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Short-Term Effect of Dietary Cholesterol on Tissue n-6 Fatty Acids in Fat-Deficient Rats (42000)

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Abstract. Weanling female rats raised on a fat-free diet for 8 weeks were then given the same diet supplemented with 0, 0.25, 0.5, or 1% by weight of cholesterol in addition to 10% of safflower oil for 3 days. Fatty acid compositions of cholesteryl esters (CE), triglycerides (TG), and phospholipids (PL) in liver and plasma were examined. Cholesterol feeding increased plasma and liver cholesterol contents and also affected the patterns of n-6 polyunsaturated fatty acids. There were no consistent changes in either plasma and liver TG which contained little 20:3n-6 and 20:4n-6. The levels of 20:3n-6 increased in plasma and liver PL, while proportions of 20:4n-6 decreased in liver and plasma CE. However, the absolute amount of 20:4n-6 in cholesteryl esters increased because of a threefold rise in cholesteryl ester levels. The changes might be attributable to an increased utilization of 20:4n-6 for cholesterol transport and/or an inhibition of Δ^5-desaturation of n-6 fatty acids by cholesterol feeding.

There is substantial evidence that polyunsaturated fatty acids (PUFA) lower plasma cholesterol levels (1–5), but there is little information available regarding the effect of cholesterol on PUFA metabolism. Several reports have demonstrated that dietary cholesterol exacerbates the symptoms of essential fatty acid (EFA) deficiency (6–8), reduces the level of arachidonic acid, and elevates the levels of linoleic acid and dihomo-γ-linolenic acid (9, 10). Recent studies suggest these changes may be at least partly attributable to an ineffective desaturation of essential fatty acids (11). Dietary linoleate is known to be rapidly metabolized (at least in the rat) via the processes of desaturation and elongation to long-chain n-6 PUFAs (12). Most of the studies (9, 10) have been based on long-term observations. It seemed to us of interest to examine the short-term effect of dietary cholesterol on levels of PUFA.

Materials and Methods. *Animals.* Twenty-five weanling female rats (3 weeks old) of the Sprague–Dawley strain were maintained on a fat-free (FF) diet (Teklad Test Diet, Madison, Wis.) and water *ad libitum*. The diet contained 60.2% sucrose, 20% vitamin free casein, 15% cellulose, 3.5% mineral mix (AIN-76), 1% vitamin mix (Teklad No. 40060) and 0.3% DL-methionine. After 8 weeks on the FF diet, the animals showed a reduced rate of growth as indicated by regular weighing and scaly skin on the feet and the tail. Five animals were sacrificed at that time and tissue lipid fatty acid compositions were examined to verify the state of EFA deficiency. The remaining animals were then randomly divided into four groups of five rats each, and given 10% safflower oil-supplemented fat-free diet with the addition of 0, 0.25, 0.5, and 1% by weight of cholesterol, respectively. Animals were killed 72 hr later. The purpose of the fat-free diet was to reduce difficulties of interpretation because of endogenous essential fatty acids. The 72-hr interval was chosen because there is evidence (12) that metabolic responses in EFA-deficient animals to linoleic acid administration are demonstratable within 5 days. Blood was obtained in ether anesthetized rats by heart puncture and collected into a test tube containing EDTA (1 mg/ml blood) and plasma separated by centrifugation. The liver and perirenal adipose tissues were excised, rinsed with ice-cold saline, blotted, weighed, and frozen.

Lipid analyses. Lipids from livers, plasma, and perirenal adipose tissue were extracted with a solvent mixture of chloroform-methanol, 2:1 (v/v) (13). The lipids of plasma and liver were fractionated on silica gel thin-layer

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chromatography (TLC) into cholesteryl esters (CE), triglycerides (TG), free cholesterol, and phospholipids (PL). Free and ester cholesterols were quantitated by gas–liquid chromatography (GLC) using 3% OV-1 column (60 cm × 2 mm i.d.) with 5α-cholestane as an internal standard (11). Fatty acid compositions of lipid fractions in plasma, liver, and adipose tissue were determined by GLC using a glass column (180 cm × 2 mm i.d.) packed with 10% Silar 10C on Gas Chrom Q as described previously (14).

