Differential Effects of Dietary Linoleic and α-Linolenic Acid on Lipid Metabolism in Rat Tissues

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Comparative effects of feeding dietary linoleic (safflower oil) and α-linolenic (linseed oil) acids on the cholesterol content and fatty acid composition of plasma, liver, heart and epididymal fat pads of rats were examined. Animals fed hydrogenated beef tallow were used as isocaloric controls. Plasma cholesterol concentration was lower and the cholesterol level in liver increased in animals fed the safflower oil diet. Feeding the linseed oil diet was more effective in lowering plasma cholesterol content and did not result in cholesterol accumulation in the liver. The cholesterol concentration in heart and the epididymal fat pad was not affected by the type of dietary fatty acid fed. Arachidonic acid content of plasma lipids was significantly elevated in animals fed the safflower oil diet and remained unchanged by feeding the linseed oil diet, when compared with the isocaloric control animals fed hydrogenated beef tallow. Arachidonic acid content of liver and heart lipids was lower in animals fed diets containing safflower oil or linseed oil. Replacement of 50% of the safflower oil in the diet with linseed oil increased α-linolenic, docosapentaenoic and docosahexaenoic acids in plasma, liver, heart and epididymal fat pad lipids. These results suggest that dietary 18:2ω6 shifts cholesterol from plasma to liver pools followed by redistribution of 20:4ω6 from tissue to plasma pools. This redistribution pattern was not apparent when 18:3ω3 was included in the diet. *Lipids 23,* 847–852 (1988).

Attempts to elucidate mechanisms by which dietary polyunsaturated fatty acids (PUFA) lower blood cholesterol level in man (1–2) and animals (3–7) have led to the hypothesis that dietary PUFA cause redistribution of cholesterol from blood to tissue pools. To test this hypothesis, several studies have examined the effect of dietary PUFA on plasma and tissue cholesterol levels (8–12), using vegetable oils rich in linoleic acid (18:2ω6). Marine oils rich in eicosapentaenoic (20:5ω3) and/or docosahexaenoic acid (22:6ω3) have been shown to be more effective against hypercholesterolemia (12–15). However, one study (16) has shown that both the vegetable oil and fish oil cause similar decreases in cholesterol levels. While vegetable oils rich in ω6 fatty acids have proaggregatory effects in thrombosis (17–19), marine oils rich in very long ω3 fatty acids may account for the enhanced bleeding tendency observed (20–22), due to their antiaggregatory effects. Some studies have shown that vegetable oils have antiaggregatory effects (23,24). However, these studies suggest one thing in common, that the number and arrangement of double bonds within a fatty acid molecule is an important factor for cholesterol-lowering and thrombotic activity. Eicosapentaenoic and docosahexaenoic acids present in marine oils are the desaturated-chain elongated products of α-linolenic (18:3ω3) acid (25). The question of whether 18:3ω3 can affect the plasma and/or tissue cholesterol and fatty acid metabolism has not been given much attention. It is conceivable that 18:3ω3 may have an intermediate effect, in terms of its antiaggregatory and hypocholesterolemic effects, between that of ω6 fatty acids of vegetable oils and very long-chain ω3 fatty acids of marine oils.

The present experiment was designed to clarify the effect of feeding 18:3ω3 on tissue cholesterol pools and fatty acid composition. Safflower- and linseed-oil-fed rats were used to identify the specificity of the action of 18:2ω6 and 18:3ω3 on the distribution of tissue cholesterol pools and levels of 20:4ω6, in comparison with animals fed a diet high in hydrogenated beef tallow.

