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# Hyperlipidemic effects of *trans* fatty acids are accentuated by dietary cholesterol in gerbils

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Trans *isomers of dietary fatty acids, generated during the commercial hydrogenation of unsaturated fats, may contribute to coronary heart disease (CHD) in humans by interfering with lipid metabolism. To examine this possibility in a fat-sensitive model, the Mongolian gerbil (*Meriones unguiculatus*) was used to compare the cholesterolemic and triglyceridemic potential of modest increments of* trans *fatty acids from partially hydrogenated soybean oil with other saturated fatty acids in the presence and absence of dietary cholesterol. Age-, dose-, and time-dependent effects were examined in weanling, 6-month-old, and 1-year-old gerbils. Although lipoprotein metabolism in weanling gerbils was initially refractory to* trans *fat, even as perturbations by saturated fatty acids were demonstrable, these gerbils eventually (after 16 weeks) developed a* trans*-induced hypercholesterolemia that was intermediate between the response to 16:0 and 12:0 + 14:0. The hepatic and plasma 18:1/18:2 cholesteryl ester (CE) ratio was depressed by* trans *in a manner similar to saturated fatty acids. The 6-month-old gerbils readily developed hypertriglyceridemia but not hypercholesterolemia, again revealing a decrease in the plasma 18:1/18:2 CE ratio. The 1-year-old gerbils revealed a dose-related (0, 5, 10%en as* trans*) elevation in total cholesterol (TC), and especially triglycerides (TG), that was accentuated by 0.04% dietary cholesterol. Increases in plasma lipids were again accompanied by a significant decrease in the mass of hepatic esterified cholesterol, particularly 18:1-cholesteryl esters. Thus, dietary* trans*-fatty acids induce age-, time-, and dose-dependent modulations in gerbil plasma lipids associated with decreased 18:1 cholesteryl esters. Further investigation with gerbils may reveal mechanisms by which* trans *fat consumption disturbs lipoprotein metabolism.* (J. Nutr. Biochem. 6:353–361, 1995.)

**Keywords:** *trans* fatty acids; Mongolian gerbil; hepatic cholesteryl esters; hypertriglyceridemia

## Introduction

*Trans* fatty acids are geometric isomers of unsaturated fatty acids formed during hydrogenation to "harden" fats for margarines, shortenings, frying oils, and other related products.[1] They were developed as a substitute for naturally saturated fats, such as butter, lard, and tallow, which generally have been considered atherogenic by comparison. The normal bend (*cis* isomer) in the carbon chain disappears, leaving a rigid structure not unlike a saturated fatty acid. In fact, hydrogenation of polyunsaturated oils increases stability and provides a more desirable, butter-like consistency to the fat.

The amounts of *trans* fatty acids available for consumption in the United States is presently estimated to account for an average of 3 to 5%en (8 to 12 g *trans*) per person per day.[1,2] This figure is controversial, as some sources imply that 6 to 8%en (15 to 20 g *trans*) is a more accurate estimate of consumption.[3,4] Since these are averages, some individuals may consume substantially more *trans* fatty acids, up to 15%en (36 g *trans*) per day.

Both epidemiologic and clinical studies in humans indicate that consumption of *trans* fatty acids adversely affects coronary heart disease (CHD) risk,[5,6] in part by increasing plasma concentrations of total cholesterol (TC) or low density lipoprotein cholesterol (LDL-C)[5–13] and triglycerides

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(TG)[10,12,13] and decreasing HDL-C.[5–7,9,10,13] On the contrary, several human studies[14–19] reported that dietary *trans* fat had no adverse effects on plasma lipids; however, the latter studies utilized diets that did not carefully exchange *trans*-18:1 for *cis*-18:1 or they contained very low or no dietary cholesterol.[17,20] In addition, previous test diets typically compared a hydrogenated oil (*trans*) with the original (unhydrogenated) oil, so that the concentrations of several other fatty acids differed, particularly replacing polyunsaturated fatty acids (POLYs) with saturated fatty acids (SATs).[14–16] Other fat blends compared a control fat (*cis*-18:1-rich) with a *trans*-rich fat that was also relatively enriched with POLYs.[17–19] The point is that in most previous *trans*-fat studies, multiple changes in fatty acids occurred along with the *trans* fatty acids so that the intrinsic effect elicited by *trans* was dubious.

Specific mechanisms by which *trans* might influence lipoprotein and cholesterol metabolism remain obscure, in part because most previous *trans* fat diets have included cholesterol, which precludes assessing the independent influence of *trans* fat from that of a dietary cholesterol synergism, a possible interaction noted previously in humans.[20] For example, a recent hamster study concluded that dietary *trans*-18:1 potentiated the hypercholesterolemic effect of dietary cholesterol by increasing plasma LDL-C secondary to decreased LDL receptor activity.[21] But both diets included an excessive cholesterol load for hamsters (0.08% in the control diet and 0.18% in test diets)[22] and *trans* was not isocalorically substituted for *cis*-18:1 or specific saturated fatty acids. Instead, 10% hydrogenated corn oil was simply added to the control diet.

A critical aspect of assessing the mechanism for a specific metabolic process is finding an animal that responds appropriately. Recent data[22] suggest that the plasma lipid response to dietary fatty acids by Mongolian gerbils is more sensitive than that of humans, both in the presence and absence of dietary cholesterol, even though the nature of the response seems identical across species.[22–27] Accordingly, the present study examined lipoprotein metabolism in gerbils consuming a moderate amount of *trans* fat (as partially hydrogenated soybean oil), both with (0.04%, equivalent to 95 mg/1,000 kcal) and without dietary cholesterol. The objective was to determine whether this species would be sensitive to *trans* fat intake and how the response might compare with diets rich in different saturated fatty acids.