Statistical comparisons were made by Student's *t* test. Linear regression and analysis of the regression line was performed by least-squares analysis.

Results and Discussion. Tissue fatty acid profiles in fat-deficient animals showed that linoleate concentrations were consistently low, confirming the state of EFA deficiency. Concomitant feeding of cholesterol and linoleate to animals subsequent to EFA deprivation enabled us to evaluate the interaction between exogenous cholesterol and linoleate without the complication of high levels of endogenous linoleate.

Table I shows the effects of dietary cholesterol supplementation on plasma and liver cholesterol contents. Both plasma and liver cholesterol concentration significantly increased in accordance with the levels of dietary cholesterol fed. The exception was the unchanged plasma cholesterol level in rats fed only 0.25% cholesterol. The cholesterol increase in both the plasma and the liver was mainly in the form of esters.

The effects of different cholesterol levels on various plasma lipid fractions are shown in Table II. The levels of 16:0, 16:1, and 18:1 in plasma CE were significantly increased as cholesterol in the diet increased, while the amount of 20:4n-6 was reduced. Cholesterol feeding produced an approximately threefold rise in plasma cholesteryl esters. It led to a change in the fatty acid composition of the cholesterol esters. This suggests that either the fatty acid composition of the pool from which CE are formed has been changed by cholesterol or that the cholesterol is selectively modifying the removal of individual fatty acids from CE. The proportion of 20:4n-6 in the CE fraction fell in significant inverse relation to the plasma cholesterol level ($r = 0.817$, Fig. 1). Absolute levels of 20:4n-6 were higher in the cholesterol-fed animals because of the rise in total cholesterol. The proportionate fall in 20:4n-6 shows that either 20:4n-6 synthesis, unlike that of other PUFAs, does not keep pace with the rising CE level or that the 20:4n-6 is being more rapidly utilized.

The fatty acid profiles in plasma TG and PL did not change consistently, except that in plasma PL, 20:3n-6 rose and 20:4n-6 fell in animals fed 1% cholesterol.

In contrast to the plasma CE, there was

TABLE I. EFFECT OF CHOLESTEROL FEEDING ON PLASMA AND LIVER CHOLESTEROL CONTENT

	Dietary cholesterol content (%)			
	0	0.25	0.5	1
Plasma cholesterol (mg/ml)	0.43 ± 0.05	0.44 ± 0.05	0.97 ± 0.27[b]	1.02 ± 0.25[b]
Free	0.12 ± 0.02	0.12 ± 0.03	0.16 ± 0.06	0.14 ± 0.05
Ester	0.31 ± 0.04	0.32 ± 0.03	0.81 ± 0.24[b]	0.88 ± 0.22[b]
Liver cholesterol (mg/g)	3.34 ± 0.77	6.45 ± 1.41[b]	12.28 ± 1.70[b]	15.26 ± 4.28[b]
Free	1.63 ± 0.54	2.74 ± 0.51[a]	3.52 ± 0.49[b]	3.89 ± 0.53[b]
Ester	1.70 ± 0.69	3.71 ± 0.51[b]	8.75 ± 1.57[b]	11.37 ± 4.07[b]

Note. Each value represents mean ± SD of five determinations. Animals were given fat-free diet and water *ad libitum* for 8 weeks, and then were given for 3 days a 10% safflower oil-supplemented diet containing by weight 0, 0.25, 0.5, and 1% of cholesterol.

[a,b] Significantly different from control values (0% cholesterol) at $P < 0.05$ and $P < 0.001$, respectively.

