60 ± 0.5[e]	84.4 ± 0.6[e]
cis-18:1	561 ± 7[d]	157 ± 5[d]	42 ± 5[d]	7.48 ± 0.6[d]	97.3 ± 0.1[d]
trans-18:1	488 ± 9[d]	148 ± 4[d]	33 ± 7[d,e]	6.80 ± 0.5[d]	94.5 ± 0.1[d]

Values represent mean ± SEM for 20 animals per group.
[a] Final body weights after 8 weeks.
[b] Feed efficiency = 8-week weight gain/8-week food intake.
[c] Based on calculation:

$$\%\ \text{absorption} = \frac{[\text{total lipid consumed (g)}] - [\text{total lipid excreted (g)}]}{[\text{total lipid consumed(g)}]} \times 100$$

[d,e] Values in a column not designated by the same superscript are significantly different at $P < 0.05$.

The plasma lipid and lipoprotein cholesterol levels and early atherosclerosis are shown in **Table 3**. As measurements of plasma lipid and lipoprotein cholesterol levels at 4, 6, and 8 weeks were not statistically different from each other, values represent the means of the three time points. The 8:0, *cis*-18:1, and *trans*-18:1 diets had significantly lower plasma TC (−21%, −26%, and −25%, respectively), LDL-C (−44%, −50%, and −50%, respectively), and HDL-C (−14%, −19%, and −37%, respectively) compared to 14:0-fed animals ($P < 0.05$) (Table 3). Plasma VLDL-C and TAG levels were not significantly altered by diet treatment (Table 3).

Aortic fatty streak area (early atherosclerosis) for *trans*-18:1-fed animals was significantly lower than that for the *cis*-18:1 (−57%), 14:0 (−70%), and 8:0 (−68%) diet treatments ($P < 0.05$) (Table 3). *Cis*-18:1-fed animals also had significantly less early atherosclerosis than animals receiving the 14:0 (−31%) and 8:0 (−27%) diet treatments ($P < 0.05$) (Table 3).

The *trans*-18:1-fed hamsters had significantly higher LDL TAG levels compared to the 14:0-fed hamsters (220%) ($P < 0.05$) (**Table 4**). No other differences were observed between groups for LDL composition (Table 4). Similarly, liver free cholesterol levels (mean ± SEM of 4.6 ± 0.2 mg/g of tissue) and cholesteryl ester levels (mean ± SEM of 5.2 ± 0.4 mg/g of tissue) were not significantly different between dietary treatments.

The fatty acid distribution of liver and plasma LDL are summarized in **Table 5** and **Table 6**, and generally reflect the fatty acid profile of the dietary fat with the exception of 8:0. Despite 8:0 composing 50% of one of the test fats, no detectable amounts of 8:0 were found in liver or LDL fatty acids. Not surprisingly, the enrichment of *trans*-18:1 in both plasma LDL and liver for *trans*-18:1-fed hamsters was at the expense of the *cis*-18:1.

Plasma LDL α-tocopherol (AT) levels and LDL oxidation parameters are shown in **Table 7**. Levels of LDL AT

TABLE 3. Effect of diets enriched in individual fatty acids on plasma lipids lipoprotein cholesterol levels (mmol/L) and early atherosclerosis (AFSA) ($\mu m^2/mm^2 \times 100$)

Diet	TC	VLDL-C	LDL-C	HDL-C	TAG	AFSA
			mmol/L			*$\mu m^2/mm^2 \times 100$*
8:0	5.4 ± 0.1[a]	0.9 ± 0.1[a]	0.9 ± 0.1[a]	3.7 ± 0.2[a]	10.5 ± 1.0[a]	16.1 ± 3.6[a]
14:0	6.8 ± 0.2[b]	1.0 ± 0.2[a]	1.6 ± 0.2[b]	4.3 ± 0.2[b]	10.9 ± 0.8[a]	17.2 ± 2.5[a]
cis-18:1	5.0 ± 0.1[a]	0.7 ± 0.1[a]	0.8 ± 0.1[a]	3.5 ± 0.2[a]	9.4 ± 1.0[a]	11.8 ± 2.5[b]
trans-18:1	5.1 ± 0.2[a]	1.1 ± 0.1[a]	0.8 ± 0.1[a]	2.7 ± 0.1[a]	8.7 ± 0.6[a]	5.2 ± 1.6[c]

Values represent mean ± SEM for 5 pools of 4 animals/group, except values for TAG and AFSA which were derived from 20 animals/group.