MATERIALS AND METHODS

Animals and diets. Male adult Sprague-Dawley rats weighing 200–250 g were allotted to individual stainless steel cages and provided water and laboratory chow for three days before starting the experimental diet treatment. After three days of feeding chow, rats were weighed and divided into three groups of five rats per group in a manner ensuring that the average body wt was similar in each group. Each group of animals was fed a semisynthetic diet enriched with either saturated or linoleic or α-linolenic acid. Semipurified diets containing 20% (w/w) fat (26) were prepared by mixing either 180 g hydrogenated beef tallow plus 20 g safflower oil or 200 g safflower oil or 100 g linseed oil plus 100 g safflower oil (Table 1) per kg of total diet. The fatty acid composition of these diets is illustrated (Table 1). The fat mixture of the hydrogenated beef tallow diet contained 26% palmitic and 41% stearic acid, the safflower oil diet was high in linoleic acid (71.2%), while the linseed oil diet provided a high content of α-linolenic acid at 24.1% (Table 1). Diets were prepared weekly and stored at −20 C. Food and water were available to the animals ad libitum for a feeding period of 28 days.

Analytical procedures. After feeding the experimental diets for four weeks, animals were killed between 0800 and 1000 hr and liver, heart and epididymal fat pads were removed and placed in ice-cold physiological saline. After decapitation, trunk blood was collected in heparinized tubes and the plasma was separated (1500 × *g* for 10 min). For lipid extraction, plasma, livers, hearts and epididymal fat pads from animals fed the fat-supplemented diets were homogenized in chloroform/methanol (2:1, v/v) (27). Aliquots of the total lipid extracts from these tissues were taken for determination of free cholesterol, esterified cholesterol and total cholesterol (28). Free cholesterol and esterified cholesterol from lipid extracts of the epididymal fat pad were separated by thin-layer chromatography (TLC) on Silica Gel G plates using a solvent system composed of petroleum ether/diethyl

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Abbreviations: PUFA, polyunsaturated fatty acids; GLC, gas-liquid chromatography; TLC, thin-layer chromatography.

TABLE 1

Fatty Acid Composition of Experimental Diets (wt % of total fatty acids)

Fatty acid[a,b]	Beef tallow[c]	Safflower oil[c]	Linseed oil[c]
14:0	3.7	0.5	0.3
15:0	0.5	—	—
16:0	26.1	8.1	6.3
16:1ω7	0.3	—	0.1
17:0	1.5	—	—
18:0	47.1	2.7	3.8
18:1ω9	5.7	12.4	18.8
18:1ω7	0.7	0.7	0.6
18:2ω6	9.0	71.2	43.3
18:3ω6	0.1	1.2	0.2
18:3ω3	0.1	0.2	24.1
18:4ω3	—	—	1.0
Others	0.2	0.3	0.3
ΣSaturated	79.9	11.6	10.8
ΣMonounsaturated	6.7	13.2	19.8
Σω6	9.2	72.2	44.2
Σω3	0.1	0.2	25.2

[a]Fatty acids are designated by the chain length, number of double bonds and the position of the first double bond from the methyl end of the molecule.

[b]Only major individual fatty acids are reported.

[c]Fats were added to the basal diet in the following proportions: 180 g hydrogenated beef tallow plus 20 g safflower oil (beef tallow diet); 200 g safflower oil (safflower oil diet); and 100 g linseed oil plus 100 g safflower oil (linseed oil diet) per kg of diet. The basal diet contained the following components (g/kg diet): casein, 270; starch, 200; glucose, 207; nonnutritive cellulose, 50; vitamin mix, 10; mineral mix, 50; choline, 2.75; L-methionine, 6.25; as described elsewhere (23).

ether/acetic acid (80:20:1, v/v/v), extracted and then assayed individually (28).

Lipid extracts were converted to fatty acid methyl esters using BF_3-methanol (14%, w/w) reagent (29). Fatty acid methyl esters were separated and quantified by automated gas-liquid chromatography (GLC) (Varian, Model-6000; 30), using a 20-m fused-silica capillary column (BP-20, S.G.E. Pty. Ltd., Melbourne, Australia). Helium was used as the carrier gas. Injector and detector temperatures were maintained at 250 C. The column temperature was programmed from 130 C to 225 C, at a rate of 20 C/min up to 175 C, and then at a rate of 5 C/min up to 225 C. These chromatographic conditions separated all major positional and geometric isomers for fatty acids from 12 to 24 carbons in chain length. Fatty acid methyl ester peaks were identified by injecting authentic standard mixtures of fatty acid methyl esters.