TABLE II. FATTY ACID COMPOSITION OF PLASMA LIPIDS IN FAT-DEFICIENT (FF) RATS AND THOSE GIVEN VARIOUS CHOLESTEROL LEVELS IN ADDITION TO 10% OF SAFFLOWER OIL FOR 3 DAYS

Dietary cholesterol (%)	Fatty acid composition (%)								
	16:0	16:1	18:0	18:1	18:2-n6	20:3-n9	20:3-n6	20:4-n6	22:5-n6
					Cholesteryl esters				
FF	10.7 ± 2.6	0.7 ± 0.2	7.3 ± 1.7	3.2 ± 0.7	2.7 ± 0.4	25.6 ± 5.2	—	25.0 ± 0.6	1.9 ± 0.5
0	6.1 ± 0.7	4.9 ± 1.8	1.4 ± 0.4	7.9 ± 3.2	18.7 ± 3.8	—	0.2 ± 0.02	56.7 ± 5.4	—
0.25	8.6 ± 2.1	7.5 ± 1.5	1.7 ± 0.07	15.4 ± 1.8[a]	18.8 ± 0.6	—	0.3 ± 0.1	45.1 ± 0.3[a]	—
0.5	12.3 ± 1.3[f]	12.9 ± 1.4[e]	2.2 ± 0.5	28.4 ± 4.1[e]	21.0 ± 1.1	—	0.3 ± 0.02[a]	20.9 ± 6.0[e]	—
1	12.6 ± 2.9[b]	12.6 ± 0.8[b]	2.6 ± 1.0	23.8 ± 3.4[e]	19.6 ± 1.8	—	0.5 ± 0.3[a]	25.3 ± 6.7[e]	—
					Triglycerides				
FF	28.6 ± 0.7	14.2 ± 3.2	2.0 ± 0.4	47.2 ± 3.1	1.7 ± 0.3	2.6 ± 0.4	—	1.3 ± 0.7	—
0	22.5 ± 2.6	6.4 ± 1.1	2.3 ± 0.4	27.3 ± 5.3	38.4 ± 8.9	—	0.5 ± 0.3	1.3 ± 0.2	—
0.25	20.8 ± 1.2	7.5 ± 1.5	1.7 ± 0.1[a]	28.6 ± 3.9	38.7 ± 4.3	—	0.4 ± 0.2	1.1 ± 0.08	—
0.5	20.7 ± 3.0	6.8 ± 1.3	1.7 ± 0.5	28.1 ± 3.3	40.4 ± 4.8	—	0.5 ± 0.2	1.2 ± 0.3	—
1	23.3 ± 2.5	9.2 ± 0.6[c]	1.5 ± 0.4[a]	26.8 ± 0.5	36.9 ± 1.9	—	0.4 ± 0.05	1.0 ± 0.1	
					Total phospholipids				
FF	19.1 ± 1.3	4.2 ± 0.7	19.1 ± 1.4	17.8 ± 1.0	5.5 ± 0.5	19.1 ± 2.1	—	9.3 ± 0.8	1.6 ± 0.3
0	12.1 ± 0.7	1.0 ± 0.1	22.1 ± 3.8	5.6 ± 0.2	24.8 ± 2.8	0.6 ± 0.4	1.3 ± 0.4	27.6 ± 2.9	3.6 ± 0.2
0.25	12.0 ± 0.8	1.0 ± 0.2	18.7 ± 1.6	6.0 ± 0.8	30.3 ± 3.3[a]	0.3 ± 0.04	1.4 ± 0.5	26.7 ± 3.8	3.1 ± 0.9
0.5	11.3 ± 1.4	1.1 ± 0.4	19.1 ± 2.1	5.6 ± 0.6	28.9 ± 3.0	0.3 ± 0.1	1.7 ± 0.4	28.7 ± 1.4	3.0 ± 0.8
1	13.4 ± 0.8	0.9 ± 0.3	27.6 ± 3.0	6.3 ± 0.2[d]	24.4 ± 2.2	0.5 ± 0.3	2.7 ± 0.4[e]	20.6 ± 1.5[c]	2.6 ± 0.3[d]

Note. Cholesterol levels were 0, 0.25, 0.5, and 1% by weight of diet. Each volume represents mean ± SD of five rats.
[a-f]Significantly different from control values (0% cholesterol) at $P < 0.05$, $P < 0.025$, $P < 0.02$, $P < 0.01$, $P < 0.005$, and $P < 0.001$, respectively.

