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Dietary trans-18:1 raises plasma triglycerides and VLDL cholesterol when replacing either 16:0 or 18:0 in gerbils

Vasuki Wijendran[1,a], Andrzej Pronczuk[a], Constantin Bertoli[b], K. C. Hayes[a,*]

[a]*Foster Biomedical Research Lab, Brandeis University, Waltham, MA, USA*
[b]*Nestlé Product Technology Center, Kemptthal, Switzerland*

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Abstract

To compare the relative impact of trans-18:1 with the two main dietary saturated fatty acids it replaces, plasma lipid response was assessed in Mongolian gerbils fed diets rich in 16:0 (24%en),18:0 (10%en), or trans-18:1 (4 or 6%en). The diets were designed such that the 18:0-rich diet substituted 7%en as 18:0 for 16:0, whereas 4%en and 6%en from trans-18:1 was substituted for 16:0 in the two trans diets. The control group was fed a diet formulated according to the fatty acid balance of American Heart Association (AHA), but provided 40%en as fat. Gerbils ($n = 10$ per dietary group) were fed one of the five diets for 8 weeks. The control diet, with 4 times the polyunsaturated fatty acids (PUFA) content and a P:S ratio about 10 times greater than the test diets, resulted in the lowest plasma TC, LDL cholesterol (LDL-C) and VLDL cholesterol (VLDL-C). Among the test diets, plasma TC and TG were lowest with the 18:0-rich diet. TC in gerbils fed the 16:0-rich diet and 4%en-trans were 20% higher than the 18:0-rich diet, while the 6%en-trans diet was 35% higher. VLDL-C was significantly higher in the 6%en-trans diet compared to all other groups at 8 weeks. Both trans fatty acid diets elevated plasma TG approximately 2- and 3-fold, respectively, compared to the 16:0-rich and 18:0-rich diets at 8 weeks. Further, plasma TG continued to rise over time with trans fatty acids compared to 16:0 or 18:0. Thus, in the fatty acid-sensitive gerbil, impaired TG metabolism represents a major aspect of the hyperlipemia caused by trans fatty acid substitution for major saturated fatty acids.

Keywords: Trans fatty acids; Palmitic acid; Stearic acid; Saturated fatty acids; Linoleic acid; Cholesterol; Triglyceride

1. Introduction

Clinical and epidemiological studies have established that trans fatty acids adversely affect the plasma lipid profile [1–4] and increase the risk for coronary heart disease (CHD) [5,6] and diabetes [7] compared to cis unsaturated fatty acids. Recent studies even indicate that the adverse effect of trans fatty acids on CHD and diabetes risk exceeds that produced by saturated fatty acids (SFA) [7–10]. However, specific trans fatty acid effects are often difficult to discern because multiple dietary factors, particularly other fatty acids, were varied simultaneously in diets comparing trans fats with other natural fats.

Individual SFA vary in their metabolic effects on plasma lipids and lipoproteins [11–13]. Among saturated fats, those rich in myristic acid (14:0) are most cholesterolemic [14,15]. Palmitic acid (16:0), the most abundant saturated fatty acid in the diet, raises plasma cholesterol at low PUFA intake when dietary cholesterol intake is high [14,16–18], but it can be neutral when these conditions do not apply [12,19]. Stearic acid (18:0), on the other hand, is generally considered to be neutral in its effects on lipoproteins [11,17,20,21]. Few studies [10,22,23] have investigated the lipid and lipoprotein metabolic response to an exchange between trans fatty acids and individual SFA when other fatty acids, particularly linoleic acid (18:2n-6) and cholesterol intake were held constant. Thus, the present study compared the effects of diets rich in specific SFA namely, 16:0 (24%en) or 18:0 (10%en) to two trans fatty acid rich diets providing 4%en or 6%en as trans-18:1, while dietary

Abbreviations: CHD, coronary heart disease; CE, cholesterol ester; EC, esterified cholesterol; EDTA, ethylenediaminetetraacetic acid; FC, free cholesterol; GC, gas chromatography; HDL-C, HDL cholesterol; HPLC, high performance liquid chromatography; LDL-C, LDL cholesterol; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TC, total cholesterol; TG, triglycerides.

