

Dietary Fat Saturation and Hepatic Acylcoenzyme A:Cholesterol Acyltransferase Activity

Effect of n-3 Polyunsaturated and Long-Chain Saturated Fat

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The acylcoenzyme A:cholesterol acyltransferase (ACAT) activity in liver microsomes from rats fed a diet containing 14% menhaden oil (M_p) for 11 days was 117% higher than that in microsomes from rats fed a corresponding diet containing 14% cocoa butter (M_s). There were no differences in the cholesterol and phospholipid contents of M_p and M_s or in the activities of palmitoyl coenzyme A hydrolase and NADPH cytochrome c reductase. NADPH-dependent lipid peroxidation was higher in M_p , whereas glucose 6-phosphatase activity was higher in M_s . These findings indicate that the ACAT response to differences in dietary fat saturation is not due to a nonspecific effect of these diets on microsomal enzymes. When 1% cholesterol was added to the diets, the cholesterol content and ACAT activity of both microsomal preparations increased, but the ACAT activity of M_p remained 60% higher than that of M_s . Addition of cholesterol by incubation of the microsomes with liposomes also increased ACAT activity. At corresponding cholesterol contents, however, the ACAT activity of M_p remained 50% to 70% above that of M_s . There was no difference in the plasma cholesterol concentration in the two groups of rats, indicating that the ACAT effect probably is not due to a difference in the amount of circulating cholesterol available to the liver. M_p contained 40% more polyunsaturated fatty acids and five times more n-3 polyunsaturates than M_s . These findings suggest that the increase in ACAT activity in M_p is due, at least in part, to the difference in the fatty acid composition of the microsomes. (Arteriosclerosis 3:242-248, May/June 1983)

Liver microsomes from rats fed a diet containing 14% sunflower seed oil, which is rich in the linoleic acid (n-6) class of polyunsaturates, have a higher acylcoenzyme A:cholesterol acyltransferase (ACAT) activity than those from rats fed a corresponding diet containing coconut oil, a saturated fat.¹ A similar effect on ACAT activity has been reported in intestinal

microsomes when rabbits are fed a diet enriched with safflower oil, which also is high in linoleic acid.² ACAT plays an important role in regulating intracellular cholesterol metabolism,³⁻⁸ in particular, by catalyzing the esterification of the cholesterol that is released during the lysosomal degradation of plasma low density lipoproteins.⁹ In addition, ACAT is present in the arterial wall, where it appears to mediate the synthesis of the cholesteryl esters that accumulate intracellularly in the atherogenic process.³⁻⁵ Furthermore, hepatic ACAT activity may be involved in regulating cholesterol availability for lipoprotein production, or in facilitating the deposition of circulating cholesterol in the liver. Because of the important role of ACAT in cholesterol metabolism, it was of interest to further explore the effects of dietary fat saturation on ACAT activity.

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In the present study, we investigated whether a diet rich in the linolenic acid (n-3) class of polyunsaturates would, like a diet rich in n-6 polyunsaturates, increase hepatic ACAT activity relative to a diet rich in saturated fat. This information was necessary to determine if the increase in ACAT activity produced by feeding sunflower seed oil might be mediated specifically by linoleic acid or arachidonic acid, both of which accumulate in hepatic microsomal phospholipids when the diet is high in linoleic acid.^{1, 10} A second question that we examined was the effect of dietary fatty acid chain length on ACAT activity. Coconut oil, which contains predominantly medium-chain fatty acids, was used as the saturated fat in our previous ACAT studies.¹ In similar work involving rat liver hydroxymethylglutaryl coenzyme A reductase, it was found that the shorter chain length, rather than the greater degree of saturation of the fatty acids in coconut oil, was responsible for the decrease in activity of this enzyme.¹¹ Based upon this, we wondered whether the difference in chain length also might be responsible for the reduction in hepatic ACAT activity produced by feeding coconut oil.¹ To evaluate this, we tested the effect on hepatic ACAT activity of a diet enriched with cocoa butter, a saturated fat that contains long-chain fatty acid.

