NOTE

Fish Oil Added to Biscuits is a Potent Hypolipidemic Agent in Hypercholesterolemic Rats[1]

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ABSTRACT

The results of an eight-week experiment, using young rats as the test model, showed that a diet containing biscuits made with fish oil exerted a persistent and potent lowering effect on serum total cholesterol (CH) and triglyceride (TG) in hypercholesterolemic rats; initially, it also caused an increase in high-density lipoprotein CH. A diet of biscuits containing linseed oil also substantially lowered both serum total CH and TG levels in hypercholesterolemic rats, but less than the fish oil diet. The canola oil diet lowered total CH but not TG levels. The fish oil diet and, to a lesser extent, the linseed oil diet also lowered serum total CH in normocholesterolemic rats.

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Intervention trials have now presented direct clinical evidence that lowering elevated blood cholesterol (CH) and probably triglyceride (TG) levels decreases the incidence of cardiovascular disease (LRC 1984). Diet modification that includes moderating intakes of CH and saturated fatty acids is widely recommended to lower blood CH. The CH-lowering effect of edible oils high in polyunsaturated fatty acids (PUFAs) is also well recognized. These oils, which are primarily of plant origin, are rich in omega-6 acids and/or the omega-3 acid linolenic acid (LNA, 18:3). How they may compare with fish oil, which is also rich in omega-3 acids but of a kind different from LNA (eicosapentaenoic acid [EPA] 20:5 and docosahexaenoic acid [DHA] 22:6), is largely unknown. These studies were designed to examine this. Rats were fed a fish oil-based diet (containing 5.1% fish oil) that earlier showed (Ranhotra and Gelroth 1989) a most favorable lipidemic response among the various fish oil-based diets tested. Other groups of rats were fed diets containing either canola oil (PUFA content similar to fish oil) or linseed oil (PUFA and omega-3 content much higher than in fish oil); canola oil is also high in the omega-9 (18:1) fatty acid oleic acid, an important hypolipidemic agent (Mattson and Grundy 1985).

A great deal of similarity exists (Herold and Kinsella 1986) between animals and man in serum lipid response when omega-3s are consumed. In this continuation study on fish oil, rats were again used as the test model.

MATERIALS AND METHODS

Biscuits

Baking powder biscuits using vegetable shortening alone or in combination with a test oil, were made using the following ingredients (in grams): pastry flour, 100; baking powder, 3; salt, 1; nonfat dry milk, 3; water, 50; shortening (or shortening plus oil), 25. After the ingredients were mixed, biscuits were baked at 215°C for 23 min.

Four large batches of biscuits were made and then used to formulate diets. The all-shortening biscuits contained 25% shortening (flour basis), and the oil-based biscuits contained 15% shortening and 10% oil—either fish, canola, or linseed oil. Whereas shortening contained only 0.5% omega-3 acids, fish oil contained 26.2% (EPA, 15.5%; DHA, 9.1%; LNA, 1.6%), canola oil contained 9.5% (as LNA), and linseed oil contained 56.6% (as LNA).

Diets

Biscuits, rapidly air-dried and finely ground, were used to formulate two control diets (A and AA, contained no oil) and six test diets. Test diets contained either fish oil (diets B and BB), canola oil (diets C and CC), or linseed oil (diets D and DD). Diets A–D (Table I) differed from diets AA–DD in that they contained CH and cholic acid to induce hypercholesterolemia in rats; CH and cholic acid were added at the expense of cornstarch. All diets were frozen until needed and were complete in all nutrients required by the rats (NRC 1987), including vitamin E. The content and distribution of energy from fat, protein, and carbohydrates were equalized among all diets. About 97% of the fat in diets originated from shortening (alone or in combination with oil) used in the biscuits; pastry flour contributed the remaining 3%. Whereas the control diets were virtually free of omega-3s, test diets contained various amounts. The omega-3 levels were highest in diets D and DD (source: LNA) intermediate in diets B and BB (source: EPA, DHA, and LNA), and lowest in diets C and CC (source: LNA).

Animals

Eight groups of male, weanling rats (10 rats/group) of Sprague-Dawley strain (Harlan Sprague-Dawley, Indianapolis, IN) were housed individually in mesh-bottom stainless steel cages in a controlled environment. Each rat was allowed to consume an adequate, but identical, amount of total diet during the eight-week test period; deionized water was offered ad libitum. Body weight records were maintained.

