

Lipidemic Response in Rats Fed Flaxseed or Sunflower Oils¹

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ABSTRACT

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Flaxseed oil, a rich source of omega-3 fatty acids, and sunflower oil (SO), a rich source of omega-6 fatty acids, and their blends were evaluated for their hypolipidemic effects as compared to that of a hard fat. Hypercholesterolemic rats were used as the test model. Hypercholesterolemia was induced through dietary means. Serum total cholesterol (CH) levels were highly elevated throughout the six-week study period in rats fed hard fat. In comparison to those in rats fed hard fat, CH levels averaged only 18-26% (week 2), 19-28% (week 4), and 21-40% (week 6) in rats fed oil-based diets. Within these ranges, flaxseed oil, but more significantly

its blends with SO, showed a more noticeable serum CH-lowering effect than did SO. All oil-based diets also showed lower serum triglyceride levels than did the diet formulated with hard fat. Although liver CH levels from oil-based diets were also significantly lower than those from the hard fat diet, liver lipid levels were lower only for diets that contained significant levels of SO. Viewed collectively, the results suggest that a diet with a proper balance between omega-3 and omega-6 fatty acids may be more desirable than a diet skewed heavily toward omega-6 fatty acids.

Fish oil, a rich source of omega-3 polyunsaturated fatty acids, is associated with a low incidence of cardiovascular disease, probably, in part, through its effect on blood lipids (Balasubramanian et al 1985, Herold and Kinsella 1986, Norum and Drevon 1986, Ranhotra et al 1990, Simopoulos 1991, Wallingford and Yetley, 1991). For use in food products, including bakery foods, fish oil is routinely hydrogenated to improve its stability and functionality characteristics. This, however, adversely affects the omega-3 content of the oil.

Flaxseed oil (FO), a cholesterol (CH)-free product, is also rich in omega-3s (Nettleton 1991). In fact, FO contains about twice as much omega-3 as fish oil. FO has a pleasing flavor and is relatively more stable, especially if packaged properly (it is quite stable in its unextracted form). FO is routinely used as a cooking oil in Europe but is not currently available for food use in North America.

FO and other newer oils such as canola oil and sunflower oil (SO) represent new sources of oils for food use. FO and SO are unique in that they both contain nearly the same levels of saturated, monounsaturated, and polyunsaturated fatty acids. However, the polyunsaturates in the two oils differ in their contents of omega-3 and omega-6 fatty acids. This difference may evoke different lipidemic responses. Blending the two oils would change the ratios of omega-3 to omega-6 fatty acids; this may also affect blood lipid levels differently. This study, using hypercholesterolemic rats as the test model, was undertaken to examine such effects.

MATERIALS AND METHODS

Test Samples

Three fats—FO, SO, and a hard fat—were used as test fats in this study (Table I). The fully saturated hard fat (fat flakes) is a commercial product prepared using soybean oil as the starting material.

Test Diets

A hard fat-based diet (diet A) and five oil-based diets (diets B-F) were prepared. Each contained 14.8% fat (Table II). Diets

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B and F contained only one oil source—FO or SO. The three other oil-based diets (diets C–E) contained blends of FO and SO. This blending involved replacing 25, 50, and 75% of FO with SO and resulted in different ratios of omega-3 to omega-6 fatty acids (Table II). All diets were complete in nutrients required by the rats (NRC 1987), and they contained added CH and cholic acid to induce hypercholesterolemia in rats. Diets were kept frozen and were withdrawn only in amounts needed for daily feeding.

Animals

Six groups of male, weanling rats (10 rats per group) of the Sprague-Dawley strain (Harlan Sprague-Dawley, Indianapolis) were housed individually in mesh-bottomed stainless steel cages in a controlled environment. Although food intake of rats was restricted to achieve nearly identical intakes between groups, each rat consumed an adequate diet; on a per-day basis, diet intake also varied minimally as the experiment progressed. Deionized water was offered ad libitum. Body weight records were maintained.

TABLE I
Fatty Acid Composition

Fatty Acid	Flaxseed Oil ^a	Sunflower Oil ^b	Hard Fat
Saturated, %	9	11	98
Monounsaturated, %	20	20	2
Polyunsaturated, %	71 ^c	69 ^d	...

^aSupplied by the Flax Institute, Fargo, ND.

^bFrom a commercial source.

