NOTE

Lipidemic Responses in Rats Fed Flaxseed Oil and Meal

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ABSTRACT

In a six-week study, diets prepared with full-fat flaxseed meal (FM), defatted-FM (restored to full-fat level with a hard fat or isolated flaxseed oil [FO]), or just FO were compared for their effect on blood lipid levels. Hypercholesterolemic rats were used as the test model. FM greatly negated the adverse effect of hard fat (hydrogenated soybean oil) on serum total cholesterol (CH) and serum non high-density-lipoprotein CH. FM also accentuated the CH-lowering effect of isolated FO. Liver CH levels were quite elevated, especially in rats fed the diet not containing FM. Liver lipid levels ranged between 16 and 31%. Serum triglyceride levels were in the normal range and no lowering effect due to FM or FO was observed.

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In many countries, especially in Asia, flaxseed has been used as food for centuries. Flaxseed oil (FO) has also been used as a cooking oil in some countries. Although cultural and other changes have resulted in an extensive replacement of flaxseed by other foods, new knowledge of the role omega-3 fatty acids may play in health (lowering blood lipid levels, for example) has generated renewed interest in flaxseed (Herold and Kinsella 1986, Simopoulos 1991). The oil in flaxseed contains twice as much omega-3 (as linolenic acid) as fish oil, another rich source of omega-3 (as eicosapentaenoic and docosahexaenoic acids).

In a recent study (Ranhotra et al 1992), it was shown that FO, alone or in combination with sunflower oil, exerted a significant lowering effect on serum cholesterol (CH) in rats in comparison with a hard fat.

FO has a pleasing flavor; however, like fish oil, it is not a very stable oil. Hydrogenation improves stability, but it also adversely affects the omega-3 content of these oils. FO is quite stable in its unextracted form. For this reason, and because the possibility exists that nonlipid fractions of flaxseed may also exhibit a hypolipidemic effect, a study, using hypercholesterolemic rats as the test model, was undertaken to test this hypothesis.

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MATERIALS AND METHODS

Test Samples and Diets

Full-fat flaxseed meal (FM), defatted FM, refined FO, and a hard fat were used as the test materials in this study. Compositional information on FM is presented in Table I. Two types of FM from the same lot were obtained from Vitamins Inc. (Chicago, IL). FO, a refined product, was supplied by the Flax Institute (Fargo, ND). The hard fat (flakes) was a commercial product made from soybean oil.

Four diets were prepared (Table II). Diet A contained full-fat FM. Diets B and C contained defatted FM with fat restored to the full-fat level with isolated FO (diet B) or hard fat (diet C). Diet D contained no FM but had the same level of fat (added as FO) as did the other diets. All diets were complete in nutrients required by the rat (NRC 1987), and they contained 1% CH and

0.2% cholic acid to induce hypercholesterolemia in rats. Diets were kept frozen and were withdrawn only in amounts needed for daily feeding.

Animals

Four groups of male, weanling rats (10 rats per diet) of the Sprague-Dawley strain (Harlan Sprague-Dawley, Indianapolis, IN) were housed individually in mesh-bottomed stainless steel cages in a controlled environment. Each rat consumed an adequate diet, although food intake was slightly restricted to achieve identical intake for the four groups. Deionized water was offered *ad libitum*. Body weight records were maintained.

TABLE I
Composition (%) of Flaxseed Meal

Component	Flaxseed Meal	
	Full Fat	Defatted
Moisture	5.6	1.7
Protein (N × 5.7)	19.7	37.1
Fat (ether extract)	35.0	0.8
Ash	3.8	7.4
Total dietary fiber	29.8	45.4
Soluble fiber	2.3	6.2
Carbohydrates[a]	6.1	7.6

[a]Calculated values.

