

Effect of Shortenings Containing Stearic Acid on Blood Lipids and Fat Digestibilities in Hamsters

G. S. Ranhotra,^{1,2} J. A. Gelroth,¹ S. D. Leinen,¹ and T. W. Ricklefs¹

ABSTRACT

Cereal Chem. 75(4):557–559

Tristearin (TS), a stearic acid-rich hard fat, and soybean oil (SO) were blended in different ratios to produce four functional shortenings (blends) for use in foods. Groups of hamsters were then fed diets containing TS, SO, and the four blends for four weeks. After four weeks, serum total cholesterol (CH) levels were measured: the group fed SO had 219 ± 19 mg/dL, and the groups fed four blends had a range of 214 ± 14 to 222 ± 15 mg/dL. Thus, TS in the blends exerted no hypercholesterolemic effect; it even lowered serum

triglycerides (SO vs. blends). Liver CH levels were significantly lower only in the group fed the blend containing the highest level (60%) of TS. While SO was nearly completely digested (97.7%), digestibility of TS in the blends was low with a range of 10.2–26.3%, which was inversely related to the level of TS in the blend. Thus, functional shortenings produced by blending TS with edible oils may not only not raise blood CH levels, but they would be free of *trans* fatty acids and may be classified as reduced-calorie fats.

Shortenings and margarines, partially hydrogenated vegetable oil products, are important ingredients in many grain-based and other foods. They are also the major source of *trans* fatty acids in our diet (Hunter and Applewhite 1991). The *trans* fats are now implicated as adversely affecting blood lipid profiles (Mensink and Katan 1990, Troisi et al 1992, Judd et al 1994, Almendingen et al 1995, Hernandez and Lusas 1997). A recent study, which involved 80,082 women, reported that the replacement of 2% of energy from *trans* fats with energy from unhydrogenated, unsaturated fats would reduce the risk of coronary heart disease by 53% (Hu et al 1997).

Unhydrogenated oils are usually less functional in processed foods and, thus, less desirable as ingredients when compared to partially hydrogenated vegetable oil products. However, functional shortenings and margarines free of *trans* fatty acids may be produced by interesterification and transesterification of oils (Majumdar and Bhattacharyya 1986, Miller et al 1991, Hernandez and Lusas 1997), by use of nonisomerizing catalysts during hydrogenation of oils (Ariaansz and Okonek 1995), and by other means. Blending fully hydrogenated (hard fats containing no *trans* fatty acids) oils with edible oils such as soybean, canola, or corn oil may be a more practical approach, however.

Because they are usually hypercholesterolemic, most hard fats may be unsuitable for such blending. A stearic fat (tristearin [TS]) may be an exception. It apparently does not raise blood cholesterol (CH) (Kritchevsky 1994) and provides fewer calories than traditional fats and oils because of incomplete digestibility (Ranhotra et al 1998).

We recently showed a blend of 30% TS and 70% SO to be as functional as regular baker's shortenings when tested in three bakery products (Ranhotra et al 1997). This blend did not raise blood CH in hamsters. In expanding the scope of this study, we have now prepared four blends of TS and SO. The focus was to examine the effect of increasing levels of TS (in the blends) on blood lipid profile and TS digestibilities. A subsequent study will evaluate functional attributes of these blends.

MATERIALS AND METHODS

Fats and Blends

A stearic hard fat (91.3% stearic acid), an edible SO (no preservatives), and four blended fats were tested. Blends were prepared by adding stearic fat (TS) to SO at increasingly higher levels: 15,

30, 45, or 60% (Table I). The 30:70 and 45:55 blends matched textural characteristics of commonly used baker's shortenings. The 15:85 blend, a somewhat free-flowing blend, may be suitable for use in bread-type products, while the harder blend (60:40) may find applications in icings, glazes, and fillings.

Test Diets

The six fats were used to make test diets (Table II). All diets contained the same level (16.9%) of fat. Thus, only the source of fat differed. At the level used, fat provided 35% of total calories. An additional 1% SO was added to ensure adequacy of essential fatty acids (NRC 1987), especially in the all TS-based diet (diet F). All diets contained the same levels of protein (15%), fiber (5%), and added CH (0.25%). Their sources did not differ.