STATISTICAL ANALYSIS

All data are presented as the mean ± standard deviation. The effect of dietary fat supplements was analyzed using analysis of variance procedures and the effect of individual diets was compared for statistical significance ($p < 0.05$) using a Duncan's multiple range test (31).

RESULTS

The average body wt, liver wt, liver wt to body wt percentages, heart wt and amount of food consumed per day for the animals fed the fat-supplemented diets for a four wk period are presented (Table 2). Rats fed the linseed oil diet had significantly higher body wt compared with those fed the hydrogenated beef tallow diet. Animals consumed similar amounts of food per day irrespective of the fat supplement. The wet wt of the livers, liver wt to body wt ratios and heart wt were also unaffected by the dietary fatty acid composition (Table 2).

Feeding the safflower oil diet for 28 days reduced plasma cholesterol content compared with control animals fed beef tallow (Table 3). This reduction was detected in the esterified-cholesterol content while the unesterified-cholesterol content was increased in the plasma of rats fed the safflower oil diet. Feeding the linseed oil diet was more effective in lowering the plasma cholesterol level (Table 3). Total cholesterol content of liver tissue was significantly increased following feeding of the safflower oil diet. This increase was due to accumulation of both free and esterified cholesterol in the liver. Replacement of 50% of the safflower oil by linseed oil reduced accumulation of cholesterol in liver tissue. Cholesterol levels in heart and epididymal fat pads were unchanged by dietary treatments. Neither the free- nor the esterified-cholesterol content of heart or the epididymal fat pad was affected by changes in the dietary fat composition (Table 3).

Essential fatty acid (18:2ω6 and 20:4ω6) content of plasma was elevated by feeding the safflower oil diet, with an accompanied decrease in 16:0, 18:0 and 18:1 content when compared with animals fed diets containing beef tallow (Table 4, Fig. 1). Feeding the linseed oil diet resulted in accumulation of 18:3ω3, 22:5ω3 and 22:6ω3 in the plasma lipids, but failed to alter the 20:4ω6 content. In liver tissue, both the safflower oil and the linseed oil diets lowered the 20:4ω6 content (Table 4, Fig. 1) to the

TABLE 2

Effect of Dietary Fat Treatments on the Liver Weight, Body Weight, Heart Weight, Liver Weight to Body Weight Ratios and Food Consumption[a]

Diet	Body wt (g)	Liver wt (g)	LW/BW (%)	Heart wt (g)	Food consumed (g/day)
Beef tallow	370 ± 14	14.1 ± 0.6	3.8 ± 0.2	1.1 ± 0.1	21.8 ± 1.9
Safflower oil	401 ± 20	14.5 ± 0.7	3.6 ± 0.2	1.2 ± 0.1	20.4 ± 1.8
Linseed oil	421 ± 22[b]	16.1 ± 1.5	3.8 ± 0.2	1.2 ± 0.1	22.9 ± 1.8

[a]Values given are the mean ± standard deviation of 5 rats.

[b]Significantly different from the beef tallow-fed animals at $p < 0.05$.