TABLE III. FATTY ACID COMPOSITION OF LIVER LIPIDS IN FAT-DEFICIENT (FF) RATS AND THOSE GIVEN VARIOUS CHOLESTEROL LEVELS IN ADDITION TO 10% OF SAFFLOWER OIL FOR 3 DAYS

Dietary cholesterol (%)	Fatty acid composition (%)								
	16:0	16:1	18:0	18:1	18:2-n6	20:3-n9	20:3-n6	20:4-n6	22:5-n6
					Cholesteryl esters				
FF	18.9 ± 1.7	16.0 ± 2.2	3.5 ± 0.7	42.4 ± 6.7	5.7 ± 7.4	1.3 ± 0.4	1.5 ± 0.3	—	—
0	25.3 ± 1.5	16.1 ± 3.8	6.9 ± 2.6	27.9 ± 5.1	10.2 ± 1.9	—	1.9 ± 0.8	8.7 ± 4.0	—
0.25	24.3 ± 4.9	16.8 ± 0.6	6.4 ± 2.2	35.1 ± 3.8[a]	12.4 ± 0.9	—	0.5 ± 0.3[f]	3.1 ± 1.1[f]	—
0.5	20.6 ± 1.5[d]	13.8 ± 1.0	7.4 ± 2.7	37.5 ± 0.9[f]	14.4 ± 1.9[f]	—	1.1 ± 0.4	2.4 ± 0.4[f]	—
1	22.9 ± 1.6	15.0 ± 1.7	7.2 ± 1.5	34.8 ± 2.0[a]	13.8 ± 1.2[f]	—	1.0 ± 0.9	3.2 ± 2.2[f]	—
					Triglycerides				
FF	29.4 ± 0.9	10.7 ± 1.8	2.6 ± 0.4	52.7 ± 2.0	1.3 ± 0.2	1.7 ± 0.4	—	0.3 ± 0.03	—
0	33.0 ± 3.0	10.8 ± 1.5	3.0 ± 0.6	34.6 ± 2.4	16.1 ± 2.1	—	—	0.5 ± 0.07	—
0.25	31.2 ± 1.2	11.1 ± 0.5	2.3 ± 0.3	35.7 ± 3.2	18.0 ± 3.7	—	—	0.5 ± 0.2	—
0.5	31.6 ± 1.6	10.1 ± 0.7	2.3 ± 0.4	36.4 ± 1.9	17.3 ± 2.6	0.2 ± 0.06	0.2 ± 0.06	0.4 ± 0.09	—
1	32.6 ± 3.0	10.6 ± 1.2	2.3 ± 0.5	33.5 ± 0.7	16.4 ± 3.9	0.2 ± 0.06	0.2 ± 0.06	0.4 ± 0.2	—
					Total phospholipids				
FF	14.5 ± 0.7	3.8 ± 0.8	24.8 ± 1.6	16.2 ± 0.7	4.1 ± 0.3	15.2 ± 1.4	—	12.2 ± 1.0	2.2 ± 0.4
0	11.9 ± 0.3	2.4 ± 0.4	23.2 ± 1.9	6.0 ± 0.9	14.6 ± 1.2	1.0 ± 0.1	1.7 ± 0.3	30.0 ± 2.0	5.8 ± 0.4
0.25	12.0 ± 0.4	2.9 ± 0.4	18.9 ± 2.3[a]	6.3 ± 1.1	17.9 ± 1.3[c]	1.0 ± 0.1	2.2 ± 0.6	30.9 ± 3.6	4.8 ± 1.2
0.5	11.5 ± 0.6	2.7 ± 0.7	21.5 ± 3.6	6.5 ± 0.4	16.4 ± 1.7	1.0 ± 0.2	2.5 ± 0.4[b]	29.4 ± 1.6	5.0 ± 1.4
1	12.2 ± 0.6	2.6 ± 0.5	23.2 ± 2.5	6.3 ± 0.6	17.6 ± 1.4[c]	0.8 ± 0.3	2.9 ± 0.3[f]	27.2 ± 1.3	4.0 ± 0.3[f]

Note. Cholesterol levels were 0, 0.25, 0.5, and 1% by weight of diet. Each value represents mean ± SD of five rats.
[a-f] Significantly different from control values (0% cholesterol) at $P < 0.05$, $P < 0.025$, $P < 0.02$, $P < 0.01$, $P < 0.005$, and $P < 0.001$, respectively.