Blood and Liver Sampling

At two-week intervals, the rats were fasted overnight (14 hr), then lightly anesthetized, and about 2.0 ml of blood was withdrawn by heart puncture. The blood was allowed to clot and was then centrifuged prior to obtaining the serum. Lipid analyses were run on the refrigerated serum the next day. After the final blood sampling, the 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 CH determination.

Analytical Methods

The standard AACC methods (AACC 1983) were used to analyze biscuits for protein (Kjeldahl), fat (acid hydrolysis), and

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ash. Moisture was determined under vacuum (16 hr, 70°C, 24 mm Hg). Total CH and high-density lipoprotein (HDL) CH in serum were determined enzymatically using kit no. 352 from Sigma Chemical Co. (St. Louis, MO); HDL-CH was determined following magnesium dextran sulfate precipitation of nonHDL-CH. Total CH in liver was determined by the method of Abell et al (1952). Serum TG was also determined enzymatically using kit no. 336 from Sigma.

Statistical Analyses

Mean comparisons were made with Duncan's multiple-range test using the Statistical Analysis System (SAS 1982).

RESULTS

Body Weight Gain

All eight groups of rats were fed an identical, 720 ± 1 g, amount of diet during the eight-week test period (Table II). After eight weeks, body weight gains of these animals averaged between 202 and 221 g. Animals fed the CH-added diets (diets A–D) gained slightly less weight, and thus showed higher diet-gain ratios than animals fed diets with no CH added.

Serum Total Cholesterol

Adding CH to the diet of the animals to induce hypercholesterolemia resulted in a sharp and pronounced increase in their serum total CH (TCH) levels (diets A–D vs. AA–DD). This increase tapered off as the study progressed, but differences between the two sets of diets (CH added or not added) were still substantial by week 8 (Table II).

In hypercholesterolemic rats, all oil-based diets caused a significant ($P < 0.05$) reduction in serum TCH compared with the control diet (diets B–D vs. diet A). This occurred at all sampling intervals with the exception of one measurement, diet C at week 6. By week 8, serum TCH levels in rats fed the oil-based diets were 27–49% lower compared with the control diet. The fish oil diet (diet B) exhibited the most pronounced CH-lowering effect followed, in descending order, by the linseed oil diet and the canola oil diet (Table II, Fig. 1).

In normocholesterolemic animals, fish oil and linseed oil diets also showed a CH-lowering effect (diets BB and DD vs. diet AA). However, this effect was not statistically significant (Table II). The canola oil diet showed no CH-lowering effect.

Serum HDL Cholesterol

In hypercholesterolemic rats, HDL-CH represents a much smaller fraction of the TCH compared with that of normocholesterolemic animals, where HDL-CH alone constitutes two-thirds or more of the TCH concentration. In hypercholesterolemic rats, the serum HDL-CH level (as a percentage of TCH) tended to be higher in rats fed the fish oil diet compared with the control diet. This effect was, however, significant ($P < 0.05$) only at week 4 and did not persist as the study progressed. In rats fed the two other oil-based diets, no significant increase in serum HDL-CH was observed. In normocholesterolemic rats, none of the oil-based diets showed any increase in HDL-CH level compared with the corresponding control diet.

Serum TG

In hypercholesterolemic rats, triglyceridemic responses greatly paralleled the cholesterolemic responses. Serum TG levels were significantly ($P < 0.05$) lower in rats fed the fish oil diet compared with the control diet (Table II). This effect persisted throughout the test period. Serum TG levels were also significantly ($P < 0.05$) lower in animals fed the linseed oil diet. This effect also persisted throughout the test period, although it was somewhat less pronounced than with the fish oil diet. With one exception (week 4), the canola oil diet did not show a TG-lowering effect in rats. In normocholesterolemic rats, none of the oil-based diets showed any TG-lowering effect.

Liver Cholesterol

In contrast to body weight gains, which differed little, liver weight gains in rats fed CH were significantly ($P < 0.01$) higher than in rats not fed CH. Higher liver CH content contributed little to this higher weight, which was probably the consequence of fatty infiltration of hepatic tissue and/or higher water and protein content.