[a,b,c] Values in a column not designated by the same superscript are significantly different at $P < 0.05$.

between the *trans*-18:1- and *cis*-18:1-fed hamsters was comparable and significantly greater than the 8:0 (44% and 33%, respectively) and 14:0 (41% and 30%, respectively) dietary treatments ($P < 0.05$) (Table 7). The lag phase of conjugated diene formation were comparable between *trans*-18:1- and *cis*-18:1-fed hamsters and significantly greater than the 8:0 (26% and 28%, respectively) and 14:0 (19% and 21%, respectively) dietary treatments ($P < 0.05$) (Table 7). The propagation phase of the conjugated diene formation for the 14:0-fed hamsters was significantly greater than the *trans*-18:1 (121%), *cis*-18:1 (158%), and 8:0 (72%) dietary treatments ($P < 0.05$) (Table 7). Maximum dienes formed were comparable between the *trans*-18:1 and *cis*-18:1 groups and significantly reduced relative to the 8:0 (−34% and −18%, respectively) and 14:0 (−32% and −17%, respectively) dietary treatments ($P < 0.05$) (Table 7).

Correlations on pooled LDL samples were performed on combined diet treatments to provide adequate sample size and are presented in **Table 8**. Early atherosclerosis was significantly correlated with the lag phase ($r = -0.51$, $P < 0.03$), rate of LDL conjugated diene formation ($r = 0.52$, $P < 0.02$), and maximum dienes formed ($r = 0.43$, $P < 0.04$). The correlation between early atherosclerosis and LDL-C ($r = 0.39$, $P < 0.1$) just missed statistical significance.

DISCUSSION

The aim of this study was to evaluate the independent effects of individual fatty acids on plasma lipids, LDL antioxidant (AT) levels, LDL oxidative susceptibility, and early atherosclerotic responses of hamsters fed test fats enriched in *trans*-18:1, *cis*-18:1, 8:0, 14:0, and 18:0 fatty acids. TAG enriched with 14:0 was chosen as the representative saturated fat because its hypercholesterolemic response is either equal to 12:0 and 16:0 (41) or greater than 12:0 and 16:0 (42). TAG enriched in 18:0 was chosen to represent neutral fats as previous studies in humans (41, 42) and animals (12–14) indicated it to be neutral or even hypocholesterolemic (43). TAG enriched in 8:0 was also chosen as neutral fat because of the experimental studies in hamsters (12–14).

The finding that % lipid absorption, body weight, and fecal cholesterol excretion were significantly different for the 18:0-fed animals relative to the other groups is in disagreement with studies in humans (43) and animals (14), but is consistent with other studies as reviewed by Kritchevsky (44). A possible explanation for the differences in dietary lipid and cholesterol absorption for the 18:0 group in the present hamster study and the results of Woollett et al. (14) may be housing conditions. Unlike the present study in which animals were individually caged to reduce coprophagy and permit individual determinations of food consumption, weight gain, and fecal lipid and cholesterol absorption, the studies of Woollett et al. (14) utilized dietary groups, which comprised single boxes of 6 animals housed together. Not only did this treatment prevent individual determination of the parameters mentioned above, but the highly coprophagous nature of hamsters involving, in this case, feces enriched in lipid and cholesterol, possibly compromised the assessment of the magnitude of absorption versus excretion of dietary lipid and cholesterol.

In agreement with several human (1–8) and other animal studies (14, 45), the TAG enriched with *trans*-18:1 as well as the other fatty acids investigated in the present study significantly lowered plasma TC and LDL-C relative to saturates such as 14:0. Similarly, the comparable reductions of plasma HDL-C in hamsters fed *trans*-18:1 and the other TAG enriched with different fatty acids relative to the 14:0 group are consistent with data from other studies comparing saturated and unsaturated fatty acids in animals (46, 47) and humans (3, 4, 7–11). On the other hand, the finding that plasma HDL-C levels in *trans*-18:1- and *cis*-18:1-fed hamsters were similar is consistent with some human studies (4, 8, 10, 11), but not others (7, 9). The finding that TAG enriched in 8:0 fatty acids had similar plasma TC relative to *cis*-18:1 is not consistent with the