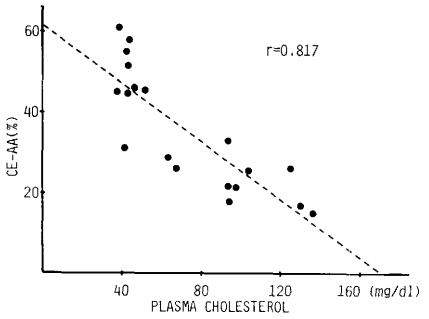

FIG. 1. Correlation between plasma cholesterol and the levels of arachidonic acid (AA, 20:4n-6) in plasma cholesteryl esters in fat-deficient rats raised on a fat-free diet supplemented with various levels of cholesterol in addition to 10% of safflower oil for 3 days.

no consistent relationship between AA levels in plasma PL or in plasma TG and the plasma cholesterol concentrations.

Table III shows the fatty acid compositions of various liver lipids. The levels of oleic acid and linoleic acid in liver CE were elevated in animals fed cholesterol, while that of 20:4n-6 was reduced. The proportions of 18:2n-6 and 20:3n-6 in liver PL were also increased significantly. 20:3n-6 in liver PL rose with the level of cholesterol fed and there was a significant positive correlation ($r = 0.719$) between liver PL 20:3n-6 and liver cholesterol concentration (Fig. 2). 20:3n-6 accumulation has also been noted in cholesterol-rich arterial fatty streaks (14). However, the AA levels in liver PL remained constant despite increasing liver cholesterol content. There were also no consistent changes in liver TG.

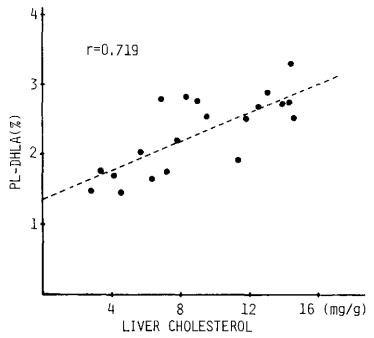

FIG. 2. Correlation between liver cholesterol concentration and the levels of dihomo-γ-linolenic acid (DHLA, 20:3n-6) in liver phospholipids in fat-deficient rats fed on a fat-free diet supplemented with various levels of cholesterol in addition to 10% of safflower oil.

TABLE IV. EFFECT OF DIETARY CHOLESTEROL ON THE RATIOS (20:4n-6 + 22:5n-6)/20:3n-6 OF PLASMA AND LIVER PHOSPHOLIPIDS

% Dietary cholesterol	Ratio	
	Plasma PL	Liver PL
0	24.0[a]	21.1
0.25	21.3	16.2
0.5	18.6	13.8
1.0	8.6	10.8

[a] Figures were calculated from the data presented in Tables II and III.

Perirenal adipose tissues in fat-deficient animals raised on 10% SFO-supplemented diet for 3 days contained 43.9% oleic acid, 27.6% palmitic acid, 14.2% palmitoleic acid, 9.4% linoleic acid, and 3% of stearic acid. Cholesterol feeding for 3 days did not change the fatty acid profiles (data are not shown).

In this study, the effects of cholesterol feeding on the plasma and liver fatty acid profiles, particularly in PL and CE, were consistent. In general, the increase of 20:3n-6 and decrease of 20:4n-6 were correlated to the levels of dietary cholesterol. Increased utilization of arachidonic acid relative to other fatty acids for cholesterol transport might contribute to the reduced proportion of 20:4n-6 but could not explain the increased 20:3n-6. When the ratios of (20:4n-6 + 22:5n-6)/20:3n-6, an index for the activity of Δ^5-desaturation, for the plasma and liver phospholipids were calculated (Table IV), there is a progressive decrease in the ratios as increasing amounts of cholesterol were added to the diet. It suggests that inhibition of Δ^5-desaturase by cholesterol is a possibility, but because of the complexity of the interactions between fatty acids in the various fractions, this must be confirmed by direct measurement.

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