Test Animals

Sixty-five three-week-old male Syrian hamsters (Harlan Sprague-Dawley, Indianapolis, IN) were housed in suspended mesh-bottomed cages in a controlled environment (24°C, 60% rh, 12-hr light and dark cycle). After four days on a CH-free diet, these animals were weighed and assigned to six groups of 10 animals each, and one group of five animals, with a mean weight of 57 ± 4 g. The smaller group was sacrificed on day 0 to obtain baseline serum lipid and liver CH values.

Feeding and Sampling

All groups of hamsters were fed test diets for four weeks, with fresh diet offered daily. Their food intake increased gradually as the study progressed, but this intake was equalized (pair feeding) among groups. Deionized water was provided ad libitum. All cages were fitted with slide-in trays underneath to allow total fecal collection for the entire four-week period. Animals were weighed weekly.

After 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. The dried fecal matter was finely ground, pooled for each animal separately, bagged, and stored frozen until analyzed for total fat.

Analytical and Statistical Procedures

SO, TS, and the four blends were analyzed for fatty acid composition (Table I) by gas chromatography as described earlier (Ranhotra et al 1996). Feces were analyzed for fat by AOAC method 922.06 (AOAC 1995). Serum total CH and triglyceride (TG) values 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 phos-

¹ Nutrition Research Program, American Institute of Baking, 1213 Bakers Way, P.O. Box 3999, Manhattan, KS 66505-3999.

² Corresponding author. Phone: 785/537-4750. Fax: 785/537-1493. E-mail: granhotra@aibonline.org

TABLE I
Fatty Acid Composition (%) of Test Fats and Blends

Fatty Acid	Test Materials ^a					
	Tristearin	Soybean Oil	15:85 Blend	30:70 Blend	45:55 Blend	60:40 Blend
16:0 (palmitic)	4.8	10.8	10.0	8.9	8.2	7.4
18:0 (stearic)	91.3	4.4	16.9	31.8	43.6	55.6
18:1 (oleic)	...	21.2	18.2	14.6	11.8	8.8
18:2 (linoleic)	...	55.0	47.0	37.6	30.3	22.6
20:0 (arachidic)	2.4	0.4	0.6	1.0	1.3	1.6
20:1 (gadoleic)	...	1.0	0.9	0.7	0.1	...
18:3 (linolenic)	...	6.7	5.7	4.6	3.7	2.8
22:0 (behenic)	1.0	0.4	0.5	0.6	0.7	0.8
24:0 (lignoceric)	0.5	0.1	0.2	0.2	0.3	0.4

^a Numbers preceding blends represent percentage of tristearin and soybean oil, respectively.

photungstic acid precipitation of other lipoproteins. Total CH in liver was determined by the method of Abell et al (1952). The data were analyzed statistically by analysis of variance using the Tukey test for means separation (SigmaStat Statistical Software, Jandel Scientific Software, San Rafael, CA).

RESULTS AND DISCUSSION

Fat Blends

The four TS and SO blends prepared (Table I) were all judged, based on textural characteristics, as suitable for use in baked goods and other foods. This study was, however, aimed at assessing the effect of progressively higher TS levels in the blends on the blood lipid profile and stearate digestibility. One blend (30:70) had lowered serum total CH in hamsters by 15.4% in an earlier study (Ranhotra et al 1997). The lowering effect in that study, however, was compared to a hypercholesterolemic (high lauric) fat tested.

Fatty Acid Profile

Although TS was quite high in the saturated fatty acid stearic acid, it also contained 8.7% nonstearic acids (Table I). Oleic and linoleic acids, reported to lower elevated blood CH levels (Chan et al 1991, Nydahl et al 1994, Fukushima et al 1996), were the predominant fatty acids in SO. As analyzed, the fatty acid profile of the four blends reflected the profile of the two fats blended, with the level of stearic acid steadily increasing as more TS was used in the blend (Table I).

Fat Intake and Weight Gains

Diets tested differed only in the source, and not the level, of fat. Thus, differences observed in serum lipid profile may be attributed to differences in fatty acid profiles of these fats, especially since total fat intake among groups of hamsters was nearly identical, and body weight gains differed minimally except in the group fed diet F (Table III).

