Effect of Tristearin and Other Functional Fats on Blood Lipids in Hamsters

G. S. RANHOTRA,[1,2] J. A. GELROTH,[1] and S. D. LEINEN[1]

ABSTRACT

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Groups of hamsters were fed diets containing tristearin (TS), a baker's shortening (BS), soybean oil (SO), a blend (BL) of TS and SO, or a hard fat (HF). Only BS contained *trans* fatty acids. After four weeks, serum total cholesterol (T-CH) levels were most elevated in hamsters fed HF. In comparison, hamsters fed BS, BL, or SO showed 13.3, 15.4, or 23.7% reduction in serum T-CH, respectively. Reduction in serum T-CH was even more pronounced in hamsters fed TS. However, this group also showed poor weight gains, apparently because only 16.2% of ingested TS was absorbed. Reduction in T-CH occurred due to reduction of both high-density-lipoprotein (HDL)-CH and non-HDL-CH, but the pattern of reductions still favored lower T-CH-to-HDL-CH ratios. Like T-CH, serum TG levels were also most elevated in hamsters fed HF, with other groups showing significant reductions. Liver CH responses did not conform to responses observed for serum CH and TG levels. Tested in selected baked products prepared in-house, blended fat (BL) was found as functional as regular BS, and it contained no *trans* fatty acids.

Fat consumption data in the United States suggest that margarines and shortenings, which are widely used in baked goods and other grain-based products, are the major source of *trans* fatty acids in our diet (Hunter and Applewhite 1991). Although probably not as cholesterolemic as saturated fatty acids (Judd et al 1994), *trans* fatty acids may raise blood cholesterol (CH) levels enough to be of concern (Mensink and Katan 1990, Troisi et al 1992).

trans Fatty acids in shortenings and margarines can be reduced by more selectively increasing the content of stearic acid, a saturated fatty acid that is less cholesterolemic than saturated fatty acids of shorter chain length (Kritchevsky 1994). Interesterification of fatty acids or the use of nonisomerizing catalysts during hydrogenation have been suggested as other means of reducing the level of *trans* fatty acids in shortenings and margarines. Blending tristearin with edible oils may offer another possibility to obtain fat blends that are free of *trans* fatty acids but show texture and functionality characteristics similar to shortenings and margarines. In this study, we took this latter approach to prepare a functional fat and tested it (and other fats) for its effect on blood and liver lipids levels using hamsters as the animal model.

MATERIALS AND METHODS

Test Materials

Four commercial sources of fat were used: soybean oil (no preservative), a baker's shortening (partially hydrogenated soybean and cottonseed oils), hard fat flakes (hydrogenated palm kernel oil), and tristearin. An additional fat tested was a blend of soybean oil (70%) and tristearin (30%). Fatty acid composition of these fats is shown in Table I. The blended fat matched textural and stability characteristics of baker's shortenings. A limited evaluation of the functional characteristics of this blend was also undertaken.

Test Diets

Except that fat sources differed, all diets contained the same level of fat (Table II). They also contained the same level of protein and carbohydrates, and they were complete in nutrients required by the weanling hamsters (NRC 1987). At the level used, fat provided 35% of total calories. All diets also contained 0.25% cholesterol.

Animals and Feeding

Fifty-five three-week-old male Syrian hamsters (Harlan Sprague-Dawley, Indianapolis, IN) were housed in suspended mesh-bottomed cages (fecal collection trays underneath) in a controlled environment (24°C, 60% rh, 12-hr light and dark cycle). After five days on a CH-free diet, these animals were weighed and assigned by selective randomization to five groups of 10 animals each, with a mean weight of 43 ± 6 g. They were then fed test diets (Table II) for four weeks. Five hamsters were sacrificed on day 0 to obtain baseline serum lipid values. Animals were fed fresh diet daily. Their food intake increased gradually as the experiment progressed; however, this intake was equalized among groups. Deionized water was offered ad libitum. Animals were weighed weekly.