## Methods and materials

### *Animals, diets, and basic design*

The composition of all purified diets fed in the three experiments is delineated in *Table 1*. The first experiment directly compared a reasonable intake of *trans* fat with saturated fatty acids in weanling gerbils, each bled three times throughout the 16-week period. The *trans* fat in these studies was prepared by partial hydrogenation of soybean oil using a killed catalyst and slow hydrogenation in order to yield an iodine value of 84, i.e., close to that of olive oil. The fatty acid profile is shown in *Table 2*. In Experiment 1, 50 weanling male gerbils (45 to 55 g at the start of the study) were fed four cholesterol-free diets: one rich in *cis*-18:1, a second containing 7.5%en as *trans*-18:1 exchanged for *cis*-18:1 (with 0.4%en from *trans*-18:2 isomers), a third rich in 16:0, and a fourth rich in 12:0 + 14:0 fatty acids.

Since *trans* appeared to have significant effects on hepatic lipids but showed only a relative increase in plasma cholesterol after prolonged feeding (16 weeks) in these young gerbils, the second experiment aimed to determine whether 6-month-old gerbils would respond to *trans* fatty acids more quickly. Thus, 20 6-month-old male (80 to 100 g) gerbils were fed cholesterol-free diets, one rich in *cis*-18:1 or another containing 7.5%en as *trans*-18:1 exchanged for *cis*-18:1.

A third experiment in still older gerbils included two modest levels of *trans* fat in the presence or absence of dietary cholesterol. This experiment was undertaken to examine the dietary cholesterol effect as well as the progressive sensitivity of the response to *trans* with increasing gerbil age that was observed in the first two experiments. Accordingly, 72 1-year-old male (80 to 100 g) Mongolian gerbils (Tumblebrook Farm, West Brookfield, MA) were divided among six diet treatments containing 0, 5, or 10%en as *trans*-18:1. The *trans*-18:1 was isocalorically exchanged for *cis*-18:1, and diets contained either 0.04% cholesterol (95 mg/1,000 kcal, i.e., approximately 200 mg/day human equivalent on a caloric basis or 1,300 mg/day on a relative body weight basis) or no dietary cholesterol.

All gerbils were given free access to water and fed 7 g of diet (185 kcal/kg of body wt for adults, based on an average body wt of 90 g; 325 kcal/kg of body wt for growing gerbils, with body wt averaging 50 g) per day, which was predetermined as adequate for maintenance of body weight between 80 to 100 g in adult gerbils and for growth in younger gerbils. If fed ad libitum, approximately one-third of the animals will overindulge and develop severe hypertriglyceridemia and cholesterolemia. Each diet provided 42%en as fat and approximately 4.2%en from polyunsaturates (*Table 1*). Diets were formulated such that all levels of vitamins, minerals, fiber, protein, carbohydrates, and fat were equal between diets, the only difference being the exchange between particular fatty acids according to experimental protocol. By fatty acid analysis of the diets, total saturates, monos, and polys in the *cis*- and *trans*-18:1 diets were, indeed, similar. In the 16:0-rich and 12:0 + 14:0-rich diets, those fatty acids were also substituted specifically for *cis*-18:1 (monounsaturate). Animals were fasted individually overnight in hanging cages for 16 hr prior to obtaining blood, which was achieved by cardiac puncture under $O_2/CO_2$ (1:1) anesthesia. Each gerbil was bled at each time point as indicated, being terminally exsanguinated at the end of a study.

### *Fatty acid analysis*

Fatty acid analysis of fats (and formulated diets as fed) utilized the one-step transesterification method of Lapage and Roy.[28] Gas-liquid chromatography (GLC) of fatty acid methyl esters was performed on a Hewlett-Packard 5790A GLC (Hewlett-Packard Co., Avondale, PA USA) using fused silica capillary columns (SP-2330 and SP-2430, Supelco Inc., Bellefonte, PA USA). Content of *trans* fatty acids in partially hydrogenated soybean oil (PHSBO) was determined by GLC of fatty acid methyl esters on a Shimadzu GC9-AM GLC using a 100-m fused silica capillary column (SP-2560, Supelco Inc.).

### *Plasma lipid analyses*

Total plasma cholesterol (TC), high density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured by an enzymatic/colorimetric assay, TC and TG using Sigma kits (procedures 352 and 336, respectively; Sigma Diagnostics Co., St. Louis, MO USA). HDL-C was assayed in the supernatant after sodium phosphotungstate-$Mg^{2+}$ precipitation of apoE- and apoB-containing lipoproteins from plasma samples (procedure 543004; Boehringer Mannheim Diagnostics, Indianapolis, IN USA). This procedure does not always correlate exactly with lipoproteins separated by ultracentrifugation (described below) but can be used as a crude estimate of relative changes in HDL-C (unpublished data).