TABLE 3

Effect of Dietary Fat Treatment on the Cholesterol Content of Rat Plasma, Liver, Heart and Epididymal Fat Pad[a]

Tissue	Cholesterol fraction	Beef tallow	Safflower oil	Linseed oil
Plasma	Free	29.8 ± 2.5	34.2 ± 1.2[b]	30.0 ± 3.0
(mg/100 ml)	Esterified	39.4 ± 2.0	28.1 ± 2.9[c]	25.2 ± 3.4[c]
	Total	69.2 ± 1.6	62.1 ± 1.9[b]	55.2 ± 1.7[c]
Liver	Free	130 ± 11	156 ± 7[b]	137 ± 7
(mg/100 g)	Esterified	70 ± 6	95 ± 12[b]	76 ± 14
	Total	200 ± 15	251 ± 15[b]	214 ± 12
Heart	Free	108 ± 7	107 ± 4	117 ± 11
(mg/100 g)	Esterified	26 ± 1	26 ± 3	29 ± 2
	Total	135 ± 7	133 ± 6	146 ± 14
Adipose tissue	Free	133 ± 3	135 ± 4	136 ± 6
(mg/100 g)	Esterified	32 ± 2	31 ± 2	32 ± 4
	Total	165 ± 3	166 ± 3	168 ± 6

[a]Values are the mean ± standard deviation for 5 animals.

[b]Significantly different from beef tallow-fed animals at $p < 0.05$.

[c]Significantly different from beef tallow-fed animals at $p < 0.01$.

TABLE 4

Effect of Dietary Fat Treatments on the Fatty Acid Composition of Rat Plasma and Liver Total Lipids (wt % of total fatty acids)[a]

	Plasma			Liver		
Fatty acid	Beef tallow	Safflower oil	Linseed oil	Beef tallow	Safflower oil	Linseed oil
14:0	2.3 ± 0.5[a]	0.4 ± 0.1[b]	0.3 ± 0.0[b]	—	—	—
16:0	22.3 ± 1.0[a]	15.5 ± 1.1[b]	13.1 ± 1.1[b]	15.4 ± 0.8	14.9 ± 0.8	13.5 ± 0.7
16:1ω7	1.6 ± 0.5[a]	0.5 ± 0.1[b]	0.3 ± 0.1[c]	1.2 ± 0.3[a]	0.4 ± 0.1[b]	0.3 ± 0.0[b]
17:0	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.6 ± 0.1[a]	0.2 ± 0.0[b]	0.3 ± 0.0[c]
18:0	21.8 ± 0.9[a]	13.0 ± 1.4[b]	14.3 ± 2.6[b]	25.7 ± 0.8[a]	14.7 ± 1.8[b]	19.4 ± 3.9[c]
18:1ω9	15.2 ± 1.4[a]	8.0 ± 0.8[b]	10.2 ± 1.8[b]	8.2 ± 0.5	6.9 ± 0.6	7.7 ± 1.9
18:1ω7	2.2 ± 0.3[a]	1.8 ± 0.4[a]	1.3 ± 0.1[b]	2.5 ± 0.4	2.2 ± 0.4	1.8 ± 0.1
18:2ω6	18.5 ± 1.3[a]	40.3 ± 4.1[b]	32.8 ± 2.8[c]	11.2 ± 0.8[a]	32.4 ± 3.6[b]	23.3 ± 4.0[b]
18:3ω6	0.2 ± 0.1[a]	0.3 ± 0.1[a]	0.1 ± 0.0[b]	0.2 ± 0.0[a]	0.5 ± 0.1[b]	0.2 ± 0.1[a]
18:3ω3	T[b,a]	—	8.3 ± 1.9[b]	0.3 ± 0.2[a]	0.1 ± 0.0[a]	3.8 ± 1.4[b]
20:1ω9	0.2 ± 0.1	—	—	0.1 ± 0.0[a]	0.2 ± 0.0[b]	0.2 ± 0.1[b]
20:2ω6	0.1 ± 0.0[a]	0.9 ± 0.1[b]	0.4 ± 0.1[c]	0.3 ± 0.1[a]	1.5 ± 0.2[b]	0.8 ± 0.2[c]
20:3ω9	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.1 ± 0.0[b]	—	—	—
20:3ω6	0.4 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.1
20:4ω6	11.4 ± 0.4[a]	14.7 ± 1.2[b]	11.8 ± 2.8[a,b]	25.0 ± 1.4[a]	18.0 ± 1.5[b]	18.4 ± 2.4[b]
20:5ω3	—	—	—	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
22:4ω6	—	0.7 ± 0.2[a]	0.1 ± 0.0[b]	0.4 ± 0.1[a]	1.0 ± 0.2[b]	0.2 ± 0.0[c]
22:5ω6	0.6 ± 0.2[a]	0.9 ± 0.2[a]	T	1.3 ± 0.2	1.6 ± 0.2	—
22:5ω3	0.3 ± 0.2[a]	0.2 ± 0.1[a]	0.6 ± 0.1[b]	0.1 ± 0.0[a]	—	0.9 ± 0.1[b]
22:6ω3	0.9 ± 0.1[a]	0.7 ± 0.2[a]	2.1 ± 0.4[b]	3.1 ± 0.2[a]	1.6 ± 0.2[b]	4.3 ± 0.6[c]
ΣSat.	47.7 ± 1.4	30.2 ± 2.8	28.5 ± 3.8	42.7 ± 0.9	30.6 ± 2.5	33.7 ± 4.3
ΣMUFA[c]	19.6 ± 1.6	10.8 ± 1.2	12.5 ± 1.8	12.7 ± 0.9	10.5 ± 0.5	11.2 ± 1.9
Σω6	31.2 ± 1.8	57.9 ± 3.2	46.8 ± 1.2	38.6 ± 1.1	55.1 ± 3.0	43.2 ± 1.4
Σω3	1.5 ± 0.3	1.1 ± 0.2	11.4 ± 1.5	3.6 ± 0.3	1.8 ± 0.2	9.4 ± 1.0