TABLE I
Experimental Diets

Parameter	Diet[a] A(AA)	B(BB)	C(CC)	D(DD)
Oil source	none	fish	canola	linseed
Ingredients, g/100 g				
Biscuits[b]	67.52	67.42	66.60	67.59
Vitamin mix[c]	2.2	2.2	2.2	2.2
Mineral mix[d]	5.0	5.0	5.0	5.0
Cholesterol[e]	1.00	0.97	1.00	1.00
Cholic acid	0.2	0.2	0.2	0.2
Casein[f]	8.19	8.26	8.16	8.13
Cornstarch	15.89	15.95	16.84	15.88
Composition, %				
Fat	13.2	13.2	13.2	13.2
From shortening	12.8	7.7	7.7	7.7
From oil source	...	5.1	5.1	5.1
Protein	11.5	11.5	11.5	11.5
Energy distribution, %				
Fat	30.6	30.6	30.1	30.5
Protein	11.8	11.8	11.7	11.8
Carbohydrates	57.6	57.6	58.3	57.7
Fatty acids,[g] g/100 g				
Total	2.72	3.33	3.23	5.40
Omega-6[h]	2.65	1.94	2.70	2.46
Omega-3[i]	0.07	1.39	0.53	2.94
Ratio	1:0.0	1:0.7	1:0.2	1:1.2

[a] Diets AA–DD contained no cholesterol or cholic acid.
[b] Finely ground
[c] Vitamin diet fortification mixture from ICN Biochemicals, Cleveland, OH.
[d] Contained (in sucrose base) the following mineral elements (mg/5 g of mixture): calcium, 500; phosphorus, 400; iron, 3.5; magnesium, 40; potassium, 360; sodium, 50; chromium, 0.03; copper, 0.5; iodine, 0.015; manganese, 5; selenium, 0.01; and zinc, 1.2.
[e] From Sigma Chemical Co., St. Louis, MO. Fish oil (diets B and BB) contributed some cholesterol.
[f] Contained 83.4% protein (N × 6.25).
[g] Fatty acid composition is based on information provided by the manufacturers. Shortening used was a partially hydrogenated soybean and cottonseed oil shortening (PVO Foods, St. Louis, MO). Fish oil (Menhaden oil) was obtained from Zapata Haynie Corporation, Reedville, VA. Canola oil was supplied by CSP Foods, Saskatoon, Canada, and linseed oil was supplied by ADM Company, Redwing, MN.
[h] From linoleic (18:2, omega-6) and arachidonic (18:4, omega-6) acids.
[i] From linolenic acid (18:3, omega-3), EPA (20:5, omega-3), and DHA (22:6, omega-3).

DISCUSSION

Fish oil was compared with two unusual oils—canola oil, which is now finding wide acceptance in food use in the United States, and linseed oil, an edible oil routinely used in east European countries.

Canola oil was selected because its PUFA content closely matches that of the fish oil; in addition, it is also quite high in the monounsaturated fatty acid oleic acid, an important hypolipidemic agent (Mattson and Grundy 1985). Linseed oil was chosen because the content of omega-3 acid (LNA) in this oil well exceeds the content of omega-3s (EPA and DHA) found in fish oil.

All three oils—fish, canola, and linseed—were used in biscuits at a level of 10%. As such, test diets made with these biscuits (Table I) also reflected a fatty acid profile typical of the oil used. For example, the content of PUFAs (omega-6 plus omega-3) in fish oil and canola oil diets matched closely. This content also matched closely with the PUFAs in the control diets (diets A

TABLE II
Physiological Responses in Rats Fed Test Diets for Eight Weeks[a]