**Table 1** Composition of purified diets and fatty acid profile

| Ingredient | Diet | | | | | |
|---|---|---|---|---|---|---|
| | *cis*18:1 rich | *trans*-18:1 (5.0% en) | *trans*-18:1 (7.5% en) | *trans*-18:1 (10.1% en) | 16:0 rich | 12:0+14:0 rich |
| Casein (g/kg) | 222 | 222 | 222 | 222 | 222 | 222 |
| Cornstarch (g/kg) | 230 | 230 | 230 | 230 | 230 | 230 |
| Glucose (g/kg) | 133 | 133 | 133 | 133 | 133 | 133 |
| Cellulose (g/kg) | 150 | 150 | 150 | 150 | 150 | 150 |
| Mineral mix* (g/kg) | 50 | 50 | 50 | 50 | 50 | 50 |
| Vitamin mix** (g/kg) | 12 | 12 | 12 | 12 | 12 | 12 |
| Choline chloride (g/kg) | 3 | 3 | 3 | 3 | 3 | 3 |
| Cholesterol (g/kg) | 0 or 0.4 | 0 or 0.4 | 0 | 0 or 0.4 | 0 or 0.4 | 0 |
| **Total fat (g/kg)** | **200** | **200** | **200** | **200** | **200** | **200** |
| Olive oil | 136 | 136 | 64 | 88 | — | — |
| PHSO◇ | — | 48 | 74 | 96 | — | — |
| Safflower oil | — | — | 12 | 8 | 15 | 25 |
| Coconut oil | — | 8 | — | 8 | — | 175 |
| Tallow† | 48 | 8 | 30 | — | — | — |
| Palm stearin | — | — | — | — | 185 | — |
| Butter fat† | 16 | — | 20 | — | — | — |
| **Fatty acids (% en)** | | | | | | |
| **≤12:0** | 0.2 | 0.9 | 0.4 | 0.9 | 0.1 | 21.0‡ |
| 14:0 | 0.5 | 0.4 | 0.5 | 0.3 | 0.4 | 7.4 |
| 16:0 | 8.7 | 6.5 | 7.6 | 5.5 | 24.3 | 3.9 |
| 18:0 | 1.7 | 1.3 | 1.7 | 1.3 | 1.3 | 0.8 |
| **Total SFAs** | **11.1** | **9.1** | **10.2** | **8.0** | **26.1** | **33.1** |
| 16:1 | 0.6 | 0.4 | 0.3 | 0.2 | 0.0 | 0.0 |
| *cis*-18:1 | 23.7 | 20.4 | 16.8 | 15.2 | 9.6 | 2.5 |
| *trans*-18:1 | 0.0 | 5.0 | 7.5 | 10.1 | 0.0 | 0.0 |
| **Total MONOs** | **24.3** | **25.8** | **24.6** | **25.5** | **9.6** | **2.5** |
| *cis*-18:2 | 4.1 | 4.0 | 3.9 | 3.8 | 4.1 | 4.2 |
| *trans*-18:2 | 0.0 | 0.3 | 0.4 | 0.5 | 0.0 | 0.0 |
| 18:3n3 | 0.1 | 0.1 | 0.1 | 0.1 | 0.0 | 0.0 |
| **Total PUFAs** | **4.2** | **4.4** | **4.4** | **4.4** | **4.1** | **4.2** |

Diets were fed as gel blocks, prepared by withholding from the formulation 60 g/kg of cornstarch and premixing it with 800 ml of boiling water, to form a gel to which the remaining ingredients were added.
*Ausman-Hayes mineral mix F 8530, BioServe (Frenchtown, NJ USA).
**Hayes-Cathcart vitamin mix (Hayes et al., J. Nutr. 1989, 199, 1776–1736).
◇PHSO = partially hydrogenated soybean oil; see *Table 2* for fatty acid composition.
†Tallow and butterfat are cholesterol stripped (Omega Source Corp., Burnsville, MN, USA).
‡Amount of C12:0 = 18.5% en, with remainder <12:0.

### *Lipoprotein profiles*

Plasma lipoprotein fractions were separated by discontinuous gradient ultracentrifugation according to the method of Redgrave et al.[29] with modifications.[30] The samples were centrifuged for 24 hr in an SW-41 Ti rotor (Beckman Inc., Palo Alto, CA USA) at 35,000 rpm and 15°C in a Beckman L5-50 Preparative Ultracentrifuge. Lipoprotein fractions were collected at pre-established densities[31]: Very low density lipoprotein (VLDL), $d < 1.006$; low density lipoprotein (LDL), $1.009 < d < 1.055$; HDL, $1.063 < d < 1.21$ g/ml. These fractions were dialyzed for at least 24 hr against 3 L of saline (0.15 M NaCl, 1 mM ethylene diaminetetracetate [EDTA] at pH 7.4).

### *Plasma and hepatic cholesteryl ester determination*

Plasma-free and esterified cholesterol were extracted by adding 0.2 mL of plasma to 5.0 mL of a solution of isopropanol and 0.75 M NaOH (2:1, vol/vol) and vortexing for 15 sec. After 2 min, 2 mL of n-octane was added and vortexed again for 60 sec. After brief centrifugation at 500*g*, a 1 mL aliquot of the *n*-octane (upper) layer was evaporated to dryness under a gentle stream of $N_2$, and the residue was redissolved in isopropanol for HPLC injection.[32] Hepatic cholesterol was extracted[33] by grinding a 100 mg piece of liver with anhydrous sodium sulfate and extracting three times with chloroform:methanol (2:1, vol/vol). The liquid phase was evaporated to dryness and redissolved in isopropanol, from which

**Table 2** Fatty acid composition of partially hydrogenated soybean oil (trans fat) used in these gerbil experiments*

| Fatty acid (% distribution) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 14:0 | 16:0 | 18:0 | 18:1(c)† | 18:1(t) | 18:1(i) | 18:2(c/c) | 18:2(c/t) | 18:2(t/t) | 18:3 | 20:0 |
| 0.6 | 10.6 | 5.3 | 12.7 | 49.9 | 17.0 | 1.1 | 0.7 | 1.1 | 0.5 | 0.5 |

*See methods for details of hydrogenation.
†Parentheses denote type of isomer: (c) = *cis*, (t) = *trans*, (i) = intermediate (i.e., *cis* and *trans* peaks overlapping).

an aliquot was taken and analyzed enzymatically/colorimetrically for total cholesterol exactly like plasma lipids (Sigma Kit no. 352). Another aliquot of the hepatic extract was used for HPLC analysis of free and esterified cholesterol by redissolving in isopropanol. Both plasma and hepatic cholesterol extracts were resolved on a Waters Radial-Pak Resolve C18 cartridge (8 mm × 10 cm, part 84720) in a Radial Compression Module, eluting isocratically with acetonitrile:isopropanol (45/55). The absorbance was measured at a wavelength of 210 nm, and free cholesterol as well as individual cholesteryl esters were calculated in reference to known standards.[32] Esterified cholesterol (EC) was calculated by subtracting the FC (HPLC) from TC (enzymatic kit). Percentages and ratios of individual cholesteryl esters (CE) were determined by comparing the areas of individual esters resolved on the chromatogram.