^cContained 16% linoleic (omega-6) and 55% linolenic (omega-3) acids (manufacturer's data).

^dContained 68% linoleic and 1% linolenic acids (manufacturer's data).

TABLE II
Composition of Test Diets^a

	Diet					
	A	B	C	D	E	F
Components, %						
Hard fat	14.8
Flaxseed oil	...	14.8	11.1	7.4	3.7	...
Sunflower oil	3.7	7.4	11.1	14.8
Other ^b	85.2	85.2	85.2	85.2	85.2	85.2
Polyunsaturates, %						
Omega-3	...	8.1	6.1	4.1	2.1	0.1
Omega-6	...	2.4	4.3	6.2	8.1	10.1

^aAll diets contained 12.5% protein and had 30% of the total calories from fat.

^bIncluded 9.5% casein, 5.7% gluten, 1% vitamin mix (American Institute of Nutrition [AIN] vitamin mix 76), 3.5% mineral mix (AIN mineral mix 76), 1% cellulose, 1% cholesterol, 0.2% cholic acid, 0.16% choline chloride, and 63.14% cornstarch.

TABLE III
Physiological Responses in Rats^a

Rats ^b	Diet					
	A	B	C	D	E	F
Diet intake, g	429 ± 0	421 ± 7	425 ± 1	427 ± 1	428 ± 0	428 ± 0
Body weight gain, g	131 ± 8 a	133 ± 8 a	132 ± 10 a	137 ± 5 a	136 ± 5 a	137 ± 7 a
Liver weight, g	9.9 ± 0.5 a,b	9.1 ± 0.9 c	9.3 ± 0.7 a-c	9.1 ± 0.7 b,c	9.6 ± 0.7 a-c	10.0 ± 0.8 a
Serum total cholesterol, mg/dl						
Week 2	727 ± 143 a	147 ± 35 b	150 ± 29 b	150 ± 32 b	128 ± 24 b	191 ± 30 b
Week 4	580 ± 174 a	121 ± 25 b	111 ± 28 b	135 ± 31 b	116 ± 21 b	165 ± 20 b
Week 6	388 ± 71 a	110 ± 32 c	88 ± 27 c	93 ± 17 c	83 ± 19 c	154 ± 34 b
Serum triglycerides, mg/dl						
Week 2	54 ± 11 a	32 ± 5 c	24 ± 3 d	34 ± 6 c	38 ± 7 c	45 ± 7 b
Week 4	51 ± 6 a	40 ± 4 c,d	36 ± 5 d	37 ± 5 d	45 ± 6 b,c	45 ± 6 b
Week 6	36 ± 3 a	28 ± 4 b	23 ± 3 c	27 ± 4 b	28 ± 2 b	35 ± 5 a
Liver cholesterol, mg/g	131 ± 11 a	115 ± 8 b	83 ± 14 c	75 ± 8 c	78 ± 14 c	85 ± 9 c
Liver fat, %	24 ± 2 a	23 ± 2 a,b	25 ± 2 a	21 ± 3 b,c	20 ± 3 b,c	19 ± 3 c

^aValues are average ± SD (8–10 rats per diet). Within a row, means not followed by the same letter are significantly different ($P < 0.05$).

^bInitial values: body weight, 35 g; serum total cholesterol, 109 ± 12 mg/dl, and serum triglycerides, 41 ± 3 mg/dl.

Blood and Liver Sampling

At two-week intervals, all rats were fasted overnight (14 hr) then lightly anesthetized, and about 2 ml (1 ml at week 2) of blood was withdrawn by cardiac puncture. The blood was allowed to clot and was then centrifuged before the serum was obtained. Lipid analyses were run on the refrigerated serum over the next two days. At week 6, all animals were sacrificed, and their livers were removed, blotted dry, weighed, and homogenized. The homogenate volume was recorded, and the samples were saved (frozen) for determinations of CH and total fat.

Analytical Methods

The casein and gluten used in the test diets were analyzed for protein (Kjeldahl) using the standard AACC method (AACC 1983). Total serum CH and triglyceride (TG) levels were determined enzymatically using kits 352 and 336, respectively, from Sigma Chemical Co., St. Louis, MO. Total CH in liver was determined by the method of Abell et al (1952). Total liver fat was determined by freeze-drying an aliquot of liver homogenate and then ether-extracting the sample using the standard AACC method (AACC 1983).