Methods

Animals and Diets

Weanling male Sprague-Dawley rats weighing 74 ± 2 g were fed a semisynthetic diet consisting of 54% sucrose, 27% casein, 1% vitamin mix, and 4% mineral mix (Teklad Mills, Madison, Wisconsin) sup-

plemented with either 14% menhaden oil (Zapata Haynie Corporation, Reedville, Virginia) or 14% cocoa butter (Ruger Chemical Company, Incorporated, Irvington, New Jersey). The lipid composition of the supplemented diets is shown in Table 1. In terms of fatty acid classes, the menhaden oil semisynthetic diet contained about 30% polyunsaturated fat; 80% of the polyunsaturates were of the n-3 class. The cocoa butter semisynthetic diet contained only 4% polyunsaturated fat and 70% saturated fat, the latter mostly as stearic and palmitic acid. Additional groups of rats were fed the menhaden oil and cocoa butter diets supplemented with cholesterol so that the total cholesterol content was 1% in each case. The animals were housed in a room maintained at 20° to 22°C lighted from 0700 to 1900 hours.

Preparation of Microsomes

The rats were killed between 0800 and 0900 by decapitation. They had free access to food up to the time of death. The livers were removed; they were perfused with an ice-cold phosphate buffer solution containing 137 mM NaCl, 3 mM KCl, 8 mM Na₂HPO₄, and 1.5 mM KH₂PO₄ (pH 7.4); they were blotted dry, weighed, and minced into a sucrose buffer containing 0.25 M sucrose, 0.04 M NaCl, 0.1 M KCl, 0.02 M Tris, and 5 mM MgSO₄ (pH 7.4).¹² The livers were then homogenized using a Potter-Elvehjem tissue grinder equipped with a motor-driven Teflon pestle. All manipulations were carried out at 4°C. An aliquot of the homogenate was centrifuged at 10,500 *g* for 10 minutes to remove heavier particles, and the supernatant fluid was then centrifuged at 12,000 *g* for 10 minutes. The resulting supernatant fluid was aspirated without removing the floating lipid layer, and it was then centrifuged at 105,000 *g* for 60 minutes. The microsomal pellet was resuspended in a solution containing 0.1 M K₂HPO₄ and 1 mM dithiothreitol (pH 7.2), and the microsomes were again sedimented by centrifugation at 105,000 *g* for 60 minutes. The washed microsomal pellet was resuspended in 20 ml of this buffer, resulting in a protein concentration of 5 to 15 mg/ml.

Chemical Analyses

Protein estimations were done by a modification of the Lowry method in which 1% sodium dodecylsulfate is added in order to solubilize lipids.¹³ Bovine serum albumin was used as the standard. Lipids were extracted from a 0.5 ml aliquot of microsomes with 20 ml chloroform/methanol 2:1 (vol/vol),¹⁴ and aliquots of the washed chloroform phase were taken for analysis. Phospholipid content was measured by the method of Raheja et al.¹⁵ Phospholipids and neutral lipids were separated by thin-layer chromatography on silica gel G plates developed in a solvent system containing hexane/ether/acetic acid/methanol, 170:40:4:4 (vol/vol/vol/vol). The fractions were eluted from the silica gel with chloroform/methanol/water (1:1:0.9) for subsequent fatty acid analysis.

Table 1. Lipid Composition of Menhaden and Cocoa Butter Diets

Lipid	Menhaden (%)	Cocoa butter (%)
Major fatty acids*		
16:0	18.3	18.2
16:1	8.8	ND
18:0	7.8	49.2
18:1 n-9	17.2	28.0
18:2 n-6	2.4	0.8
18:3 n-3	1.7	2.2
20:4 n-6	1.6	0.6
20:5 n-3	10.3	ND
22:4 n-6	1.6	ND
22:5 n-3	2.7	ND
22:6 n-3	6.2	ND
Cholesterol (mg/100 g diet)	60.5	ND

Diets that the rats were fed: menhaden = semisynthetic diet containing 14% menhaden oil; cocoa butter = semisynthetic diet containing 14% cocoa butter.