[a]Values given are mean ± standard deviation of 5 separate determinations (n = 5). Values without a common superscript are significantly different at $p < 0.05$.

[b]Trace amount (<0.1).

[c]Monounsaturated fatty acid.

same extent (ca. 28% compared with the group fed beef tallow). Consumption of the safflower oil diet increased deposition of 18:2ω6, 20:2ω6 and 22:4ω6 and depletion of 22:5ω3 and 22:6ω3 in the liver lipids. On the other hand, 18:3ω3, 22:5ω3 and 22:6ω3 in the liver tissue increased following the feeding of the linseed oil diet (Table 4).

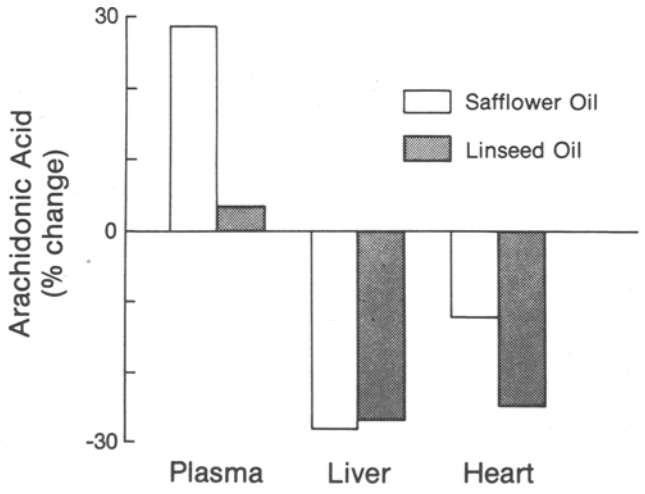

FIG. 1. Percentage change in the arachidonic acid content of plasma, liver and heart lipids compared with the hydrogenated beef tallow group.