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* Corresponding author. Tel.: +1-617-736-2051; fax: +1-617-736-2054.

E-mail address: kchayes@brandeis.edu (K.C. Hayes).

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Table 1
Composition of experimental diets

Ingredients	Diets				
	Control[1]	24%en 16:0[2]	10%en 18:0[3]	3.7%en trans[4]	5.8%en trans[5]
			g/kg		
Casein	200	200	200	200	200
Dextrose	200	200	200	200	200
Cornstarch	234	234	234	234	234
Cellulose	100	100	100	100	100
Mineral mix*	50	50	50	50	50
Vitamin mix**	12	12	12	12	12
Choline chloride	3	3	3	3	3
Cholesterol	0.8	0.8	0.8	0.8	0.8
Fat	200	200	200	200	200
Fatty acids			percent energy[6]		
Myristic acid (14:0)	0.25	0.46	0.42	0.42	0.38
Palmitic acid (16:0)	9.4	24.5	17.6	19.9	17.4
Stearic acid (18:0)	1.4	2.1	10.7	4.9	4.7
Total saturated fatty acids	11.3	27.3	29.1	25.5	22.8
Oleic acid (cis-18:1n-9)	16.4	9.8	8.1	8.3	8.7
Total cis MUFA	16.7	9.8	8.1	8.3	8.7
Linoleic acid (cis-18:2n-6)	10.2	2.6	2.4	2.3	2.2
Linoleic acid (cis-18:3n-3)	1.6	0.12	0.08	0.18	0.33
Total cis PUFA	11.8	2.7	2.5	2.5	2.6
trans-18:1 isomers	0.00	0.08	0.00	3.7	5.8
trans-18:2 isomers	0.09	0.09	0.00	0.04	0.04
Total trans fatty acids	0.09	0.17	0.00	3.7	5.8

* Ausman-Hayes mineral mix F 8650, Bioserve (Frenchtown, NJ USA).
**Hayes-Cathcart vitamin mix (Hayes et al., J Nutr 1989, 199, 1776-1736).
[1] American Heart Association blend.
[2] Blend of palm stearin and soybean oil.
[3] Blend of fully hydrogenated palm oil/palm oil, chemically interesterified, and soybean oil.
[4] Blend of palm stearin/palm oil, partially hydrogenated, and soybean oil.
[5] Blend of partially hydrogenated palm oil and soybean oil.
[6] Percent of energy from each fatty acid was calculated based on the fatty acid composition of fat blends and analyzed by GC (Table 2).

18:2n-6 and cholesterol content were held relatively constant. The study was conducted in gerbils, the species most responsive to the dietary fatty acid manipulation of cholesterol metabolism [24].

2. Methods

2.1a. Animals, diets and study design. Fifty mongolian gerbils (Meriones unguiculatus) were used in the study. Gerbils (10 per each dietary group) were fed one of the five experimental diets for 8 weeks. Table 1 shows the composition of the five experimental diets. The control diet was formulated according to the fatty acid balance of American Heart Association (AHA), but providing 40%en as fats and containing 0.08% cholesterol (185 mg/1000 kcal). The four test fats and the control diet blend were prepared by Nestlé Product Technology Center (Kemptthal, Switzerland) using palm oil products with varying degree of hydrogenation. The fatty acid composition of these diets varied only in their 16:0, 18:0 and trans-18:1 fatty acid content. Soybean oil was blended into all test fats to equalize EFA content of the diets at 2.2 to 2.6%en from 18:2n-6. The low level of 18:2n-6 and moderate amount of cholesterol (0.08%) were designed to maximize the impact of the individual fatty acids without the distortion that results from excessive dietary cholesterol [12,25,26]. Fatty acid composition of the diets, determined by gas chromatography (GC), is shown in Table 2. Relative to the 16:0-rich diet, the 18:0-rich diet substituted 8%en as 18:0, whereas 3.7%en and 5.8%en from trans-18:1 was substituted in the two trans-rich diets compared to the 16:0-rich diet. Triglyceride (TG) molecular species of the 16:0-rich diet and the interesterified 18:0-rich diet was analyzed by HPLC linked to an evaporative light scattering detector. The 16:0-rich diet contained about twice the PPP (~24%wt) and PPO/POP (~30%wt) content of the 18:0-rich diet, which substituted PSO/POS and PPS/PSP (data not shown).