Blood and Liver Sampling

At the end of four weeks, animals were fasted overnight, lightly anesthetized (under ether), and 2 mL of blood was withdrawn by cardiac puncture. The clotted blood was centrifuged (8 min) to obtain serum for lipid analyses that were run over the next two days. Livers were excised, rinsed, blotted dry, weighed, homogenized, the volume recorded, and the samples frozen until needed for CH determinations.

Analytical and Statistical

Test fats were analyzed for fatty acid profile (Table I) by gas chromatography. Details of this method are presented elsewhere (Ranhotra et al 1996). Feces, collected for the entire four-week period, were finely ground, and a sample was analyzed for fat by the AOAC method 922.06 (1995). Serum total CH and triglyceride (TG) levels were determined enzymatically using kits 352 and 336, respectively, from Sigma Chemical Co. (St. Louis, MO). High-density lipoprotein (HDL)-CH was determined (using kit 352) after phosphotungstic acid precipitation of other lipoproteins. Total CH in liver was determined by the method of Abell et al (1952). The data were subjected to analysis of variance (Tukey's multiple range test) using SigmaStat Statistical software (Jandel Scientific Software, San Rafael, CA).

RESULTS AND DISCUSSION

Blended Fat

Soybean oil and tristearin were blended in various ratios to obtain a fat with functional (plasticity and processing characteristics) and storage characteristics similar to that of partially hydrogenated vegetable oils (margarines and shortenings). A blend resulting from a 70:30 (soybean oil-to-tristearin) ratio met these criteria as judged based on scores we obtained for products (muffins, cookies, and crackers) baked in-house.

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[1] Nutrition Research Group, American Institute of Baking, 1213 Bakers Way, Manhattan, KS 66502.

[2] Corresponding author. Phone: 913/537-4750. Fax: 913/537-1493.

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TABLE I
Fatty Acid Composition of Test Materials

Fatty Acid	Test Material Hard Fat Flakes	Baker's Shortening	Blend	Soybean Oil	Tristearin
6:0–10:0[a]	8.1 ± 0.0	. . .	. . .	. . .	. . .
12:0 (lauric)	47.7±0.1	. . .	. . .	. . .	. . .
14:0 (myristic)	15.6 ± 0.0	0.2 ± 0.0	. . .	. . .	. . .
16:0 (palmitic)	8.2 ± 0.0	12.4 ± 0.1	8.9 ± 0.1	10.9 ± 0.0	4.2 ± 0.1
18:0 (stearic)	20.2 ± 0.1	13.9 ± 0.1	31.5 ± 1.0	4.4 ± 0.0	92.1 ± 0.1
18:1 (elaidic)	. . .	17.8 ± 0.0	. . .	. . .	. . .
18:1 (oleic)	. . .	32.7 ± 0.1	15.9 ± 0.3	22.9 ± 0.0	. . .
18:2 (linoleic)	. . .	22.0 ± 0.1	37.4 ± 0.6	54.2 ± 0.1	. . .
18:3 (linolenic)	. . .	0.3 ± 0.0	4.7 ± 0.1	6.8 ± 0.0	. . .
20:0 (arachidic)	0.2 ± 0.0	0.4 ± 0.0	1.0 ± 0.0	0.4 ± 0.0	2.3 ± 0.0
22:0 (behenic)	. . .	0.4 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	1.0 ± 0.0
24:0 (lignoceric)	. . .	. . .	. . .	. . .	0.4 ± 0.0

[a] Medium chain acids.

TABLE II
Composition of Test Diets

	Diet A (Hard Fat)	B (Shortening)	C (Blend)	D (Soybean Oil)	E (Tristearin)
Component, g/100 g					
Hard fat flakes	16.5	. . .	. . .	. . .	. . .
Baker's shortening	. . .	16.5	. . .	. . .	. . .
Blend[a]	. . .	. . .	16.5	. . .	. . .
Soybean oil	. . .	. . .	. . .	16.5	. . .
Tristearin	. . .	. . .	. . .	. . .	16.5
Constants[b]	83.5	83.5	83.5	83.5	83.5
Calories from fat, %	35	35	35	35	35

[a] Contained 30% tristearin and 70% soybean oil.