Ingestion of the safflower oil diet increased 18:2ω6 and 22:5ω6 content in cardiac lipids and was accompanied by decreased 20:4ω6 and 22:6ω3 content (Table 5). Feeding the linseed oil diet decreased the 20:4ω6 levels in the heart more effectively than observed for the safflower oil diet (12.2% vs 24.9%, respectively; Fig. 1). The linseed oil diet also increased 18:3ω3, 22:5ω3 and 22:6ω3 in heart lipids (Table 5). The fatty acid composition of the epididymal fat pad resembled that of the dietary fat treatment, e.g., the safflower oil diet increased 18:2ω6 content, whereas the linseed oil diet elevated 18:2ω6 and 18:3ω3 content at the expense of saturated (14:0, 16:0 and 18:0) and monounsaturated (16:1 and 18:1) fatty acids (Table 5).

DISCUSSION

The results suggest that dietary 18:2ω6 and 18:3ω3 act differentially to alter lipid metabolism of rat tissues. Animals fed the diet containing linseed oil grew faster than those fed the beef tallow diet (Table 2). The amount of food consumed per day, although not statistically different ($p > 0.05$), was somewhat higher in the linseed diet fed group. Therefore, it is conceivable that the increase in body wt after linseed oil consumption may be due to increased food intake. Liver wt, heart wt, and liver wt to body wt ratios, however, remained unchanged by the dietary fat treatments (Table 2). Therefore, the changes observed in cholesterol content and fatty acid composition of tissues apparently are a result of dietary fat treatment and do not stem from differences in growth rate.

TABLE 5

Effect of Dietary Fat Treatments on the Fatty Acid Composition of Rat Heart and Epididymal Fat Pads (wt % of total fatty acids)[a]

	Heart			Epididymal fat pad		
Fatty acid	Beef tallow	Safflower oil	Linseed oil	Beef tallow	Safflower oil	Linseed oil
14:0	1.1 ± 0.1[a]	0.6 ± 0.2[b]	0.5 ± 0.2[b]	3.7 ± 0.3[a]	0.7 ± 0.4[b]	1.0 ± 0.3[b]
16:0	12.5 ± 0.6[a]	10.0 ± 0.6[b]	9.5 ± 0.7[b]	24.9 ± 0.6[a]	13.6 ± 0.9[b]	14.5 ± 1.3[b]
16:1ω7	0.7 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	4.7 ± 0.3[a]	1.6 ± 0.2[b]	2.1 ± 0.4[b]
17:0	0.4 ± 0.0[a]	0.2 ± 0.0[b]	0.2 ± 0.0[b]	0.7 ± 0.0[a]	0.1 ± 0.1[b]	0.2 ± 0.0[c]
18:0	24.9 ± 0.8	23.4 ± 0.7	24.3 ± 0.8	10.7 ± 0.6[a]	3.0 ± 0.6[b]	4.8 ± 1.0[b]
18:1ω9	3.5 ± 0.2	3.1 ± 0.1	4.3 ± 0.8	25.6 ± 1.7[a]	16.4 ± 0.6[b]	22.0 ± 0.9[a]
18:1ω7	3.1 ± 0.2[a]	2.6 ± 0.3[a,b]	2.4 ± 0.1[b]	3.3 ± 0.2[a]	1.7 ± 0.2[b]	1.7 ± 0.4[b]
18:2ω6	17.1 ± 1.0[a]	26.6 ± 1.0[b]	23.6 ± 1.9[c]	21.2 ± 1.5[a]	58.1 ± 2.0[b]	35.1 ± 2.2[c]
18:3ω6	—	—	—	—	—	—
18:3ω3	—	—	1.8 ± 0.5	0.8 ± 0.2[a]	0.6 ± 0.3[a]	14.3 ± 1.3[b]
20:3ω6	—	0.3 ± 0.1	—	—	—	—
20:4ω6	23.7 ± 0.6[a]	20.8 ± 0.5[b]	17.8 ± 0.4[c]	—	—	—
22:4ω6	0.3 ± 0.3	0.2 ± 0.0	0.4 ± 0.1	—	—	—
22:5ω6	1.9 ± 0.4[a]	4.7 ± 0.5[b]	T[b,c]	—	—	—
22:5ω3	0.9 ± 0.1[a]	0.5 ± 0.2[b]	2.9 ± 0.2[c]	—	—	—
22:6ω3	7.8 ± 0.4[a]	4.8 ± 0.6[b]	9.7 ± 1.1[c]	—	—	—
Others	—	—	—	0.9 ± 0.3[a]	2.1 ± 0.4[b]	1.6 ± 0.3[b]
ΣSat.	39.5 ± 0.7	34.4 ± 0.5	35.2 ± 1.1	40.6 ± 0.7	17.7 ± 1.6	21.0 ± 2.0
ΣMUFA[c]	8.1 ± 0.5	6.9 ± 0.3	7.9 ± 0.9	34.0 ± 1.6	18.4 ± 1.2	25.6 ± 1.1
Σω6	43.4 ± 0.8	53.1 ± 0.8	42.5 ± 1.3	21.9 ± 1.5	59.9 ± 2.4	35.1 ± 2.2
Σω3	8.9 ± 0.8	5.3 ± 0.5	14.5 ± 1.2	0.8 ± 0.2	0.6 ± 0.3	14.3 ± 1.3