Parameter	Diet							
	A	B	C	D	AA	BB	CC	DD
Oil source	none	fish	canola	linseed	none	fish	canola	linseed
Body weight gain,[b] g	202 ± 9 c	208 ± 14 bc	208 ± 17 bc	209 ± 7 bc	208 ± 7 bc	214 ± 7 ab	221 ± 9 a	217 ± 7 ab
Liver weight gain, g	12.3 ± 1.0 a	11.9 ± 1.2 a	11.6 ± 1.4 a	12.6 ± 0.6 a	5.0 ± 0.3 b	5.2 ± 0.5 b	4.5 ± 0.3 b	4.1 ± 0.3 b
Diet intake, g	720 ± 2 a	721 ± 2 a	718 ± 7 a	721 ± 1 a	720 ± 3 a	718 ± 5 a	718 ± 7 a	720 ± 3 a
Cholesterol intake, mg	7.20	7.21	7.18	7.21	...	0.21	...	...
Diet/gain ratio	3.6 ± 0.2 a	3.5 ± 0.2 ab	3.5 ± 0.3 ab	3.4 ± 0.1 ab	3.5 ± 0.1 ab	3.4 ± 0.1 bc	3.2 ± 0.1 c	3.3 ± 0.1 bc
Serum total cholesterol,[c] mg/dl								
Week 2	574 ± 77 a	247 ± 81 c	408 ± 103 b	285 ± 67 c	105 ± 16 d	92 ± 11 d	121 ± 7 d	99 ± 10 d
Week 4	481 ± 97 a	188 ± 44 d	370 ± 70 b	276 ± 77 c	106 ± 10 e	96 ± 13 e	115 ± 8 e	98 ± 10 e
Week 6	351 ± 88 a	175 ± 38 c	371 ± 95 a	287 ± 59 b	93 ± 13 d	88 ± 9 d	104 ± 7 d	87 ± 9 d
Week 8	292 ± 65 a	148 ± 26 c	212 ± 56 b	179 ± 35 c	77 ± 10 d	68 ± 9 d	88 ± 8 d	75 ± 9 d
Serum HDL-cholesterol,[c] % of total								
Week 2	6 ± 3 c	11 ± 6 c	7 ± 3 c	7 ± 9 c	71 ± 5 ab	73 ± 4 a	73 ± 3 a	67 ± 4 b
Week 4	5 ± 3 d	16 ± 5 c	6 ± 2 d	5 ± 2 d	78 ± 4 a	73 ± 5 b	74 ± 4 b	73 ± 4 b
Week 6	8 ± 6 de	12 ± 6 d	8 ± 5 de	4 ± 2 e	75 ± 5 a	66 ± 5 c	72 ± 6 ab	67 ± 4 b
Week 8	14 ± 15 c	13 ± 6 cd	8 ± 4 d	7 ± 5 d	79 ± 6 a	72 ± 4 ab	72 ± 6 ab	67 ± 5 b
Serum triglycerides,[c] mg/dl								
Week 2	41 ± 4 a	31 ± 3 bc	40 ± 6 a	32 ± 5 bc	31 ± 6 bc	28 ± 5 c	41 ± 8 a	35 ± 8 b
Week 4	54 ± 9 bc	35 ± 8 e	46 ± 6 cd	43 ± 6 de	64 ± 15 ab	67 ± 17 a	58 ± 13 ab	55 ± 9 bc
Week 6	61 ± 8 ab	46 ± 6 d	62 ± 10 ab	51 ± 7 cd	54 ± 15 bc	68 ± 14 a	65 ± 9 a	61 ± 9 abc
Week 8	65 ± 11 a	46 ± 9 b	66 ± 14 a	46 ± 8 b	53 ± 8 b	65 ± 9 a	68 ± 13 a	50 ± 8 b
Liver total cholesterol, mg/g	8.8 ± 2.9 a	11.1 ± 3.0 b	11.3 ± 1.0 b	11.2 ± 1.9 b	3.5 ± 0.4 c	4.2 ± 0.3 d	4.1 ± 0.6 d	4.8 ± 1.0 d

[a]Values are averages (8–10 rats/diet) ± standard deviations. Averages not followed by a common letter in a line are significantly different ($P < 0.05$).
[b]Initial (0 day) body weight averaged 39 g (all groups).
[c]Initial (0 day) serum lipid profile: total cholesterol, 90 ± 12; high-density lipoprotein (HDL)-cholesterol, 63 ± 7; triglycerides, 37 ± 3.