TABLE II
Percent Composition of Test Diets[a]

Component	Diet			
	A	B	C	D
Flaxseed meal (full-fat)	42.4	...	...	...
Flaxseed meal (defatted)	...	27.6	27.6	...
Flaxseed oil	...	14.6	...	14.8
Hard fat	...	...	14.6	...
Casein	1.5	1.5	1.5	7.5
Gluten	4.1	1.5	1.5	8.9
Cellulose	...	...	...	12.6
Constant ingredients[b]	5.86	5.86	5.86	5.86
Corn starch	46.14	48.94	48.94	50.34

[a]All diets contained 12.5% protein, 14.8% fat (providing 35% of the total calories), and 12.6% total dietary fiber. Diets A, B, C, and D contained 1.0, 1.7, 1.7, and 0% soluble fiber, respectively.
[b]Included 1% vitamin mix (American Institute of Nutrition mix 76), 3.5% mineral mix (AIN mix 76), 1% cholesterol, 0.2% cholic acid, and 0.16% choline chloride.

Blood and Liver Sampling

At the end of week 6, all rats were fasted overnight and lightly anesthetized. About 2 ml of blood was withdrawn by cardiac puncture. The blood was allowed to clot and the serum obtained. Lipid analyses were run on the refrigerated serum the next day. All rats were sacrificed at week 6; their livers were removed, blotted dry, weighed, and homogenized. The homogenate volume was recorded, and the samples were frozen until needed for CH and fat determinations.

Analytical

FM, gluten, and casein used in the test diets were analyzed for moisture, protein (Kjeldahl), fat (ether extract), and ash using the standard AACC methods (AACC 1983). Total dietary fiber and soluble fiber in FM were determined by the enzymatic-gravimetric method of the AACC (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). High-density-lipoprotein (HDL) CH was determined (using kit 352) following phosphotungstic acid precipitation of non-HDL-CH fractions. HDL-CH values subtracted from total CH values represent the non-HDL-CH values. Total CH in liver was determined by the method of Abell et al (1952). Total fat in liver was determined by ether-extracting the fat using a freeze-dried liver sample.

Statistical

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

RESULTS AND DISCUSSION

Test Materials and Diet Composition

Compositional values in Table I indicate that full-fat FM is high in fat (35%). In contrast, defatted FM is virtually free of fat; this characteristic allowed restoring defatted FM to full-fat level entirely with test fats—the refined FO or hard fat. FM is also high in dietary fiber, but it is not a significant source of soluble fiber. The FO tested contained (*supplier's data*): 71% polyunsaturated fatty acids (55% omega-3 and 16% omega-6). Monounsaturated and saturated fatty acids accounted for 20% and 9%, respectively, of the total fatty acid content in FO. The hard fat tested was nearly (98%) fully saturated fat.

Besides fat, a variety of other dietary factors also affect blood lipid levels. To minimize the effect of other factors, all diets were formulated to contain the same levels of protein, fiber, and fat (Table II). The fat level of 14.8% was identical to the level maintained in an earlier experiment (Ranhotra et al 1992) to enable a comparison of the results of the two studies.

TABLE III
Physiological Responses in Rats[a,b]

Parameter[c]	Diet			
	A	B	C	D
Fat source				
FM (full-fat)	+	−	−	−
FM (defatted)	−	+	+	−
FO	−	+	−	+
Hard fat	−	−	+	−
Diet intake, g	350 ± 0 a	350 ± 1 a	350 ± 0 a	350 ± 1 a
Body weight gain, g	104 ± 5 a	89 ± 10 b	93 ± 6 b	100 ± 8 a
Liver weight, g	6.7 ± 0.6 b	6.2 ± 0.9 b	6.8 ± 0.7 b	7.6 ± 0.6 a
Serum cholesterol, mg/dl				
Total	173 ± 33 a,b	95 ± 16 c	197 ± 55 a	142 ± 32 b
Non-HDL	117 ± 22 b	52 ± 16 c	171 ± 55 a	113 ± 30 b
Serum triglycerides, mg/dl	27 ± 5 b	27 ± 5 b	29 ± 5 b	35 ± 3 a
Liver cholesterol mg/g	92 ± 8 b	81 ± 13 b	81 ± 16 b	117 ± 18 a
Liver lipid, %	31 ± 7 a	16 ± 3 c	17 ± 3 c	22 ± 1 b