*The fatty acids are abbreviated as number of carbon atoms:number of double bonds. The n-x notation indicates the number of carbon atoms the first double bond is removed from the methyl terminus of the acyl chain.

ND = None detected.

After saponification with ethanolic KOH and methylation with 14% BF_3 in methanol,¹ the fatty acid methyl esters were separated using a Hewlett-Packard 5710 A gas chromatograph equipped with a flame ionization detector. A 1.9 m \times 2 mm ID glass column containing SP-2340 on Chromasorb WAW was used, and N_2 (20 ml/min) served as the carrier gas. Peak areas were measured with a Hewlett-Packard 3380 A integrator. Individual fatty acids were identified by comparing their retention times with those of standards obtained from either Supelco, Incorporated (Bellefonte, Pennsylvania) or Nu-Chek Prep (Elysian, Minnesota).

Unesterified cholesterol was measured by gas-liquid chromatography using a modification of the method described by Driscoll et al.¹⁶ with cholestane (Supelco, Incorporated) as an internal standard. Lipids were extracted as above from an aliquot of microsomes containing 1 to 3 mg of protein mixed with 30 μg of cholestane. The column contained SP 2250 on 100/120 Supelcoport (Supelco Incorporated).

Enzyme Assays

ACAT activity was assayed isotopically using 1- ^{14}C palmitoyl CoA as the radioactive substrate.¹ The incubation mixture consisted of 0.2 mg microsomal protein, 0.1 M K_2HPO_4 adjusted to pH 7.2, 1 mM dithiothreitol, and 30 μM bovine serum albumin. Approximately 7×10^4 dpm of 1- ^{14}C palmitoyl CoA (New England Nuclear, Boston, Massachusetts) and 7.5 nmol of palmitoyl CoA (P-L Biochemical Incorporated, Milwaukee, Wisconsin) were added. Palmitoyl CoA concentrations were determined using the adenosine molar extinction coefficient of 15.4×10^3 at 260 nm. The total volume of the incubation was 0.5 ml, and the reaction proceeded for 10 minutes at 37°C in a water bath with shaking. The incubation was terminated by adding 2 ml of chloroform/methanol (2:1), immediately followed by vigorous agitation. After the phases separated, an aliquot of the chloroform phase was taken for measurement of lipid radioactivity. Additional aliquots of the chloroform solution were taken for thin-layer chromatography on silica gel G in a solvent system consisting of hexane/diethyl ether/methanol/acetic acid (170:40:2:2). Lipids were visualized by exposure of the chromatogram to I_2 vapor. After sublimation of the I_2 , the outlined segments of silica gel were scraped directly into liquid scintillation vials containing 10 ml of a scintillation mixture (Budget Solve; Research Products International, Elk Grove Village, Illinois). Measurements of radioactivity were made with a Beckman LS 7000 liquid scintillation spectrometer, and quenching was monitored using the ^{137}Cs external standard. Acyl CoA hydrolase activity also was measured in this assay by determining the radioactivity present as free fatty acid in the chromatogram.