### *Statistical analysis*

Statistical analysis was performed on a Macintosh LCIII (Apple Computer Inc., Cupertino, CA USA) using StatView SE+Graphics software package (Brain Power Inc., Calabasas, CA USA). One-way factorial analysis of variance (ANOVA) was utilized for Experiments 1 and 2, while two-way ANOVA was utilized for Experiment 3 to assess both the fatty acid and cholesterol effects.

## Results

### Trans *effect in weanling gerbils (Experiment 1)*

In the first experiment *trans* fatty acids (7.5%en) were compared with specific saturated fatty acids (16:0, 12:0+14:0) and to *cis*-18:1 (control) in weanling gerbils. Consumption of *trans* fat led to a gradual rise in TC which was ultimately intermediate between the TC response to the 12:0+14:0-rich and 16:0-rich diets. The true relative effects between the dietary fatty acids became evident only after 16 weeks (*Table 3*) because the TC and %HDL-C varied considerably between 4 and 8 weeks for most diet groups before stabilizing. After 16 weeks, TC in the saturated fatty acid–rich groups was 20 to 35 mg/dL lower on average than the 4 week values, whereas TC in the *trans* fat group became progressively elevated (+26 mg/dL) with time. It is noteworthy that in these young gerbils fed *trans*, essentially all the increase in TC was attributable to HDL-C (precipitation method) in comparison to the *cis*-fed animals. The 18:1/18:2 ratio for plasma CE also reflected *trans* intake, being depressed in the *trans* group (0.62) almost as much as that in the 12:0+14:0 group (0.39), compared with the *cis*-18:1 control (1.30) or 16:0 group (1.04) (*Table 3*). Although individual variation in TG values precluded significant differences between the *cis* and *trans* groups after 16 weeks, the average TG for gerbils on the *trans* diet was 64% higher than the *cis* group. Thus in young *trans*-fed gerbils, VLDL-C contributed more to the plasma cholesterol value than was the case in *cis*-fed controls.

After 16 weeks, liver total cholesterol was slightly lower in gerbils fed either the *trans* or the 12:0+14:0-rich diet relative to the *cis*-18:1 diet (*Table 4*). Although this was attributed entirely to lower CE concentrations (specifically to *cis*-18:1-CE), the mass of hepatic 18:2-CE was un-

**Table 3** Plasma lipids after 4, 8, and 16 weeks from 1-month-old gerbils fed diets containing *cis* or *trans* 18:1, 16:0, or 12:0+14:0 without cholesterol (Experiment 1)

| | Diets | | | |
|---|---|---|---|---|
| Plasma lipids | *cis* | *trans* (7.5% en) | 16:0 rich | 12:0+14:0 rich |
| **4 weeks** | | (mg/dL) | | |
| TC | $97 \pm 15^{ab}$ | $94 \pm 14^{cdx}$ | $137 \pm 17^{acex}$ | $165 \pm 20^{bde}$ |
| TG | $61 \pm 17^{a}$ | $81 \pm 29$ | $86 \pm 28^{x}$ | $106 \pm 34^{a}$ |
| HDL-C† | $68 \pm 13^{ab}$ | $71 \pm 10^{cd}$ | $94 \pm 19^{acexy}$ | $129 \pm 18^{bde}$ |
| | | (percent) | | |
| HDL-C | $72 \pm 7$ | $77 \pm 9^{ax}$ | $66 \pm 4^{abx}$ | $80 \pm 6^{bx}$ |
| **8 weeks** | | (mg/dL) | | |
| TC | $94 \pm 22^{a}$ | $92 \pm 15^{by}$ | $123 \pm 22^{c}$ | $160 \pm 37^{abc}$ |
| TG | $108 \pm 76$ | $111 \pm 47$ | $78 \pm 29$ | $137 \pm 80^{x}$ |
| HDL-C† | $64 \pm 23^{a}$ | $62 \pm 8^{bx}$ | $68 \pm 13^{cx}$ | $98 \pm 43^{abc}$ |
| | | (percent) | | |
| HDL-C | $70 \pm 10^{a}$ | $68 \pm 5^{bx}$ | $54 \pm 4^{abcx}$ | $66 \pm 12^{cx}$ |
| **16 weeks** | | (mg/dL) | | |
| TC | $87 \pm 11^{a}$ | $120 \pm 34^{xy}$ | $102 \pm 26^{bx}$ | $145 \pm 25^{ab}$ |
| TG | $55 \pm 19$ | $90 \pm 49$ | $53 \pm 7^{x}$ | $65 \pm 16^{x}$ |
| HDL-C† | $51 \pm 2^{ab}$ | $85 \pm 15^{ax}$ | $60 \pm 9^{cy}$ | $94 \pm 12^{bc}$ |
| | | (percent) | | |
| HDL-C | $59 \pm 5^{a}$ | $71 \pm 3^{ab}$ | $60 \pm 2^{b}$ | $65 \pm 2$ |
| | | (ratio) | | |
| 18:1/18:2 CE§ | $1.30 \pm 0.54^{a}$ | $0.62 \pm 0.02$ | $1.04 \pm 0.12$ | $0.39 \pm 0.03^{a}$ |

Values are mean ± SD ($n$ = 8 to 10).
$^{a,b,c,d,e}$Means in a row sharing a common superscript are significantly different ($P < 0.05$) using one-way ANOVA and Sheffe's $F$-test.
$^{x,y}$Means in a column sharing a common superscript are significantly different ($P < 0.05$) using one-way ANOVA and Sheffe's $F$-test.
†HDL-C obtained by PTA-Mg precipitation and are considered relative estimates of HDL-C, at best.
§$n$ = 4.