Table 2
Fatty acid composition (% by wt) of fat blends used in the study

Fatty acids	Diets				
	Control	24%en 16:0	10%en 18:0	3.7%en trans	5.8%en trans
SFA					
12:0	0.23	0.15	0.40	0.16	0.20
14:0	0.62	1.2	1.0	1.1	0.96
16:0	23.5	61.2	44.0	49.7	43.5
18:0	3.4	5.3	26.7	12.3	11.8
Total	28.3	68.3	72.7	63.7	57.1
MUFA (cis)					
18:1 n-9	41.1	24.5	20.3	20.7	21.7
Total	41.7	24.5	20.3	20.7	21.7
PUFA (cis)					
18:2n-6	25.5	6.4	6.0	5.8	5.6
18:3n-3	4.0	0.29	0.19	0.46	0.82
Total	29.5	6.7	6.2	6.3	6.4
Trans					
18:1 trans isomers	ND*	0.19	ND	8.8	14.5
18:2 trans isomers	0.23	0.23	ND	0.34	0.1
Total trans isomers	0.23	0.42	ND	9.1	14.6

* Not detectable.

Gerbils were housed in groups of 2 to 3 per cage and kept in a controlled environment with a 12hr light/dark cycle with free access to water. Diets were provided daily in an amount sufficient for normal growth.

After 4 and 8 weeks of treatment, gerbils were fasted overnight and blood was collected by cardiac puncture using an EDTA-wetted syringe and #30 needle under light anesthesia. After 8 weeks, gerbils were exsanguinated and liver, cecum and adipose tissues were excised and weighed. An aliquot of liver was stored at –20°C until lipid analysis. Plasma was separated by centrifugation at 1200*g* for 15 min and stored at 4°C for 1 to 3 days until analyzed.

2.1b. Plasma lipid analysis. Plasma total cholesterol (TC) and TG were determined by enzymatic assay using Sigma kits #362 and #336, respectively (Sigma Diagnostics Co, St. Louis, MO). Plasma HDL-C was assayed after sodium phosphotungstate-Mg^{2+} precipitation of apoB and apoE containing lipoproteins with reagent #543004 (Boehringer Mannheim Diagnostic, Indianapolis, IN). Plasma lipoproteins at 8 weeks were determined by discontinuous gradient ultracentrifugation according to the method of Chapman et al. [27], described in detail previously [24].

2.1c. Liver lipid analysis. Liver cholesterol was extracted by grinding 100 mg piece of liver with anhydrous sodium sulfate and extracting three times with 2:1 chloroform methanol. Hepatic free cholesterol (FC) and esterified cholesterol (EC) were determined by HPLC based on the method of Kim and Chung [28], as described previously [29]. For analysis of liver CE fatty acid profile, cholesterol esters (CE) were separated from other lipid classes by thin layer chromatography. The CE band was scraped off and transesterified to fatty acid methyl esters (FAME) using acetyl chloride as the catalyst. FAME were analyzed by GC-FID using CP-Sil 88 capillary column. FAME were identified based on retention times of authentic standards (Nu-Check Prep, Elysian, MN).

2.1d. Statistical analysis. Statistical analysis was performed on a Macintosh LCIII (Apple Computer Inc., Cupertino, CA) using StatView SE+Graphics software package (Brain Power Inc., Calabasas, CA). Diet effects were assessed using one-way ANOVA and Scheffe's F-post-hoc test.