TABLE 4. Effect of triacylglycerols enriched in individual fatty acids on plasma LDL composition

Diet	FC	CE	TAG	PL	Prot
			%		
8:0	8.3 ± 0.4	49.3 ± 3.1	2.9 ± 0.8[a,b]	23.1 ± 1.6	16.3 ± 1.1
14:0	9.6 ± 0.3	43.3 ± 1.4	1.5 ± 0.7[a]	26.2 ± 1.2	19.4 ± 1.0
cis-18:1	8.4 ± 0.5	46.6 ± 1.9	2.1 ± 0.8[a,b]	25.5 ± 1.1	17.4 ± 1.3
trans-18:1	8.5 ± 0.4	46.2 ± 1.6	4.8 ± 1.1[b]	23.3 ± 1.1	17.2 ± 1.3

Values represent mean ± SEM for 5 pools of 4 animals per group; FC, free cholesterol; CE, cholesteryl ester; TAG, triacylglycerols; PL, phospholipid; Prot, protein.

[a,b] Values in a column not designated by the same superscript are significantly different at $P < 0.05$.

TABLE 5. Hepatic total fatty acid composition

Diet	14:0	16:0	18:0	*trans*-18:1	*cis*-18:1	18:2	Other
				%			
8:0	0.2 ± 0.01	13 ± 0.2	13 ± 0.2	ND	42 ± 0.6	15 ± 0.1	16 ± 0.1
14:0	2.0 ± 0.01[a]	14 ± 0.1[b]	13 ± 0.2	ND	36 ± 0.6	17 ± 0.2	18 ± 0.1
cis-18:1	0.2 ± 0.01	11 ± 0.1	11 ± 0.2	ND	53 ± 0.8[a]	11 ± 0.2[a]	9 ± 0.1[a]
trans-18:1	0.2 ± 0.01	11 ± 0.1	13 ± 0.2	8 ± 0.1[a]	31 ± 0.5	19 ± 0.1	16 ± 0.1

Values represent mean ± SEM for 20 animals/group; ND, not detected; other, 16:1, 18:3, 20:1, 20:3, 20:4, and 22:6.

[a] $P < 0.05$ when compared to other groups.

[b] $P < 0.05$ when compared to *cis*-18:1 and *trans*-18:1.

recent human studies of Cater, Heller, and Denke (48) which demonstrated the cholesterol-raising properties of 8:0 and 10:0 relative to high oleic sunflower oil.

Our finding that feeding TAG enriched in *trans*-18:1 did not significantly elevate plasma TC, VLDL-C, and LDL-C relative to *cis*-18:1 is in disagreement with several human studies (7–9), one rabbit study (15), and the recent hamster studies of Woollett et al. (14). On the other hand, our findings of comparable plasma lipid responses between *cis* unsaturated fatty acids and *trans*-18:1 are similar to other studies in hamsters (45), rabbits (17), swine (19), and monkeys (20). While an explanation for the disparity in results is not readily apparent, one possibility is that most animal and human studies substitute the cholesterol-lowering *cis* unsaturated fatty acids for *trans*-18:1 making it difficult to determine whether the effects of *trans*-fatty acids on plasma lipids occur independently or are the result of reducing the dietary content of cholesterol-lowering unsaturated fatty acids. For example, in a previous study by Mensink and Katan (7) the increase in *trans* fatty acid content (0–11% of energy) was also associated with a 45% decrease in oleate content (49). Support for this notion comes from the human studies of Zock and Katan (9) which showed that *trans*-18:1 raised plasma TC and LDL-C compared to 18:2 but not to 18:0, considered to be a neutral fatty acid, i.e., neither raising nor lowering plasma TC and LDL-C levels. It should also be mentioned that in this study (9), the *trans* fatty acid diet also had 68% less linoleate relative to the linoleate content. Similarly, the reason for the incongruence of some of the results, i.e., similar plasma LDL-C response between *cis*-18:1 and *trans*-18:1 and the increased fecal lipid excretion of 18:0 relative to the other fatty acids of the present study and those of Woollett et al. (14), is equally puzzling as the same hamster species and diet treatments were used in both studies. Possible explanations could include *a*) biochemical parameters were performed in fasted animals in the present study compared to the non-fasted state in the studies of Woollett et al. (14) and *b*) housing conditions in which animals in the present study were individually housed in hanging cages versus group housing (14). Animals in grouped housing conditions eat more and develop greater hyperlipidemia, especially hypertriglyceridemia (50). This provides an explanation for the higher plasma TC and LDL-C levels in the hamster studies of Woollett et al. (14) relative to the present study.