[b] Contained (g/100 g): casein, 18.4; cellulose, 5.0; mineral mix, 4.0; vitamin mix, 2.2, dl-methionine, 0.3; choline chloride, 0.16; cholesterol, 0.25; and cornstarch, 53.19.

Diet Intake and Weight Gains

Diets differed only in the source of fat used. Furthermore, all groups of hamsters consumed equal amounts (Table III). Thus, differences observed in their serum lipid levels may be attributed to differences in the fatty acid profiles of the test fats. This assumption, while apparently valid for hamsters fed diets A–D, remains in doubt for hamsters fed diet E (tristearin) because of differences in body weight gains (diet E vs. diets A–D).

Serum Cholesterol

Compared to the baseline level (134 ± 5 mg/dl), serum total CH levels were quite elevated in all groups of hamsters after four weeks except in the group fed tristearin (Table III). In tristearin-fed hamsters, CH levels showed virtually no elevation. CH levels were most elevated in hamsters fed hard fat (diet A). In comparison, CH levels were 13.3% lower in the group fed baker's shortening (diet B), 15.4% lower in the group fed the fat blend (diet C), and 23.7% lower in the group fed soybean oil (diet D). Body weight gains among these four groups did not differ significantly ($P > 0.05$). Thus, the CH-lowering effect observed on diets B–D may be ascribed to favorable fatty acid makeup of the fats used (Table I). Compared to diet A, CH levels were least elevated in hamsters fed diet E (tristearin). This may be due to lower absorption of tristearin (Table III), due to significantly ($P < 0.05$) lower weight gains of hamsters fed tristearin (vs. hard fat), or both.

The hard fat tested was high in lauric acid (Table I), which is associated with a pronounced cholesterolemic effect (Kritchevsky 1994). Elaidic acid, a *trans* fatty acid, is also associated with a cholesterolemic effect (Mensink and Katan 1990). However, the presence of elaidic acid in the baker's shortening did not render this fat any more cholesterolemic than blended fat, which contained no *trans* fatty acids. This could be because the level of elaidic acid in the shortening-based diet (diet B) was modest (<3%). Conceivably, the high level of oleic acid in diet B may have exerted some CH-lowering effect (Grundy 1990), thus, negating the cholesterolemic effect of the elaidic acid in diet B. Tristearin used in this study was >92% stearic acid (Table I). Stearic acid is not implicated in raising CH levels. This effect may be a direct effect (specific effect) of stearic acid or the consequence of its ability to lower caloric density of the diet because of poor absorption (Table III), or both.

Compared to the hard fat, the CH-lowering effect due to the other fats resulted from a lowering of both HDL and non-HDL-CH (Table III). For various fats, this lowering followed the pattern observed for total CH. However, the magnitude differed, with non-HDL-CH levels decreasing more substantially than HDL-CH levels. This consistently favored a desired shift in total CH-to-HDL-CH ratios (Table III). As observed for total CH, tristearin lowered HDL and non-HDL-CH levels the most.

Serum Triglycerides

Elevated serum TG levels are considered an independent risk factor in cardiovascular disease (Austin 1991). Like total CH, serum TG levels were also quite elevated after four weeks, with the group fed hard fat (diet A) showing the most elevation (Table III). Compared to this group, those fed shortening (diet B) showed 13.8% reduction, those fed the blend (diet C) showed 25.5% reduction, those fed soybean oil (diet D) showed 52.8% reduction, and those fed tristearin (diet E) showed 55.2% reduction in TG. Presuming that tristearin lowered TG levels, in part, through its adverse effect on body weight gains, soybean oil may appear as the most potent TG-lowering fat, since it exerted no adverse effect on body weight gains but lowered TG to the same extent as tristearin.