3. Results

Body weight and weight gain were similar for all diet groups (Table 3), indicating that diets were adequate and food consumption similar. Adipose and cecum weights were comparable, but gerbils fed trans fat diets had higher liver weights than the AHA controls.

The control diet, with 4 times the 18:2-n-6 and a P:S ratio about 10 times greater than the test diets, resulted in the lowest plasma TC, LDL cholesterol (LDL-C) and VLDL cholesterol (VLDL-C) after 8 weeks, with the difference apparent at 4 weeks (Table 3). Among the other test diets, the 18:0-rich diet resulted in the lowest plasma TC. After 4 weeks, only TC in gerbils fed the 6%en trans diet was significantly higher than the18:0-rich diet, whereas after 8 weeks, TC in gerbils fed the 16:0-rich and 4%en-trans diets were 20% higher than the 18:0-rich diet, while the 6%en-trans diet was 35% higher. LDL and HDL cholesterol increased proportionally in all test diets such that the LDL-C/HDL-C and TC/HDL-C ratios did not differ among diets. However, VLDL-C was significantly higher in the high-trans group (6%en from trans fatty acids) compared to all other groups at 8 weeks.

The most striking change in response to trans fatty acids was an increase in plasma TG. Compared to 16:0 and 18:0-rich diets, the moderate-trans (4%en as trans fatty acids) and high-trans diets elevated TG approximately 2- and 3-fold, respectively. Plasma TG continued to rise over time with trans fatty acid-enriched diets relative to the18:0-rich diet, whereas triglyceridemia caused by the 16:0-rich diet was modest and complete by 4 weeks and was similar to that produced by the 18:0-rich diet after 8 weeks.

Hepatic cholesterol accumulation was greatest in the control group, but was equally reduced 40 to 50% by all 4 test diets, with essentially all the reduction accounted for by the CE fraction (Table 4). The liver CE profile indicated that oleic acid (cis-18:1n-9) predominated among all diet groups, the percentage being identical for the control and 18:0-rich diet groups. The 16:0-rich diet and the two trans diets depressed the percentage of cis-18:1n-9 CE somewhat,

Table 3
Body weight, organ weight and plasma lipids in gerbils fed different fat blends

	Diets				
	Control	24%en 16:0	10%en 18:0	3.7%en trans	5.8%en trans
Body Weight (g)					
Initial	54 ± 2	54 ± 3	54 ± 3	53 ± 2	53 ± 2
Final	73 ± 4	72 ± 4	72 ± 5	72 ± 2	74 ± 5
Organ Weights (%BW)					
Liver	2.8 ± 0.24^{a}	2.9 ± 0.30^{a}	2.9 ± 0.20^{a}	3.1 ± 0.24^{b}	3.1 ± 0.36^{b}
Adipose	0.53 ± 0.14	0.61 ± 0.22	0.53 ± 0.21	0.53 ± 0.23	0.56 ± 0.15
Cecum	3.9 ± 0.66	3.5 ± 0.84	3.8 ± 0.77	4.6 ± 3.73	3.7 ± 0.63
Plasma					
TC (mg/dl)					
4 weeks	108 ± 11^{a}	175 ± 20^{b,c}	165 ± 21^{b}	165 ± 26^{b}	190 ± 33^{c}
8 weeks	111 ± 19^{a}	201 ± 28^{b}	167 ± 25^{c}	199 ± 26^{b}	226 ± 40^{b}
TG (mg/dl)					
4 weeks	47 ± 7^{a}	132 ± 77^{b,d}	85 ± 36^{b,c}	75 ± 23^{c}	147 ± 82^{d}
8 weeks	43 ± 10^{a}	120 ± 71^{a,b}	123 ± 108^{a,b}	208 ± 161^{b}	322 ± 135^{c}
HDL-C (mg/dL)*					
4 weeks	48 ± 5^{a}	73 ± 15^{b,c}	64 ± 15^{b}	62 ± 17^{b}	84 ± 18^{c}
8 weeks	44 ± 9^{a}	74 ± 7^{b,c}	68 ± 9^{b}	76 ± 15^{b,c}	86 ± 12^{c}
TC/HDL-C Ratio*					
4 weeks	2.3 ± 0.36	2.5 ± 0.35	2.7 ± 0.61	2.8 ± 0.76	2.3 ± 0.32
8 weeks	2.7 ± 0.44	2.7 ± 0.42	2.5 ± 0.30	2.8 ± 0.77	2.7 ± 0.44
Plasma (8 weeks)					
VLDL-C**	27 ± 5^{a}	47 ± 9^{b}	39 ± 4^{b}	44 ± 5^{b}	60 ± 2^{c}
LDL-C**	30 ± 8^{a}	64 ± 21^{b}	50 ± 3^{b}	68 ± 21^{b}	60 ± 6^{b}
HDL-C**	52 ± 6^{a}	94 ± 10^{b,c}	79 ± 8^{b}	98 ± 18^{c}	108 ± 10^{c}
LDL-C/HDL-C ratio**	0.60 ± 0.19	0.69 ± 0.28	0.64 ± 0.09	0.75 ± 0.40	0.56 ± 0.07
TC/HDL-C ratio**	2.1 ± 0.33	2.2 ± 0.23	2.2 ± 0.11	2.2 ± 0.43	2.1 ± 0.09