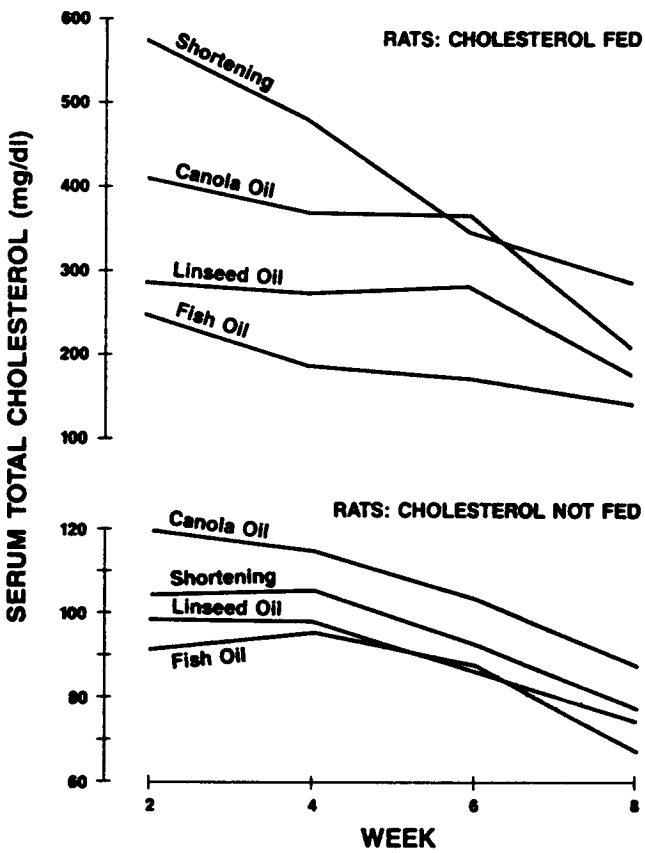

Fig. 1. Serum cholesterol levels in hypercholesterolemic (cholesterol fed) and normal (cholesterol not fed) rats measured at biweekly intervals.

and AA), although the control diets were virtually free of omega-3s (Table I). The linseed oil diets (diets D and DD) were much higher, 50% or more, in PUFA content than the other diets. This was primarily due to the high LNA content in linseed oil.

Rats fed these diets showed widely different lipidemic responses. These responses were assessed both in hypercholesterolemic (CH-fed) and normocholesterolemic (CH, not fed) rats, although lowering circulating lipid levels is primarily targeted only when levels are elevated enough to pose a health risk.

Of the three oil-based diets tested, the fish oil diet lowered serum CH levels the most; compared with the corresponding control diet, this lowering effect averaged, by week 8, 49.3% in hypercholesterolemic rats and 11.7% in normocholesterolemic rats (Table II, Fig. 1). Although substantially higher in the omega-3 acid LNA, the linseed oil diet showed a less pronounced hypocholesterolemic effect than the fish oil diet. This was true both in hypercholesterolemic and normocholesterolemic rats and may be because of the limited capacity of the animal to convert LNA to EPA and DHA, as shown in humans (Sanders and Younger 1981). The CH-lowering effect of the canola oil diet was even less than that of linseed oil even though canola oil also contained a high level of the monounsaturated oleic acid, reported (Mattson and Grundy 1985) to be an important hypolipidemic agent. In normocholesterolemic rats, in fact, the canola oil diet showed no CH-lowering effect.

Unlike human subjects, HDL-CH is the major CH fraction in normocholesterolemic rats (Table II). No increase in HDL-CH was observed when these animals were fed either of the three oil-based diets (diets BB-DD vs. diet AA). In hypercholesterolemic rats, HDL-CH level (as a percentage of the TCH) increased only in rats fed the fish oil diet, and this increase was significant ($P < 0.05$) only in week 4.

It is now agreed that the consumption of fish oil lowers plasma TG levels in both normal and hypertriglyceridemic animal and human subjects (Sanders 1986, Herold and Kinsella 1986). In the present study, fish oil lowered serum TG levels persistently and significantly ($P < 0.05$), but only in hypercholesterolemic rats; the linseed oil diet also showed a TG-lowering effect (Table II). In contrast, none of the oils tested showed any TG-lowering effect in normocholesterolemic rats. Sanders and Hochland (1983) earlier reported the failure of vegetable oils to lower plasma TG levels.

In general, the results of this study agree well with a recent report by Zak et al (1989) where the administration of fish oil decreased total plasma CH and TG concentrations and increased the HDL-CH level in hypertriglyceridemic men.

This study did not examine the probable mechanism for the hypolipidemic effect of oils tested. However, hepatic CH levels, which were significantly ($P < 0.01$) elevated in rats fed oil-based diets compared with control diets, suggest that the liver played a significant role, probably mediated through receptor sites and excretary mechanisms.

In conclusion, these studies showed that fish oil is quite unique in its capacity to lower both the TCH and TG levels in blood. The unique effect of fish oil may be due to its high content of EPA and DHA. If so, this may mean that some increase in omega-3s (marine source) compared with current levels of intake may be warranted. As we learn of the different, and sometimes opposing, effects of omega-6s and omega-3s, all PUFAs cannot be considered as a single class.

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