Statistical Analysis

The data were subjected to analysis of variance. Mean comparisons were made with Duncan's multiple-range test using the Statistical Analysis System (SAS Institute 1982).

RESULTS AND DISCUSSION

Dietary Fat Level

The 14.8% fat level chosen in this study (Table II) represents 30% of the calories from fat, a level now widely recommended in the American diet (McNutt 1980, USDA/USDHHS 1990).

Diet Intake and Weight Gain

To minimize variables (other than the fat source) that might affect blood lipid levels, rats were offered diets in amounts to ensure adequate but nearly identical consumption (Table III). This way, the CH intakes between groups of rats were identical and their body weight gains differed only minimally (Table III).

Serum Total CH

Serum CH levels in normocholesterolemic rats generally measure around 100 mg/dl (Ranhotra et al 1990). Feeding CH and cholic acid, however, elevates serum CH levels quite profoundly (Story et al 1974, Ranhotra et al 1991). Although this occurred in this study also, CH levels were consistently most elevated in rats fed hard fat (diet A) (Table III, Fig. 1). This was probably the consequence of the complete saturation of fat in hard fat. However, the possible presence of *trans* fatty acids in hard fat may have contributed to this effect also. In rats fed oil-based

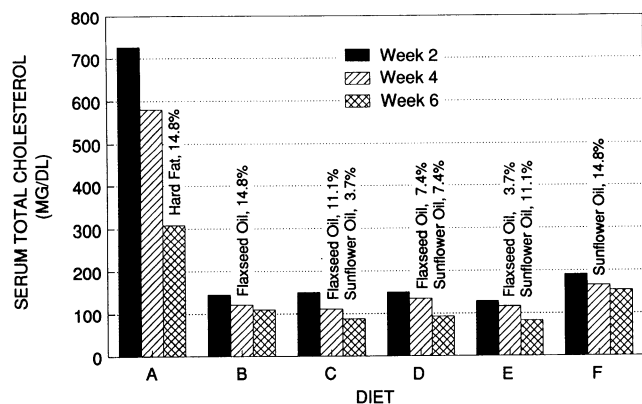


Fig. 1. Serum cholesterol levels in rats fed diets (A-F) containing hard fat, oil, or blends of oils. Diets are described in Table II.

diets, CH levels averaged (at week 2) only a fraction, 18–26%, of the CH levels observed in rats on diet A. This may mean that FO, SO, and their blends exerted a profound CH-lowering effect. This may be the effect of omega-3s and omega-6s.

FO—alone or in blends—contained appreciable levels of omega-3s. In human clinical studies, patients have not always responded favorably to diets containing omega-3s. This is attributed, in part, to differences in the saturated fat content of the diet, the omega-3 dose level tested, and the type of lipidemic condition being investigated (Simopoulos 1991).

Although the magnitude of differences varied, serum CH responses at weeks 4 and 6 showed a pattern similar to that observed at week 2 (Table III, Fig. 1). The persistence of observed differences between hard fat and test oils beyond week 6 was not examined, but it is likely that differences in CH levels may have been of a lesser magnitude.

All through the studies, the FO-based diet (diet B) persistently showed a more pronounced CH-lowering effect than the SO-based diet (diet F), although the difference was statistically significant ($P < 0.05$) only at week 6 (Table III). The most revealing observation, however, was that diets that contained both FO and SO (diets C–E) showed an even more pronounced CH-lowering effect (by week 6) than did just FO (diet B) or just SO (diet F). Blending FO and SO probably provided the balance between omega-3s and omega-6s (Table II) most conducive to favorable serum CH responses. Alternatively, proportional abundance of either omega-3s (diet B) or omega-6s (diet F) may be less effective. In either case, the results are suggestive of the need to strike a balance between the two types of polyunsaturates. The current North American diet is heavily skewed toward omega-6s (Simopoulos 1986, Nettleton 1991).

Serum TGs

Elevated serum TG levels are viewed by some (Austin 1991) as an independent risk factor in heart disease, and omega-3s seem to be consistently effective in lowering TG levels (Phillipson et al 1985, Herold and Kinsella 1986).

In this study, TG levels were in the normal range throughout the six-week test period. Some differences between diets were noted nevertheless (Table III). TG levels were significantly lower ($P < 0.05$) in rats fed oil-based diets than in those fed diet A (weeks 2 and 4 only). By week 6, TG levels tended to be lower on diets that contained some or all FO.