Values are means ± SD (n = 9–10).
^{a,b,c,d} Means in a row with different superscripts are significantly different ($p < 0.05$) using one-way ANOVA and Scheffe's F-test.
* HDL-C after PTA-Mg^{2+} precipitation
**VLDL-C, LDL-C and HDL-C obtained by discontinuous gradient ultracentrifugation, n = 3–4 (2 gerbils per sample).

while increasing 16:0 CE and trans-18:1 CE, respectively. All test diets significantly reduced the percentage of hepatic cis-18:2n-6 CE relative to control diet, reflecting the low dietary 18:2n-6 intake. Liver trans-18:2 CE percentage increased with trans fatty acid diets relative to other diets.

4. Discussion

4.1a. Plasma lipids. The present study compared the lipoprotein response of gerbils to exchanges of specific dietary SFA (namely 16:0 and 18:0), with trans-18:1. Dietary 18:2n-6 was consistently low (2.2 to 2.6%en) for all four test fats. Not surprisingly, the control diet resulted in the lowest plasma TC, TG and lipoprotein cholesterol, as predicted from its 4-fold greater 18:2n-6 and 10 times greater PUFA:SFA ratio compared to test diets [15,25].

4.1b. Trans vs. SFA. By applying low18:2n-6 and modest cholesterol intake to accentuate the effects of fatty acids on lipid metabolism [25,26], it was possible to distinguish the hyperlipemic effects of trans-18:1 from the two major SFA (16:0 and 18:0) that it typically replaces in the diet. Specifically, the trans fat diets increased plasma TC compared to 18:0 rich diet and elevated plasma TG substantially compared to both 16:0-rich and18:0-rich diets. The marked rise in plasma TG and VLDL-C suggests that a major aspect of the lipemia caused by trans fatty acids reflected impaired TG metabolism in gerbils. The progressive increase in plasma TG associated with trans fatty acid consumption was evident, even at a modest trans fat intake of 4%en. Previous studies by Rudel et al. [8] in LDL receptor null, apoB over-expressed (LDLr-/-, apoB+/+) transgenic mice and by Dictenberg et al. [29] in gerbils, also described severe hypertriglyceridemia when high trans fatty acid intake (5 to 10%en) was compared to SFA or cis-MUFA at low 18:2n-6 intake (<4%en) in the presence of dietary cholesterol.