One previous study (11) has reported the possible differences in LDL oxidative susceptibility between groups fed either *trans*-18:1 or *cis*-18:1. In this study (11) and the present study, no differences were observed in any measured parameter of LDL oxidation between diets containing *trans*-18:1 and *cis*-18:1. It is not surprising that *trans*-18:1 and *cis*-18:1 show comparable oxidative resistance, as there are numerous studies which have demonstrated that LDL enriched in monounsaturated fatty acids are resistant to oxidation compared to polyunsaturated fatty acids (51, 52). These results may be related to the increased levels of LDL AT levels of both the *trans*-18:1 and *cis*-18:1 relative to the 8:0 and 14:0 groups as AT is considered to be a major antioxidant in LDL (53). However, the finding that the propagation phase was greatly increased in the 14:0-fed animals, especially relative to the 8:0 which had similar LDL AT levels, was unexpected. It could be related to fatty acid chain length although we are unaware of any studies investigating this possibility. On the other hand, there are studies that have demonstrated that LDL AT concentration is not apparently associated with increased resistance to LDL oxidation in individuals not consuming AT supple-

TABLE 6. LDL total fatty acid composition of major fatty acids

Diet	14:0	16:0	18:0	*trans*-18:1	*cis*-18:1	18:2	Other
				%			
8:0	0.23 ± 0.03	20.9 ± 1.4	15.1 ± 1.4	ND	25.8 ± 1.2	27.7 ± 0.8	10.1 ± 0.4
14:0	1.80 ± 0.20[a]	19.3 ± 2.6	12.7 ± 0.5	ND	23.2 ± 1.8	32.1 ± 0.8[b]	10.7 ± 0.2
cis-18:1	0.18 ± 0.03	19.0 ± 0.5	14.3 ± 0.4	ND	32.2 ± 1.9[a]	23.7 ± 0.7	10.5 ± 0.4
trans-18:1	0.26 ± 0.03	17.0 ± 0.7	13.2 ± 0.1	7.8 ± 0.4[a]	24.4 ± 1.0	30.1 ± 1.3	7.2 ± 0.3[a]

Values represent mean ± SEM for 5 pools of 4 animals per pool per group; ND, not detected; other, 16:1, 20:4, and 22:6.

[a] $P < 0.05$ when compared to other groups.

[b] $P < 0.05$ when compared to *cis*-18:1.

TABLE 7. Effect of triacylglycerols enriched in individual fatty acids on LDL α-tocopherol (AT) and various parameters of conjugated diene formation

Diet	LDL AT	Lag Phase	Propagation Phase	Maximum Dienes Formed
	ng/μg LDL protein	*min*	*nmol/min/mg LDL protein*	*nmol/mg LDL protein*
8:0	5.8 ± 0.4[a]	135 ± 9[a]	1.8 ± 0.2[a]	730 ± 25[a]
14:0	6.1 ± 0.4[a]	148 ± 7[a]	3.1 ± 0.4[b]	715 ± 16[a]
cis-18:1	8.7 ± 1.0[b]	188 ± 3[b]	1.2 ± 0.1[a]	595 ± 39[b]
trans-18:1	10.4 ± 2.0[b]	182 ± 9[b]	1.4 ± 0.3[a]	484 ± 25[b]

Values represent mean ± SEM for 5 pools of 4 animals/group.
[a,b] Values in a column not designated by the same superscript are significantly different at $P < 0.05$.

ments (54, 55). This may partially explain the lack of a significant correlation between early atherosclerosis and LDL AT levels observed in the present study. The apparent increase in LDL AT and greater resistance to oxidation of both *cis*-18:1 and *trans*-18:1, relative to 8:0 and 14:0, is not easily explained. It might be related to the hypothesis of Spritz and Mishkel (56) suggesting that LDL lipids enriched in unsaturated fatty acids occupy more space than the tightly compacted LDL particles composed of saturated fatty acids. Thus, this may allow more AT to accumulate in LDL particles with unsaturated lipids thereby increasing resistance to oxidation. However, this hypothesis does not explain the greater susceptibility of polyunsaturated versus monounsaturated fatty acids (51, 52). Another possibility, although speculative, for the decreased early atherosclerosis in the *trans*-18:1-fed animals may be the result of AT effects on gene expression, which may be independent from its reduction in LDL oxidation.