Serum CH

In comparison to the baseline level (94 ± 7 mg/dL), serum total CH levels were quite elevated, likely in part due to feeding the CH-containing diet, in all but one group of hamsters including the group fed SO (Table III). SO and other unsaturates have repeatedly been shown to lower CH (Chan et al 1991, Nydahl et al 1994, Fukushima et al 1996), and this effect due to SO probably occurred in spite of CH elevations observed in this group. CH levels in man and animals usually increase with age, regardless of moderating influences of some dietary components.

Serum CH levels in hamsters fed SO (diet A) averaged 219 ± 19 mg/dL (Table III). This level closely matched CH levels observed on diets B–E, even though these diets contained increasingly higher levels of TS (Tables II and III). This is strongly suggestive that TS is not hypercholesterolemic, irrespective of the use level in the diet. This may be particularly so since body weight gains of animals fed diets B–E differed minimally and, thus, were not a significant contributory factor affecting serum CH levels. There are some indications that stearic acid may even lower serum CH. This was not

TABLE II
Composition (%) of Test Diets

	Blends					Tristearin F
	Soybean Oil A	15:85 B	30:70 C	45:55 D	60:40 E	
Fat source	16.9	16.9	16.9	16.9	16.9	16.9
Constants ^a	83.1	83.1	83.1	83.1	83.1	83.1

^a Contained (%): casein, 17.75; cellulose, 5; soybean oil, 1; mineral mix (Hegsted mix), 4.0; vitamin mix (ICN 904654), 2.2; *dl*-methionine, 0.3; choline chloride, 0.16; cholesterol, 0.25; and pregelatinized cornstarch, 52.44.

observed, probably because a known hypercholesterolemic fat was not included as a control. The aim was to compare TS with SO, a non-hypercholesterolemic fat. A significant ($P < 0.05$) lowering of CH was observed on diet F where TS was the only source of fat. This, however, may have resulted from the poor growth response of this group of animals and not necessarily due to a CH-lowering effect of TS.

Irrespective of the use level of TS in the blends tested (diets B–E), HDL CH levels in TS-fed groups tended to be somewhat lower, and the non-HDL CH somewhat higher, as compared to levels in the SO-fed group (Table III). This slight shift in HDL versus non-HDL CH ratios, but without affecting total CH levels, may be a meaningful observation. However, additional studies are needed to validate this observation and, if confirmed, to assess its physiological significance with regard to cardiovascular disease.

Serum TG

Elevated serum TG levels are viewed by some as an independent risk factor in cardiovascular disease (Austin 1991, McNamara 1992, Stensvold et al 1995, Hu et al 1997). Like serum CH, serum TG levels were also elevated in most groups (Table III). However, levels were most elevated in the group fed SO, with levels being lower on other diets, significantly so ($P < 0.05$) on diets D–F (Table III). Thus, while TS showed no CH-lowering effect in comparison to SO diet, it did show a noticeable TG-lowering effect.

Liver CH

Like serum CH and TG levels, liver total CH levels were also quite elevated in most groups of animals (Table III). Compared to the SO diet (diet A), liver CH concentrations were not significantly ($P > 0.05$) different in groups fed diets B–D, while diet E did show a significant ($P < 0.05$) lowering effect. No elevation was observed in the group fed just TS (diet F), but physiological significance of this is difficult to assess because of the poor growth response of animals in this group.

Fat Digestibility

In a recent study with rats, we reported stearate digestibility of 37% (Ranhotra et al 1998). When hamsters were used as the test model, apparent digestibility of an all-stearate fat was 16.2% (Ranhotra et al 1997). In this study, stearate digestibility ranged between 9.4 (diet F, the all-stearate diet) and 26.3% (15:85 blend), and it was inversely related to the level of stearate in the diet (Tables I and IV).