The mechanism underlying trans fatty acid induced-hypertriglyceridemia is not well characterized. One possibility is impaired TG clearance due to depressed hepatic receptor mediated uptake of TG-rich lipoproteins and/or impaired VLDL-TG removal by adipose and skeletal muscle relative to 18:0 or 16:0. Trans-18:1 isomers were inferior to cis-18:1 for regulating hepatic LDL receptor activity and plasma lipid clearance in hamsters [30,31]. A second possibility is that trans-18:1 impaired insulin action in skeletal muscle and adipose and depressed acylation stimulating protein (ASP) activity to induce futile recycling of fatty acids,

Table 4
Liver lipids in gerbils fed different fat blends

	Diets				
	Control	24%en 16:0	10%en 18:0	3.7%en trans	5.8%en trans
Liver cholesterol					
TC (mg/g)	37 ± 8^{a}	27 ± 7^{b}	25 ± 4^{b}	22 ± 5^{b}	25 ± 5^{b}
FC (mg/g)	6 ± 1	7 ± 2	6 ± 1	6 ± 1	6 ± 1
EC (mg/g)	32 ± 7^{a}	21 ± 6^{b}	19 ± 3^{b}	16 ± 4^{b}	19 ± 4^{b}
CE profile					
16:0	7 ± 2^{a}	16 ± 3^{b}	11 ± 2^{c}	$14 \pm 3^{b,c}$	11 ± 2^{c}
18:0	3 ± 0.6^{a}	4 ± 1^{b}	6 ± 1^{c}	$5 \pm 1^{b,c}$	$5 \pm 0.7^{b,c}$
cis-18:1n-9	68 ± 3^{a}	61 ± 4^{b}	67 ± 3^{a}	58 ± 4^{b}	57 ± 1^{b}
cis-18:2n-6	13 ± 1^{a}	4 ± 0.4^{b}	4 ± 0.3^{b}	4 ± 0.9^{b}	3 ± 0.4^{b}
cis-20:4n-6	0.1 ± 0.08	0.1 ± 0.02	0.1 ± 0.03	0.1 ± 0.02	0.1 ± 0.1
trans-18:1	0.05 ± 0.01^{a}	0.1 ± 0.1^{a}	0.1 ± 0.09^{a}	2 ± 0.5^{b}	3 ± 1.0^{b}
trans-18:2	0.002 ± 0.004^{a}	0.05 ± 0.03^{a}	0.04 ± 0.01^{a}	0.31 ± 0.07^{b}	0.58 ± 0.06^{b}

Values are means ± SD (n = 9–10).
[a,b,c] Means in a row with different superscripts are significantly different ($p < 0.05$) using one-way ANOVA and Scheffe's F-test.

leading to hypertriglyceridemia [32]. In fact, a recent study by Matthan et al. [33] reported that trans fatty acids impaired ASP activity, resulting in diminished VLDL TG uptake by adipose tissue and elevated plasma TG in moderately hypercholesterolemic, postmenopausal women. Additionally, Dashti et al. [34] found that trans-18:1 increased secretion of VLDL-C and LDL-C by HepG2 cells in culture compared to 16:0 and cis-18:1n-9. Further studies are needed to determine to what extent TG-rich lipoprotein clearance and/or hepatic secretion are altered by trans fatty acids and whether such effects impact post-prandial TG metabolism related to insulin resistance and atherosclerosis.

Recent large-scale epidemiological studies implicate hypertriglyceridemia as an independent risk factor in coronary artery disease [35–38]. Further, hypertriglyceridemia is characteristic of the insulin resistance syndrome, commonly referred to as the "metabolic syndrome" or "syndrome X", which is closely associated with risk for Type II diabetes mellitus [37,39]. The present study utilized a fatty acid-sensitive animal model to provide evidence that trans fatty acids induce chronic elevation of plasma TG, combined with hypercholesterolemia, when compared to the most common dietary SFAs. This metabolic relationship may underlie the particularly adverse effects of trans fatty acids on coronary vascular disease and Type II diabetes compared to SFA [7–9].

Several clinical studies have observed a significant increase in plasma TG with high trans fatty acid intake [1,3,10,40] while others reported no such effect [9,41]. In the current study, dietary 18:2n-6 was marginally low (2.2 to 2.6% en) coupled with a moderate cholesterol load (0.08%), a combination known to expose the vulnerability of lipoprotein metabolism to other dietary fatty acid stressors [25,26]. The significance of 18:2n-6 intake on modulating the hypercholesterolemic effects of SFA has been detailed previously [12,15]. The extent to which 18:2n-6 influences the lipemic effects of trans fatty acids is currently under investigation.