The finding that *trans*-18:1- and *cis*-18:1-fed animals had less severe atherosclerosis than the saturated 14:0 group is consistent with several animal studies (15–20), and may be related to the lower plasma LDL-C levels of the latter relative to the former, although a recent atherosclerosis study in monkeys did not show a protective effect of *cis*-18:1 versus SFA (47). The finding that feeding *trans*-18:1 to hamsters was not associated with increased early atherosclerosis is consistent with some human studies of *trans* fatty acid intake and cardiovascular disease in humans, but not with others as reviewed by Zock and Katan (57). The findings in hamsters need to be qualified as only the earliest events of atherosclerosis (fatty streak formation) were investigated. However, the human studies reviewed by Zock and Katan (57) that showed a positive association with *trans* fatty acid intake and cardiovascular disease must also be interpreted cautiously as it was the intake of *trans*-16:1 from animal and dairy products and not *trans*-18:1 from the hydrogenation of margarine in which the association occurred.

TABLE 8. Correlations (*r*) between early atherosclerosis, plasma and lipoprotein cholesterol, LDL α-tocopherol (AT), and various parameters of conjugated diene formation

	Early Atherosclerosis		LDL AT	
Variable	*r*	*P*	*r*	*P*
TC	0.12	NS	—	
LDL-C	0.39	<0.1	−0.25	NS
HDL-C	0.10	NS	—	
LDL AT	−0.06	NS	—	
Lag phase	−0.51	<0.03	0.22	NS
Propagation phase	0.52	<0.02	−0.34	NS
Maximum dienes formed	0.47	<0.04	−0.43	<0.07

Values are derived from all diet treatments combined, i.e., 5 pools of 4 animals each × 4 diet treatments (n = 20 pools).

The fact that swine fed hydrogenated soybean oil had less coronary artery intimal thickening than animals fed *cis*-18:1-rich safflower oil (19), despite similar plasma cholesterol levels, does not support a simple direct LDL-C versus early atherosclerosis relationship as an explanation for the lower fatty streak area for the *trans*-18:1-fed animals. The association between LDL oxidative resistance and early atherosclerosis as measured by lag phase and rate of LDL conjugated diene formation suggests a more complex interaction between plasma LDL-C levels, oxidative stress, and possibly LDL size and will require further study. The comparable atherosclerosis between the 14:0- and 8:0-fed animals has not been reported before and is not explained easily by any of the parameters measured in this study. One could speculate that the decreased levels of LDL AT and increased LDL oxidative susceptibility of the 8:0 and 14:0 groups relative to either the *trans*-18:1- or *cis*-18:1-fed animals might partially explain the difference in atherosclerosis susceptibility. The studies reported in this communication demonstrate that *trans*-18:1-fed animals had significantly less atherosclerosis than 14:0-fed animals. Moreover, the lipid response, antioxidant levels, and degree of LDL oxidative resistance between *trans*-18:1- and *cis*-18:1-fed animals were comparable. The comparable atherogenicity of the 8:0- and 14:0-fed animals is not readily explained by any of the parameters we have measured and will require further investigation.

In conclusion, there is convincing evidence that the hypercholesterolemic properties of animal fats and certain saturated vegetable oils are associated with atherosclerosis. On the other hand, the results of the present study in hamsters are consistent with numerous studies in rabbits, pigs, and monkeys and suggest little, if any, differences in early atherosclerosis incidence or severity between non-hydrogenated and hydrogenated vegetable oils containing high concentrations of *trans* fatty acids. Future studies should examine the relationship between *trans* fatty acid intake and atherosclerosis using various animal species and greater diet duration for study of more advanced atherosclerosis.

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