**Table 4** Liver lipids after 16 wks from 1-month-old gerbils fed diets containing *cis* or *trans* 18:1, 16:0 or 12:0+14:0 all without cholesterol (Experiment 1)

| Liver lipids | Diets | | | |
|---|---|---|---|---|
| | *cis* | *trans* (7 5% en) | 16:0 rich | 12:0+14:0 rich |
| | | (mg/g) | | |
| Total cholesterol | 10.7 ± 2 9 | 7.0 ± 1.4 | 9.0 ± 3.1 | 7.0 ± 0.5 |
| EC | 7.1 ± 2 1 | 3 6 ± 1.1 | 5 5 ± 2.4 | 3.4 ± 0.2 |
| FC | 3.6 ± 0.8 | 3.4 ± 0.3 | 3.5 ± 0.8 | 3.6 ± 0.5 |
| CE profile | | (percent distribution) | | |
| 20:4 | 1 ± 1 | 1 ± 1 | 2 ± 1 | 2 ± 1 |
| 18:2 | 8 ± $1^{ab}$ | 18 ± $1^{ac}$ | 10 ± $1^{cd}$ | 17 ± $2^{bd}$ |
| c18:1 | 85 ± $2^{abc}$ | 73 ± $4^{ade}$ | 60 ± $4^{bd}$ | 57 ± $5^{ce}$ |
| 16:0 | 3 ± 1 | 5 ± 1 | 26 ± 6 | 15 ± 1 |
| | | (ratio) | | |
| 18:1/18:2 | 10 7 ± $0.9^{abc}$ | 4 0 ± $0.2^{ad}$ | 5.9 ± $0.9^{bde}$ | 3.5 ± $0.4^{ce}$ |

Values are mean ± SD ($n$ = 3 to 4).
[a,b,c,d,e]Means in a row sharing a common superscript are significantly different ($P < 0.05$) using one-way ANOVA and Scheffe's $F$-test.

changed by either *trans* or 12:0+14:0 diets compared with the *cis* diet. Thus, similar to plasma, a decrease in the ratio of hepatic 18:1/18:2 CE reflected a specific decrease in the *cis*-18:1 CE pool present in gerbils fed the *trans* or the saturated fat diets.

## Trans *effect in 6-month-old gerbils (Experiment 2)*

In the first experiment, *trans* fatty acids appeared to affect lipoprotein metabolism as the gerbils matured. Thus, the second experiment utilized more mature, 6-month-old gerbils fed *trans* (7.5%en) or *cis*-18:1 diets without cholesterol for 8 weeks (*Table 5*). Although lipid values did not differ significantly between the *cis* and *trans* groups at 4 or 8 weeks, the TG value after 8 weeks was significantly elevated compared with the 4 week value in *trans*-fed gerbils. The elevations in HDL-C (by precipitation method) and TC observed in weanling gerbils (Experiment 1) were not evident in these older gerbils after 8 weeks. However, a significant decrease in the plasma 18:1/18:2 CE ratio was again observed in gerbils fed the *trans* fatty acid diet.

**Table 5** Plasma lipids after 4 and 8 weeks from 6-month-old gerbils fed diets containing *cis* or *trans* 18.1 without cholesterol (Experiment 2)

| Plasma lipids | Diets | |
|---|---|---|
| | *cis* | *trans* (7.5% en) |
| **4 weeks** | (mg/dL) | |
| TC | 85 ± 13 | 98 ± 16 |
| TG | 52 ± 12 | 65 ± $21^{x}$ |
| **8 weeks** | (mg/dL) | |
| TC | 102 ± 44 | 102 ± 20 |
| TG | 151 ± 121 | 223 ± $87^{x}$ |
| HDL-C† | 63 ± 30 | 61 ± 9 |
| | (percent) | |
| HDL-C | 60 ± 3 | 55 ± 17 |
| | (ratio) | |
| 18:1/18:2 CE§ | 1.15 ± $0.02^{a}$ | 0.73 ± $0 14^{a}$ |

Values are mean ± SD ($n$ = 8 to 10).
[a]Means in a row sharing a common superscript are significantly different ($P < 0.05$) using one-way ANOVA and Sheffe's $F$-test.
[x]Means in a column sharing a common superscript are significantly different ($P < 0.05$) using one-way ANOVA and Sheffe's $F$-test.
†HDL-C obtained by PTA-$Mg^{2+}$ precipitation.
§$n$ = 3.

## Trans *effect in fully matured gerbils (Experiment 3)*

To further explore the effect of age and the relative load of *trans* fat on the lipoprotein response, the final experiment assessed the impact of graded *trans*-fat intake over time in 1-year-old gerbils while examining the additional impact of 0.04% dietary cholesterol. Plasma lipid values were affected by all three factors, i.e., the level of dietary *trans*-18:1, the length of dietary feeding, and the addition of dietary cholesterol (*Table 6*).

Over the 8-week trial, the TC and HDL-C concentrations in gerbils fed *cis*-18:1 without cholesterol were stable. When 0.04% cholesterol was added to the control *cis*-18:1 diet, TC became significantly elevated only after 8 weeks, indicating a progressive response to dietary cholesterol. Major increases in circulating cholesterol mass occurred in VLDL-C and HDL-C.

Incremental increases in dietary *trans* intake resulted in higher plasma TC, but only after 8 weeks in the absence of dietary cholesterol. However, when cholesterol was added, gerbils fed the high-*trans* (10%en) diet developed significant TC and TG elevations within 4 weeks. Elevations in plasma TG followed the same dietary pattern, but the increases were even more striking, especially among individual gerbils. The TG increases were associated with an expansion of VLDL-C and large standard deviations in TG. LDL-C represented only 7 to 15% of TC, a percentage that was unaffected by either *trans* or cholesterol feeding. In contrast, the percentage of HDL was clearly depressed by the addition of dietary cholesterol in both *cis* and *trans* groups. Noteworthy was the telltale decrease in the plasma