[a]Values given are mean ± standard deviation of 5 separate determinations (n = 5). Values without a common superscript are significantly different at $p < 0.05$.

[b]Trace amount (<0.1).

[c]Monounsaturated fatty acid.

The reduction in plasma cholesterol level following consumption of the safflower oil diet accompanied by increased liver cholesterol content is in agreement with previous observations (8–12) that suggest PUFA of the ω6 family increase cholesterol accumulation in liver relative to feeding diets high in saturated fatty acids. Both free- and cholesterol-ester content increased following feeding of the safflower oil diet, but this increase was more pronounced in the cholesteryl-ester fraction. This observation is consistent with the fact that diets rich in linoleic acid (18:2ω6) accelerate esterification of cholesterol by acyl coenzyme A:cholesterol acyltransferase (EC 2.3.1.26) activity in the rat liver microsomes (32). This increased esterification of cholesterol following feeding of the safflower oil diet, in turn, may increase the capacity of hepatic cells to take up more free cholesterol from circulating levels in the plasma (33). Replacement of 50% of safflower oil with linseed oil restricted deposition of cholesteryl esters in the liver and concomitantly lowered the plasma cholesterol level to an even greater extent than the safflower oil diet. In the light of these results, the hypothesis that dietary PUFA shift plasma cholesterol to tissue cholesterol pools cannot be generalized for all types of PUFA and may be restricted to ω6 fatty acids only. The cholesterol levels in heart and adipose tissue of rats, however, remained unaltered by the composition of the fat fed.