TABLE III
Blood Serum and Liver Lipid Responses^a

	Fat Source and Diet					
	Soybean Oil A	15:85 Blend B	30:70 Blend C	45:55 Blend D	60:40 Blend E	Tristearin F
Diet intake, g	171 ± 2	172 ± 0	172 ± 1	172 ± 0	172 ± 0	172 ± 0
Fat intake, g	30.6 ± 0.4	30.8 ± 0.1	30.7 ± 0.1	30.8 ± 0.1	30.8 ± 0.0	30.8 ± 0.0
Body weight gain, ^b g	29 ± 3a,b	31 ± 3a	29 ± 5a,b	25 ± 3b,c	24 ± 4c	4 ± 3d
Liver weight, g	4.3 ± 0.3a	4.0 ± 0.2a	3.0 ± 0.2b	2.9 ± 0.4b	2.5 ± 0.2c	1.4 ± 0.1d
Serum cholesterol (CH) ^c						
Total CH, mg/dL	219 ± 19a	215 ± 16a	222 ± 15a	214 ± 14a	220 ± 23a	106 ± 19b
HDL CH, ^d mg/dL	184 ± 13a	161 ± 15a,b	166 ± 23a,b	149 ± 12b	157 ± 16b	91 ± 10c
Non-HDL CH, mg/dL	35 ± 10b,c	54 ± 9a,b	56 ± 24a,b	65 ± 12a	64 ± 17a	14 ± 14c
Serum triglycerides (TG), ^c mg/dL	136 ± 52a	118 ± 25a,b	102 ± 34a-c	91 ± 18b,c	86 ± 14b,c	70 ± 22c
Liver CH, ^c mg/g	76 ± 16a	77 ± 8a	83 ± 8a	83 ± 18a	35 ± 8b	7 ± 1c

^a Averages ± standard deviation of 10 hamsters per diet. Values followed by the same letter in the same row are not significantly different ($P < 0.05$).

^b Initial body weight: 57 ± 4 g.

^c Baseline (day 0) values: serum total CH, 94 ± 7 mg/dL; serum HDL CH, 54 ± 4 mg/dL; serum non-HDL CH, 40 ± 6 mg/dL; serum TG, 51 ± 20 mg/dL; and liver CH, 24 ± 2 mg/g.

^d High-density lipoprotein CH.

TABLE IV
Digestibility of Soybean Oil and Tristearin^{a,b}

	Soybean Oil in Diet A	Stearate in Diet				
		B	C	D	E	F
Fat source consumed, g	28.9 ± 0.3	4.0 ± 0.0	8.0 ± 0.0	12.0 ± 0.0	16.1 ± 0.0	26.8 ± 0.0
Apparent digestibility, ^c %	97.7 ± 0.5a	26.3 ± 6.1b	16.3 ± 7.5c	14.4 ± 5.2c	10.2 ± 3.9c	9.4 ± 5.8c

^a Values are averages ± standard deviations of 10 hamsters per diet. Values followed by the same letter are not significantly different ($P < 0.05$).

^b All diets contained some soybean oil; digestibility for soybean oil was, however, calculated for the all-soybean oil diet (diet A) only.

^c Stearate digestibility values are calculated for all tristearin-containing diets (diets B-F), but considering only the stearic acid component (91.3%).

Even at the higher level of intake, no anal leakage of TS was observed. SO was nearly completely digested (97.7%).

Because of a variety of influencing factors, it may be difficult to arrive at a precise digestibility value for incompletely digested fats such as stearic fat. However, it may logically be presumed that stearate digestibility would likely be low and inversely related to use level in the diet.

CONCLUSION

The nonhypercholesterolemic effect of stearic fats may or may not be related to their poor digestibility characteristics. However, the use of stearic fats in producing functional shortenings and margarines may have many health advantages, including the absence of *trans* fatty acids and reduced caloric density. Utilizing fat blending technologies more effectively than attempted here, would further enhance the functional characteristics of these fats.