**Table 6** Plasma lipids after 4 and 8 wks from 1-year-old gerbils fed diets containing *cis* or two levels of *trans* 18:1 with 0.04% or without cholesterol (Experiment 3)

| | Diets | | | | | |
|---|---|---|---|---|---|---|
| | No cholesterol | | | 0.04% cholesterol | | |
| Plasma lipids | *cis* | *trans* 5.0%en | *trans* 10.1%en | *cis* | *trans* 5.0%en | *trans* 10.1%en |
| **4 weeks** | | | (mg/dL) | | | |
| TC | 88 ± 28 | 87 ± 25 | 83 ± 16$^{x}$ | 87 ± 19$^{x}$ | 79 ± 23$^{x}$ | 123 ± 21$^{a}$ |
| TG | 122 ± 85 | 93 ± 42$^{x}$ | 154 ± 73$^{y}$ | 57 ± 32$^{y}$ | 96 ± 53$^{y}$ | 432 ± 204$^{a}$ |
| HDL-C† | 65 ± 19 | 60 ± 16 | 58 ± 12 | 50 ± 12 | 51 ± 11 | 71 ± 13$^{a}$ |
| | | | (percent) | | | |
| HDL-C | 75 ± 8 | 69 ± 7 | 73 ± 5 | 58 ± 7 | 66 ± 6 | 57 ± 9 |
| **8 weeks** | | | (mg/dL) | | | |
| TC$^{bc}$ | 81 ± 22 | 104 ± 20 | 145 ± 24$^{x}$ | 123 ± 18$^{x}$ | 165 ± 43$^{x}$ | 136 ± 36 |
| TG$^{b}$ | 64 ± 27 | 192 ± 131$^{x}$ | 314 ± 199$^{y}$ | 124 ± 83$^{y}$ | 261 ± 140$^{y}$ | 358 ± 296 |
| VLDL-C§ | 18 ± 1 | 27 ± 17 | 62 ± 19 | 45 ± 9 | 70 ± 53 | 63 ± 31 |
| LDL-C§ | 12 ± 1 | 9 ± 3 | 10 ± 4 | 12 ± 2 | 16 ± 1 | 16 ± 5 |
| HDL-C$^{bc}$§ | 50 ± 9 | 65 ± 3 | 83 ± 5 | 65 ± 3 | 101 ± 6 | 83 ± 11 |
| | | | (percent) | | | |
| VLDL-C | 23 ± 3 | 22 ± 14 | 39 ± 8 | 37 ± 4 | 34 ± 17 | 39 ± 4 |
| LDL-C | 15 ± 1 | 9 ± 3 | 7 ± 2 | 10 ± 1 | 9 ± 2 | 10 ± 3 |
| HDL-C | 62 ± 4 | 66 ± 9 | 54 ± 9 | 53 ± 3 | 57 ± 15 | 51 ± 7 |
| | | | (ratio) | | | |
| 18:1/18:2 CE$^{b}$‡ | 1.70 ± 0.40 | 1.06 ± 0.27 | 1.03 ± 0.11 | 1.76 ± 0.26 | 1.48 ± 0.22 | 1.13 ± 0.11 |

Values are mean ± SD ($n$ = 9 to 12).
[a]Significant ($P < 0.01$) interaction term for *trans* X dietary cholesterol revealed by two-way ANOVA.
[b]Significant ($P < 0.01$) effect of fat revealed by two-way ANOVA.
[c]Significant ($P < 0.0005$) effect of cholesterol by two-way ANOVA.
[x,y]Means in a column sharing a common superscript are significantly different ($P < 0.05$) using one-way ANOVA and Sheffe's $F$-test.
†HDL-C obtained by TPA-$Mg^{2+}$ precipitation.
§HDL-C, LDL-C and VLDL-C obtained by discontinuous gradient ultracentrifugation, $n$ = 3.
‡$n$ = 5.

18:1/18:2 CE ratio as the *trans* intake increased. Overall in this experiment the impact of dietary *trans* fatty acids on plasma lipids was more pronounced than that of 0.04% dietary cholesterol, i.e., the magnitude of the increases in TG and TC associated with *trans* feeding per se were greater than the modest exacerbations induced by the addition of dietary cholesterol alone, at least at 8 weeks. However, cholesterol supplementation was required to elicit a *trans*-fat effect after only 4 weeks.

The concentration of hepatic total cholesterol and CE decreased significantly as the percent of energy from *trans* fatty acids increased (*Table 7*). These decreases coincided with a significant depression in the 18:1/18:2 ratio for hepatic CE, reflecting a decline in the mass of *cis*-18:1 CE which was evident both with and without dietary cholesterol. By contrast, the actual mass of 18:2-CE was unchanged, or slightly increased, by increasing *trans* fat consumption.

## Discussion

Recent reports have raised concerns that *trans* fatty acids may have a negative impact on lipid metabolism and plasma lipoproteins to enhance CHD risk.[5,6,9,10] Accordingly, the present study explored the feasability of utilizing the gerbil, a species whose plasma lipids are extremely sensitive to dietary fatty acid saturation,[22–27] as a model for the study of *trans* fatty acid metabolism. The current experiments had four overall objectives. The first was to determine whether a reasonable intake of dietary *trans* fatty acids would affect plasma lipoproteins and liver lipids. A second was to assess whether age-related metabolic dynamics would affect the response. The third was to note any time-dependent aspects of *trans* fat on lipoprotein metabolism, and fourth was to determine whether dietary cholesterol would affect *trans* fat metabolism. The data indicate that even modest consumption (5 to 7%en) of *trans* fatty acids by gerbils, when compared with *cis* isomers of oleic acid, results in elevated plasma lipids (especially triglycerides) and depressed hepatic 18:1-cholesteryl esters. Furthermore, mature gerbils responded more rapidly and demonstrably than growing animals, and the *trans* fat–induced lipemia was exacerbated by dietary cholesterol.