4.1c. 16:0-rich vs. 18:0-rich diets. The 16:0-rich diet elevated plasma TC by comparison to 18:0, presumably because dietary cholesterol intake was moderately high (0.08%) in this study. We have previously demonstrated that 16:0 raised plasma cholesterol in the presence of dietary cholesterol during marginal PUFA intake [15,18]. By contrast, 18:0 is typically neutral in its effects on plasma lipids, but it can depress HDL-C at high intakes [11,20]. Enhanced LDL-C production [42] and impaired LDL receptor activity [17,43] have been reported to underlie the16:0- induced hypercholestrolemia in the presence of high dietary cholesterol and low PUFA.

TG structure also may have influenced cholesterol metabolism in the 16:0-rich diet group [44,45]. The palm stearin used to generate the16:0-rich diet contained predominantly tripalmitin (PPP), whereas the18:0- rich fat was formulated from an interesterified, fully hydrogenated palm oil/palm oil blend, which resulted in a diverse mix of triglycerides. Thus, it is possible that the monoacyl trisaturated structure of palm stearin contributed to its plasma cholesterol-raising effect compared to the chemically interesterified 18:0-rich diet. Such data beg the question of more detailed information on triglyceride molecular structure, particularly related to SFA, and its influence on cholesterol metabolism [44].

4.1d. Liver CE. Total hepatic CE (mg/g) was reduced by all test diets compared to the control diet, which is likely due to the higher PUFA content in the latter. PUFA have been shown to up-regulate liver LDL receptor activity and enhance hepatic uptake and clearance of plasma cholesterol in the presence of dietary cholesterol [46]. Further, PUFA and cis-18:1 enhance ACAT activity and increase liver CE content relative to SFA [47].

Liver CE profile (% individual CE) across the experi-

mental diets supports the fact that cis-18:1n-9 is the preferred substrate for hepatic ACAT enzyme [48,49], even in gerbils. Increased liver trans-18:1 and trans-18:2 CE and lower cis-18:1n-9 CE percent in gerbils fed trans fat diets compared to gerbils fed 18:0-rich diet indicate that ACAT utilized both trans-18:1 and cis-18:1 during trans fatty acid consumption. Previous study by Dictenberg et al. [29] also reported a decline in hepatic cis-18:1n-9 CE percent in gerbils fed 5%en as trans fatty acids, consistent with present study results. However, in that study [29] trans CEs were not separated from cis-18:2n-6 CE, and presumably co-eluted with cis-18:2n-6 CE with the HPLC methodology applied. Hence, the increased liver cis-18:2n-6 CE reported in those gerbils fed trans fatty acids most likely included trans CEs, a point demonstrated by improved CE separation in the present study. Thus, trans-18:1, and not 18:2n-6 as suggested by Dictenberg et al. [29], was substituted for cis-18:1n-9 by ACAT.

Liver weights were increased by both trans-rich diets, whereas livers from gerbils fed SFA diets did not differ from the controls. Liver cholesterol mass was lower with trans diets compared to control diet, but similar to the response to SFA, indicating that cholesterol accumulation did not account for higher liver weights. Hepatomegaly induced by trans fatty acids may reflect toxicity or altered hepatic lipid metabolism other than that involving cholesterol metabolism.

In summary, plasma TC was elevated when trans fatty acids or 16:0 were specifically exchanged for 18:0 in the diet, in the presence of dietary cholesterol and low PUFA intake. Trans fatty acids induced significant increase in plasma TG and liver weight when exchanged for either dietary 16:0 or 18:0 in gerbils. Further, the adverse lipemic effects of trans fatty acids continued to worsen over time compared to 16:0 or 18:0, indicating that prolonged intake of trans fatty acids may impair TG metabolism leading to severe hypertriglyceridemia, a known risk factor for Type II diabetes mellilus and CHD [35,37].

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