Liver Cholesterol

Liver CH responses did not parallel responses observed for serum CH (and TG) levels. Liver CH levels were quite low, not only in hamsters fed tristearin, but also in those fed the hard fat (Table III). Among the other three fats tested, the group fed the

TABLE III
Physiological Responses in Hamsters Fed Test Materials[a]

	Diet				
	A (Hard Fat)	**B (Shortening)**	**C (Blend)**	**D (Soybean Oil)**	**E (Tristearin)**
Diet intake, g	183 ± 1	183 ± 1	183 ± 1	185 ± 1	185 ± 0
Body weight gain,[b] g	41 ± 6a	45 ± 4a	46 ± 6a	41 ± 9a	18 ± 3b
Liver weight, g	3.4 ± 0.4b	4.1 ± 0.4a	3.8 ± 0.3a,b	3.9 ± 0.5a	1.9 ± 0.1c
Serum cholesterol (CH)[c]					
Total, mg/dl	297 ± 25a	257 ± 24b	251 ± 24b,c	227 ± 16c	137 ± 7d
High-density-lipoprotein (HDL), mg/dl	183 ± 15a	170 ± 9a,b	166 ± 14b	163 ± 10b	96 ± 9c
Non-HDL, mg/dl	113 ± 17a	88 ± 18b	88 ± 23b	63 ± 10c	41 ± 7d
Total/HDL, ratio	1.62 ± 0.10a	1.52 ± 0.10a,b,c	1.54 ± 0.17a,b	1.39 ± 0.05c	1.43 ± 0.11b,c
Decease in serum CH,[d] %					
Total	. . .	13.3 ± 7.9c	15.4 ± 8.0b,c	23.7 ± 5.5b	54.0 ± 2.3a
HDL	. . .	7.3 ± 5.2b	10.7 ± 7.1b	10.8 ± 5.3b	47.6 ± 4.9a
Non-HDL	. . .	22.3 ± 15.6c	22.2 ± 20.6c	44.0 ± 8.8b	64.0 ± 6.1a
Serum triglycerides					
Level[c], mg/dl	242 ± 30a	209 ± 55a,b	180 ± 52b	114 ± 31c	109 ± 31c
Decrease,[d] %	. . .	13.8 ± 22.9b	25.5 ± 21.5b	52.8 ± 12.8a	55.2 ± 12.9a
Liver CH, mg/g	8 ± 3d	75 ± 11b	58 ± 8c	111 ± 20a	7 ± 1d
Fat digestibility					
Fat consumed, g	30.3 ± 0.2	30.2 ± 0.2	30.2 ± 0.2	30.4 ± 0.2	30.5 ± 0.1
Apparent absorption, %	90.0 ± 1.9c	94.4 ± 0.9b	77.9 ± 4.2d	98.6 ± 0.2a	16.2 ± 3.8e

[a] Values are averages ± standard deviations of 10 hamsters per diet over four weeks. Within a row, values not sharing a common letter are significantly different ($P < 0.05$).
[b] Inital body weight: 43 ± 6 g.
[c] Baseline (day 0) levels (mg/dl): serum total CH, 134 ± 5; serum HDL-CH, 90 ± 7; serum non-HDL-CH, 44 ± 9; and serum triglycerides, 53 ± 19.
[d] In comparison to diet A (hard fat)..

blended fat (diet C) showed significantly ($P < 0.05$) lower liver CH values. This may be physiologically significant, but it is difficult to explain based on data at hand.

Fat Digestibility

As is well documented (Carroll 1958, Mattson et al 1979), tristearin and stearic acid are poorly absorbed. In our study, tristearin-fed hamsters absorbed only 16.2% tristearin (Table III). As part of the blend (diet C), tristearin absorption increased somewhat, but it was still quite low as compared to the other three fats. Apparent absorption of the other three fats ranged between 90.0 (hard fat) and 98.6% (soybean oil), the lower values probably being the consequence of stearic acid present in hard fat (Table I). Fat digestibility appeared to be inversely related to the amount of stearic acid present in fats tested.

CONCLUSION

Processing technology should permit blending soybean or other suitable oils with tristearin, a non-cholesterolemic hard fat, to yield functional fats that can be used in baked goods and other grain-based products. Such blends would be free of *trans* fatty acids which are associated with a cholesterolemic effect.

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