### *Triglyceridemia versus cholesterolemia*

These data revealed a disturbance in lipoprotein metabolism by *trans* fat intake on two fronts. On the one hand, a "low impact" effect was noted in young gerbils fed 7.5%en as *trans*, where the increase in TG was modest when compared with the progressive rise in TC associated with apoB + E-rich lipoproteins. The *trans* effect on hepatic cholesterol metabolism was evidenced by the distorted CE ratio (decreased 18:1 mass) and depressed hepatic CE pool. In so far as *trans* fat increased plasma CE and decreased liver CE while distorting the 18:1/18:2 CE ratio, it resembled certain

**Table 7** Liver lipids after 8 weeks from 1-year-old gerbils fed diets containing *cis* or two levels of *trans* 18:1 with or without cholesterol (Experiment 3)

| | Diets | | | | | |
|---|---|---|---|---|---|---|
| | No cholesterol | | | 0.04% cholesterol | | |
| Liver lipids | *cis* | *trans* 5.0%en | *trans* 10.1%en | *cis* | *trans* 5.0%en | *trans* 10.1%en |
| | | | (mg/g) | | | |
| Total cholesterol[a] | 11.6 ± 2.2 | 6.3 ± 1.5 | 7.0 ± 1.5 | 14.0 ± 2.3 | 11.3 ± 3.3 | 7.6 ± 1.3 |
| EC[a] | 8.7 ± 1.7 | 3.0 ± 1.4 | 3.8 ± 1.0 | 10.8 ± 2.1 | 7.8 ± 3.0 | 4.0 ± 1.2 |
| FC | 2.9 ± 0.5 | 3.2 ± 0.5 | 3.1 ± 0.5 | 3.3 ± 0.2 | 3.5 ± 0.3 | 3.6 ± 0.3 |
| CE profile | | | (percent distribution) | | | |
| 20:4 | 2 ± 2 | 3 ± 1 | 3 ± 1 | 1 ± 1 | 2 ± 1 | 3 ± 1 |
| 18:2[a] | 9 ± 2 | 12 ± 2 | 19 ± 2 | 8 ± 1 | 14 ± 1 | 18 ± 2 |
| 18:1[ab] | 82 ± 3 | 74 ± 6 | 67 ± 3 | 88 ± 1 | 80 ± 1 | 73 ± 4 |
| 16:0 | 7 ± 2 | 11 ± 5 | 12 ± 4 | 3 ± 1 | 4 ± 1 | 7 ± 3 |
| | | | (ratio) | | | |
| 18:1/18:2[a] | 9.3 ± 1 8 | 6.2 ± 1.6 | 3.6 ± 0.4 | 11.3 ± 1.0 | 5.9 ± 0.6 | 4.0 ± 0.4 |

Values are mean ± SD ($n$ = 5).
[a]Significant ($P < 0.0001$) effect of fat revealed by two-way ANOVA.
[b]Significant ($P < 0.0001$) effect of cholesterol by two-way ANOVA.

aspects of saturated fatty acids, causing less of a "saturated fat" effect than 12:0 + 14:0 but more than 16:0. In contrast to *trans* fat, the impact of the saturates was immediate but abated with time, whereas the lipemia from *trans* fat progressively worsened. Furthermore, the increase in plasma TC associated with *trans* or 12:0 + 14:0 in young gerbils (Experiment 1) was largely accounted for by HDL-C, suggesting that $LDL_r$ activity was not a major factor for either group of fatty acids in gerbils. It might be anticipated that LDL receptor modulation would play a minimal role in this model, considering that gerbil LDL-C represents only 10 to 15 mg/dL while HDL-C typically represents 50 to 100 mg/dL. However, this lack of effect on $LDL_r$ activity does not concur with certain reports of the chow-fed hamster response to saturated fatty acids[34,35] or the hamster response to *trans*,[21,36] even though the LDL/HDL ratio in hamsters is very similar to gerbils. By contrast, in humans where LDL-C predominates, saturated fat may contribute to hypercholesterolemia by down-regulating LDL receptors.[37]

On the other hand, a second scenario surfaced in the lipoprotein profile of the "high impact" *trans* effect, observed best in the fully mature gerbils (Experiment 3). In those gerbils, a plasma cholesterol increase was secondary to a striking rise in plasma TG that represented an exaggeration of the triglyceride response described in humans.[8,10,13] In fact, the hypertriglyceridemic effect of 10%en from *trans* was greater than that elicited by 28%en from the most cholesterolemic saturated fatty acids (12:0 + 14:0) (Experiment 1). The triglyceridemic effect of *trans* became evident more quickly and severely as gerbil age increased and in the presence of dietary cholesterol. Preliminary investigations suggest that an increased VLDL secretion rate may contribute to this effect. Because triglyceridemia was most striking in mature animals (up to 5-fold increase) with "full" adipose reserves and was much less apparent in young growing gerbils with expanding adipose pools, it is possible that insulin resistance and poor TG removal, much like Type IV hyperlipidemia in humans,[38] also may by involved. Lichtenstein et al.[12] recently reported that *trans* fat specifically increased VLDL-C in middle-aged to elderly, moderately hypercholesterolemic men and women. Mensink and Katan[10] elicited a significant triglyceridemic effect by feeding an 11% *trans*-18:1 diet to young men and women (mean age 25 years). It was noteworthy in the present study that *trans* fat fed to 6-month-old gerbils for 8 weeks (Experiment 2) caused the plasma TG to rise without an apparent increase in plasma TC, revealing a transition from modulation of TC (Experiment 1) to a primary effect on triglyceride and VLDL metabolism evident in fully mature gerbils (Experiment 3).

At first glance these gerbil lipoprotein results appear dissimilar in two respects to the human response to *trans*, i.e., in humans HDL-C decreases and LDL-C increases with only a modest change in VLDL.[10,11,13] Increased cholesteryl ester transfer protein (CETP) activity has been proffered as an explanation for this shift in lipoprotein cholesterol.[39] Although the absolute mass of HDL-C increased in gerbils fed *trans*, the percentage of HDL-C actually decreased relative to the apoB–rich lipoproteins (mostly VLDL-C since LDL-C did not change). In this sense, overall lipoprotein dynamics (i.e., relative expansion of the apoB lipoprotein pool) were similar to those in humans. The latter may inherently convert much more VLDL to LDL than gerbils do.