LITERATURE CITED

- Abell, L. L., Levy, B. B., Brodie, B. B., and Kendall, F. E. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* 195:357-366.
- Almendingen, K., Jordal, O., Kierulf, P., Sandstad, B., and Pedersen, J. I. 1995. Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp(a) in men. *J. Lipid Res.* 36:1370-1384.
- AOAC. 1995. Official Methods of Analysis of the Association of Official Analytical Chemists. 16th ed. Method 922.06. The Association: Arlington, VA.
- Ariaansz, R. F., and Okonek, D. V. 1995. Non-traditional catalyst applications. *Oils Fats Intl.* 11(3):16-18.
- Austin, M. 1991. Plasma triglycerides and coronary heart disease. *Arteriosclerosis* 11:1-5.
- Chan, J. K., Bruce, V. M., and McDonald, B. E. 1991. Dietary α -linolenic acid is as effective as oleic acid and linoleic acid in lowering blood cholesterol in normolipidemic men. *Am. J. Clin. Nutr.* 53: 1230-1234.
- Fukushima, M., Akiba S., and Nakano, M. 1996. Comparative hypocholesterolemic effects of six vegetable oils in cholesterol-fed rats. *Lipids* 31:415-419.
- Hernandez, E., and Lusas, E. W. 1997. Trends in transesterification of cottonseed oil. *Food Tech.* 51(5):72-76.
- Hu, F. B., Stampfer, M. J., Manson, J. E., Rimm, E., Colditz, G. A., Rosner, B. A., Heekens, C. H., and Willett, W. C. 1997. Dietary fat intake and the risk of coronary heart disease in women. *N. Engl. J. Med.* 337:1491-1499.
- Hunter, J. E., and Applewhite, T. H. 1991. Reassessment of *trans* fatty acid availability in the U.S. diet. *Am. J. Clin. Nutr.* 54:363-369.
- Judd, J. T., Clevidence, B. A., Muesing, R. A., Wittes, J., Sunkin, M. E., and Podczasy, J. J. 1994. Dietary *trans* fatty acids: Effects on plasma lipids and lipoproteins of healthy men and women. *Am. J. Clin. Nutr.* 59:861-868.
- Kritchevsky, D. 1994. Stearic acid metabolism and atherogenesis: History. *Am. J. Clin. Nutr.* 60 (suppl.):997S-1001S.
- Majumdar, S., and Bhattacharyya, D. K. 1986. *Trans* free vanaspati from palmstearin and vegetable oils by interesterification process. *Oleagineux* 41:235-240.
- McNamara, J. R., Jenner, J. L., Li, Z., Wilson, P. W. F., and Schaffer, J. R. 1992. Change in LDL particle size is associated with change in plasma triglyceride concentration. *Arterioscler. Thromb.* 12:1284-1290.
- Mensink, R. P., and Katan, M. B. 1990. Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N. Engl. J. Med.* 323:439-445.
- Miller, D. A., Prausnitz, J. M., and Blanch, H. W. 1991. Kinetics of lipase-catalysed interesterification of triglycerides in cyclohexane. *Enzyme Microb. Technol.* 13:98-103.
- NRC. 1987. Nutrient requirements of laboratory animals. Page 75 in: Nutrient Requirements of Domesticated Animals. NAS/NRC: Washington, DC.
- Nydahl, M., Gustafsson, I. B., Ohrvall, M., and Vessby, B. 1994. Similar serum lipoprotein cholesterol concentrations in healthy subjects on diets enriched with rapeseed and with sunflower oil. *Eur. J. Clin. Nutr.* 48:128-137.
- Ranhotra, G. S., Gelroth, J. A., and Leinen, S. D. 1997. Effect of tristearin and other functional fats on blood lipids in hamsters. *Cereal Chem.* 74:297-299.
- Ranhotra, G. S., Gelroth, J. A., and Vetter, J. L. 1996. Determination of fat in bakery products by three different techniques. *Cereal Foods World* 41:620-622.
- Ranhotra, G. S., Gelroth, J. A., and Leinen, S. D. 1998. Energy value of a fat high in stearic acid. *J. Food Sci.* 63:363-365.
- Stensvold, I., Tverdal, A., Urdal, P., and Graff-Iversen, S. 1993. Non-fasting serum triglyceride concentration and mortality from coronary heart disease and any cause in middle aged Norwegian women. *Brit. Med. J.* 307:1318-1322.
- Troisi, R., Willett, W. C., and Weiss, S. T. 1992. *Trans* fatty acid intake in relation to serum lipid concentrations in adult men. *Am. J. Clin. Nutr.* 56:1019-1024.

[Received December 22, 1997. Accepted March 30, 1998.]