[a]Values are average ± SD (8–10 rats per diet). Within a row, means not followed by the same letter are significantly different ($P < 0.05$).
[b]Initial values: body weight, 41 ± 5 g; serum total cholesterol, 113 ± 5 mg/dl; serum non-HDL cholesterol, 54 ± 19 mg/dl; and serum triglycerides, 29 ± 10 mg/dl.
[c]FM = flaxseed meal, FO = flaxseed oil, HDL = high-density-lipoprotein.

Diet Intake and Weight Gains

All rats were fed diets in amounts to ensure adequate but identical consumption (Table III). This resulted in identical total fat and CH intakes for all groups of rats. Protein and energy intake were also nearly identical. However, rats fed diets prepared with defatted FM (diets B and C) did show significantly ($P < 0.05$) lower weight gains than did rats fed the other two diets. This may be the consequence of the process (residual solvent) used to defat FM.

Serum Total and Non-HDL Cholesterol

Rats fed CH and cholic acid invariably show a rapid elevation in serum CH levels. This effect, although somewhat diminished as CH feeding continues, persists up to week 8 (Ranhotra and Gelroth 1989) and perhaps beyond. Week 6 was chosen as the blood sampling interval in this study. At this interval, serum CH levels were most elevated in rats fed defatted FM containing hard fat (diet C) (Table III). However, the levels were appreciably less elevated (197 ± 55 vs. 388 ± 71 mg/dl) than that of the earlier study (Ranhotra et al 1992). In that study, hard fat (same source) was fed to the rats by itself, i.e., without FM. This suggests that FM was somewhat effective in negating the CH-raising effect of hard fat. A more convincing evidence of the possible hypocholesterolemic effect of FM is apparent when diets B and D are compared; rats fed diet B (defatted FM plus FO) showed significantly lower serum CH values than rats fed diet D (FO only).

Isolated and refined FO, alone (diet D), or added to defatted FM (diet B), seems a more potent CH-lowering agent than naturally occurring FO (diet A). Whether isolated or naturally occurring, FO was most effective in keeping the level of undesirable (non-HDL) CH levels low as compared to the diet based on hard fat (diet C). In rats fed hard fat, non-HDL-CH represented the major fraction (86%) of the total serum CH. In rats fed FO diets, non-HDL-CH fraction ranged between 80 and 54%, the lowest was on diet B (containing refined FO added to defatted FM). In fact, rats fed diet B showed normal total and non-HDL-CH values, unlike other groups.

Serum TG

Elevated serum TG levels are viewed by some (Austin 1991) as an independent risk factor in heart disease. Although diets high in omega-3 have repeatedly shown a TG-lowering effect (Herold and Kinsella 1986, Ranhotra et al 1992), TG levels were in the normal range in the current study.

Liver Cholesterol and Lipid Levels

In comparison with rats not fed CH (Ranhotra et al 1990), those fed CH showed a profound elevation of liver CH. Perhaps this is the consequence of enhanced storage and impaired excretion. Liver CH levels were quite elevated in all groups of rats, but less so in those fed diets containing FM (diets A–C) than in those not containing FM (diet D) (Table III). This may again suggest a protective effect of FM, provided that elevated liver CH levels are also viewed as a risk factor in heart disease. Total lipid levels in liver ranged from 16 to 31% and did not necessarily reflect a response pattern similar to serum lipid levels

CONCLUSIONS

As information on health benefits of flaxseed accumulates, flaxseed is likely to find application in a variety of foods. It is already used in some bread and related products produced in North America.

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