Liver Cholesterol and Fat Levels

Because of enhanced CH storage and/or impaired CH excretion, liver CH levels were highly elevated in all rats, especially

those fed diet A (hard fat) and diet B (FO) (Table III). These groups of rats also showed a more pronounced fatty infiltration (visual) of the liver than did rats fed the other diets except diet C. In the absence of a clear understanding of the relationship between hepatic and blood lipid profiles, the significance of these observations is difficult to interpret. Nevertheless, increased hepatic levels of CH and/or lipids perhaps can't be viewed as protective mechanisms against heart disease.

CONCLUSIONS

Viewing serum and liver lipid responses observed in this study collectively, it becomes apparent that diets that contained both FO and SO may influence lipid responses more favorably than diets containing just FO or SO. It is also apparent that fully saturated fats are potent hypercholesterolemic agents.

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.
- AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC. Method 46-12, approved October 1976, revised October 1986; Method 30-25, approved April 1961, revised October 1991. The Association: St. Paul, MN.
- AUSTIN, M. 1991. Plasma triglycerides and coronary heart disease. *Arterioscler. Thromb.* 11:1.
- BALASUBRAMANIAN, S., SIMONS, L. A., CHANG, S., and HICKIE, J. B. 1985. Reduction in plasma cholesterol and increase in biliary cholesterol by a diet rich in n-3 fatty acids in the rat. *J. Lipid Res.* 26:684.
- HEROLD, P. M., and KINSELLA, J. E. 1986. Fish oil consumption and decreased risk of cardiovascular disease: A comparison of findings from animal and human feeding trials. *Am J. Clin. Nutr.* 43:566.
- McNUTT, K. 1980. Dietary advice to the public: 1957 to 1980. *Nutr. Rev.* 38:353.
- NRC. 1987. Nutrient requirements of laboratory animals. Page 23 in: Nutrient Requirements of Domestic Animals. National Research Council/National Academy of Sciences: Washington, DC.
- NETTLETON, J. A. 1991. Omega-3 fatty acids: Comparison of plant and seafood sources in human nutrition. *J. Am. Diet. Assoc.* 91:331.
- NORUM, K. R., and DREVON, C. A. 1986. Dietary n-3 fatty acids and cardiovascular diseases. *Arteriosclerosis* 6:352.
- PHILLIPSON, B. E., ROTHROCK, D. W., CONNOR, W. E., HARRIS, W. S., and ILLINGWORTH, D. R. 1985. Reduction of plasma lipids, lipoprotein, and apoproteins by dietary fish oils in patients with hypertriglyceridemia. *New Engl. J. Med.* 312:1210.
- RANHOTRA, G. S., GELROTH, J. A., and ASTROTH, K. 1990. Fish oil added to biscuits is a potent hypolipidemic agent in hypercholesterolemic rats. *Cereal Chem.* 67:213-216.
- RANHOTRA, G. S., GELROTH, J. A., ASTROTH, K., and BHATTY, R. S. 1991. Relative lipidemic responses in rats fed barley and oat meals and their fractions. *Cereal Chem.* 68:548-551.
- SAS INSTITUTE. 1982. SAS User's Guide: Statistics. The Institute: Cary, NC.
- SIMOPOULOS, A. P. 1986. Historical perspective, conference conclusions and recommendations, and actions by federal agencies. Pages 3-29 in: Health Effects of Polyunsaturated Fatty Acids in Seafoods. A. P. Simopoulos, R. R. Kifer, and R. E. Martin, eds. Academic Press: New York.
- SIMOPOULOS, A. P. 1991. Omega-3 fatty acids in health and disease and in growth and development. *Am. J. Clin. Nutr.* 54:438.
- STORY, J. A., TEPPER, S. A., and KRITCHEVSKY, D. 1974. Influence of synthetic conjugates of cholic acid on cholesterolemia in rats. *J. Nutr.* 104:1185.
- USDA/USDHHS. 1990. Dietary Guidelines for Americans. Home and Garden Bull. 232. U.S. Dept. Agric. and U.S. Dept. Health and Human Serv.: Washington, DC.
- WALLINGFORD, J. C., and YETLEY, E. A. 1991. Development of the health claims regulations: The case of omega-3 fatty acids and heart disease. *Nutr. Rev.* 49:323.

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