### *Dietary cholesterol impact on* trans

Dietary cholesterol exacerbated the *trans*-induced lipemia as well as causing cholesterolemia in its own right. Mature gerbils (Experiment 3) fed dietary cholesterol exhibited significant hypercholesterolemia and hypertriglyceridemia in response to *trans* fat (10%en) after only 4 weeks, whereas those fed cholesterol-free diets did not respond until 8 weeks. Previous inference that humans were unresponsive to *trans* may reflect the lack of dietary cholesterol or limited duration of study.[17,20] The accentuation of the *trans* effect by cholesterol is similar to the synergism initially reported

in humans[20] or in hamsters, where Hayashi et al.[21] demonstrated that hydrogenated corn oil (*trans*-containing fat) enhanced the cholesterolemic effect of dietary cholesterol by increasing plasma VLDL-C and LDL-C secondary to down-regulation of LDL receptors (presumably from the added cholesterol). Unfortunately, the contribution of *trans* fat alone could not be assessed in hamsters because a cholesterol-free, *trans*-rich diet was not tested.

### *Hepatic lipids*

Moderate consumption of *trans* fatty acids, both in the presence and absence of dietary cholesterol (Experiment 3), significantly decreased hepatic CE concentration and the oleate-to-linoleate (18:1/18:2) liver cholesteryl ester ratio in comparison with the *cis*-18:1 diet. The shift represented a decreased mass of *cis*-18:1 CE without affecting the 18:2-CE mass. These changes in CE due to *trans* were similar to those observed with the 12:0 + 14:0 saturated fat diet in the absence of dietary cholesterol (Experiment 1). Decreases in total hepatic CE and the 18:1/18:2 CE ratio were also described[34] for hamsters whose $LDL_r$ activity was reduced by dietary triglycerides rich (15%en) in myristic acid (14:0, the most cholesterolemic saturate) in comparison with a diet rich in a monounsaturated fatty acid (*cis*-18:1). The decrease in hepatic 18:1-CE by dietary *trans* is even more striking when one considers the minimal dietary myristate present and, more importantly, the substantial amount of *cis*-18:1 still included in our *trans* diets (>16%en). Apparently, *trans*-18:1 is not metabolized like *cis*-18:1 by the liver and, like 12:0 + 14:0,[40] markedly interferes with hepatic (and possibly plasma) CE metabolism by being a poor substrate for ACAT in animals[36] and possibly in humans, since *trans* fat also depresses the 18:1/18:2 CE ratio in human plasma.[20] Substituting *trans* for *cis* effectively nullified any beneficial impact of a high dietary *cis*-monounsaturate-to-saturate ratio on lipoprotein metabolism, a dietary ratio that tends to be inversely related to plasma lipids in hamsters as well as humans.[35]

### *Possible mechanism*

*Trans* fat consumed in modest amounts (10%en) by humans appears to increase the concentrations of atherogenic LDL-C and triglycerides while decreasing the antiatherogenic HDL-C.[5,6,10,13] The mature gerbil revealed that *trans* fatty acids not only increased plasma TC (especially the percentage of VLDL-C) and triglyceride concentrations, but these atypical fatty acids also decreased the hepatic CE concentration (specifically *cis*-18:1 esters) in comparison with dietary *cis* isomeric equivalents. Apparently the *trans* fatty acids in our hydrogenated soybean oil preparation exerted a profound effect on TG metabolism and may have raised plasma (and HDL) cholesterol levels indirectly by increasing VLDL production. Preliminary results of an increased TG secretion rate in gerbils fed *trans* strengthen this hypothesis.

Hepatic differences in CE metabolism further suggest that *trans* affected ACAT (acyl-CoA: cholesterol acyltransferase) and cholesterol esterification, a process that is driven by the intrahepatic availability of free cholesterol. As indicated earlier, the enzyme prefers *cis*-18:1 as substrate over *trans*-18:1 or saturated fatty acids[36] and in vivo hamster studies[34,36] revealed that the both *trans*-18:1 and saturated fatty acids increased plasma LDL-C associated with concomitant decreases in hepatic CE and $LDL_r$ activity when compared with *cis*-18:1. An inverse correlation was noted between increased plasma TC or TG and decreased hepatic CEs in gerbils fed diets rich in *trans* or saturated fat (12:0 + 14:0) (Experiment 1). This suggests that *trans* may depress hepatic ACAT activity in a manner similar to certain saturates. However, our observation that the time-related *increase* in TC of weanling gerbils fed *trans* was opposite to the tendency toward *decreased* TC during saturated fat intake suggests that *trans* fatty acids are not metabolized exactly like saturates, and their effect may be cumulative.

In addition to the lipoprotein effects, *trans* fatty acids have been shown to inhibit adenylate cyclase activity in cardiac membranes of rats in comparison with *cis* isomeric equivalents[47] and to adversely affect reproduction in rats[42] and mice.[43] The *trans* fatty acids in partially hydrogenated soybean oil have also been found to interfere with essential fatty acid metabolism in rats.[44] Other reports[45–47] indicate that *trans* fatty acids are less effective in mediating cell signal transduction in comparison with *cis* fatty acids.

In summary, short-term feeding of a modest level of dietary *trans* fatty acids produces substantial metabolic effects, and our sequential evaluation of lipoprotein metabolism suggests that these effects may be exaggerated during prolonged consumption. These studies in gerbils, like previous human studies of the lipoprotein response to *trans* fat, indicate that substitution of predominately *trans*-monounsaturated fatty acids for *cis*-unsaturates adversely affects lipoprotein metabolism. Subsequent studies will be required to determine which *trans* isomers are involved and whether the response in gerbils relates to the lipoprotein changes described in humans.

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