Consumption of the safflower oil diet resulted in a significant increase in plasma 20:4ω6 level, while in liver and heart lipid, the concentration of 20:4ω6 was significantly decreased in comparison with animals fed the diet high in beef tallow (Tables 4 and 5, Fig. 1). Increase in plasma 20:4ω6 content may be due to increased synthesis in the liver microsomes via desaturation and chain elongation of 18:2ω6 (34). However, this seems unlikely as the 20:4ω6 level in the liver decreased when rats were fed the safflower oil diet. Increased 20:4ω6 content in plasma lipids with concomitant depletion of 20:4ω6 in liver and heart tissue for animals fed the safflower oil diet treatment may be taken as evidence for shift of tissue 20:4ω6 to plasma pools. Some studies have reported either no change (35), or even a decrease (36), in liver microsomal Δ^6-desaturase activity, a rate-limiting enzyme in the 20:4ω6 biosynthetic pathway, following feeding of a diet rich in linoleic acid. Previous evidence indicates transfer of cholesterol from plasma to the liver pools with participation of liver phosphatidylcholine containing 20:4ω6 in the SN-2 position (37). The present paper demonstrates that this process of transfer of plasma cholesterol to liver pools and liver 20:4ω6 transport to plasma pools occurs at a faster rate when rats are fed an 18:2ω6 enriched diet. Inclusion of 18:3ω3 in the diet appears to slow this cycle by depleting the liver 20:4ω6 pool via inhibition of 20:4ω6 biosynthesis. In this context, the diet rich in 18:3ω3 did not change the 20:4ω6 content of plasma, depleted the 20:4ω6 level in the liver to the same extent as observed for animals fed the safflower oil diet, and depleted the 20:4ω6 level to an even greater extent in heart lipids. α-Linolenic present in the linseed oil diet competes with 18:2ω6 for Δ^6-desaturation, thus limiting synthesis of 20:4ω6 from 18:2ω6 (38). It is also conceivable that some of the tissue 20:4ω6 may shift to plasma pools for maintenance of circulating levels of 20:4ω6. These observations are further supported by the observation that an increase in the desaturated-chain elongated products of 18:3ω3 metabolism, i.e., 22:5ω3 and 22:6ω3, occurs in plasma, liver and heart lipids of rats fed the linseed oil diet. Interestingly, 20:5ω3 did not accumulate in any of the tissues examined even after feeding a high dietary load of 18:3ω3. In an earlier study, we noticed an increase in plasma and tissue 20:5 levels (39) following the feeding of 18:3ω3 rich diets. This contradiction may be due to the fact that the linseed oil diet in the present study contained a high level of 18:2ω6 in addition to a high 18:3ω3 content, whereas in the previous study, 18:3ω3 was fed to the rats along with saturated and monounsaturated fatty acids (39). In this regard, the balance between ω6 and ω3 fatty acids recently has been shown to be an important factor for modification of desaturase enzymes responsible for synthesis of 20:5ω3 and 22:6ω3 from 18:3ω3 (40). The decrease in monounsaturated fatty acids (16:1ω7 and/or 18:1ω9) in rat tissues by the diets containing high levels of 18:2ω6 and/or 18:3ω3 is consistent with the fact that PUFA inhibit Δ^9-desaturase activity (34,35).

The present study demonstrates that dietary 18:3ω3 is more potent than 18:2ω6 in lowering plasma cholesterol level and does not result in accumulation of cholesterol in liver tissue. However, 18:3ω3 appears to be relatively less effective for reducing cholesterol level than 20:5ω3 and/or 22:6ω3 present in fish oils (12–15). Feeding diets high in linseed oil for 28 days did not lower plasma 20:4ω6 level, but depleted tissue levels. Longer-term use of 18:3ω3 supplements may lower plasma 20:4ω6 level and, thus, may show antiaggregatory effects (19). Consumption of fish oils rich in 20:5ω3 and/or 22:6ω3 lowers plasma, platelet and tissue 20:4ω6 content more effectively even after a short-term feeding (41,42), which may account for the enhanced bleeding time observed after such diet treatments. Therefore, 18:3ω3 appears to have an effective intermediate in reducing cholesterol levels and, perhaps, platelet aggregation between that of 18:2ω6 and 20:5ω3. As ω6 PUFA shift cholesterol from blood to the liver pools, but do not lower total body cholesterol, they may not be as beneficial against cardiovascular diseases as they currently are thought to be. However, α-linolenic acid from linseed oil appears to lower plasma cholesterol without accumulation of cholesterol in the liver tissue. Dietary PUFA have also been shown to have different lipid-lowering effects in animals and humans (43,44), e.g., fish oil lowers the circulating cholesterol level in the rat (43), but not in the human (44). Therefore, the effect of α-linolenic acid should be examined in human subjects with appropriate controls, before any further conclusion can be drawn.

ACKNOWLEDGMENTS

This work was supported by grants from the Natural Sciences and Engineering Research Council of Canada, and the Medical Research Council of Canada. The authors thank Tom Wharton for his technical assistance.

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[Received January 22, 1988; Revision accepted May 18, 1988]