Microsomal NADPH-dependent lipid peroxidase activity was measured in a system containing 600–800 μg protein, 0.025 M Tris HCl buffer (pH 7.5), 1

mM ADP, 10 μM FeCl_3 , and 0.154 M KCl. Following incubation at 37°C for 5 minutes, the reaction was initiated by adding 50 μM NADPH. After 10 minutes the reaction was terminated with 0.1 ml trichloroacetic acid, and the malondialdehyde produced was measured spectrophotometrically with thiobarbituric acid using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$.^{10, 17}

Glucose-6-phosphatase activity was determined by measuring inorganic phosphate release.¹⁸ NADPH cytochrome c reductase activity was determined spectrophotometrically by measuring the reduction of cytochrome c at 550 nm.¹⁹

Enrichment of Microsomes with Cholesterol

To prepare cholesterol/dipalmitoyl phosphatidylcholine liposomes, a chloroform solution containing cholesterol and dipalmitoyl phosphatidylcholine (2:1, mol/mol) was evaporated under N_2 to dryness. A buffered sucrose solution (0.25 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4) was added to give 8.3 $\mu\text{mol/ml}$ of phospholipid. This mixture was kept at 50°C in stoppered tubes for 15 minutes, dispersed for 2 minutes with a vortex mixer, and then sonicated under N_2 at 10 watt output for 15 minutes at 50°C with a Branson sonifier. The resulting liposome solution was centrifuged at 12,000 g for 30 minutes to remove any metal particles released by the sonifier probe.

Microsomes were incubated with the cholesterol/dipalmitoyl phosphatidylcholine liposomes for 15, 30, 60, or 120 minutes at 37°C to increase the microsomal unesterified cholesterol content. The incubation mixture contained 0.8 μmol of phospholipids in liposomes, 1.6 μmol phospholipids in microsomes, and 5 mg of fatty acid-free bovine serum albumin in 1 ml of buffered sucrose solution. As a control, additional aliquots of microsomes were incubated in absence of liposomes for corresponding periods of time. After incubation, the mixture was cooled in ice, layered over 5 ml of 20% sucrose solution and sedimented at 4°C for 1 hour at 105,000 g . The resulting microsomal pellet was dispersed in 2 ml of buffered sucrose solution, layered over 5 ml of a 20% sucrose solution, and again sedimented at 105,000 g for 1 hour. The pellet was dispersed in buffered sucrose solution to give 0.8 mg/ml protein and assayed for ACAT activity and cholesterol content.

Lipid Analysis of Plasma

Rats were fed the experimental diets for 11 days. After the rats were anesthetized with ether, the thoracic cavity was opened and blood samples were withdrawn by direct cardiac puncture. The blood samples were centrifuged at 12,000 g for 10 minutes, and the plasma was analyzed for total cholesterol and triglycerides by the Technicon II analyzer method.²⁰

Statistical Analysis

Data are reported as mean \pm standard error of the mean. The paired *t* test was used to analyze differences in the means between groups, except as otherwise noted.

Results

ACAT Activity

As demonstrated previously,¹ this ACAT assay which uses 1-¹⁴C palmitoyl CoA as the radioactive substrate was linear over the 10-minute incubation period and, in addition, was linearly dependent on microsomal protein concentration in the range that was used, 0.2 mg. Furthermore, the palmitoyl CoA concentration that was employed, 15 μ M, was saturating but not inhibitory.

Table 2 contains values for ACAT and palmitoyl CoA hydrolase activities in hepatic microsomes isolated from rats fed the diet supplemented with 14% menhaden oil (polyunsaturated, M_p), or 14% cocoa butter (saturated, M_s). The rats were given these diets for 11 days before the livers were removed. The ACAT activities were 117% higher in M_p than in M_s . To confirm these ACAT results, a second study was carried out for a 10-day period. One group of four rats was fed the menhaden oil diet, another the cocoa butter diet. The ACAT activity of M_p was found to be 68.7 ± 8.3 pmol/mg protein/min, whereas that of M_s was 34.3 ± 5.6 . These values agree very closely with those for M_p and M_s shown in Table 2, and the twofold difference in the ACAT activities also is in close agreement with the difference noted in the first experiment. A third study was carried out in which the rats were fed the diets for only 3 days. This was done because previous work on hepatic microsomal peroxidation indicated that diet-induced changes in the activity of this enzyme already were apparent at this time.¹⁰ After a 3-day feeding period, the ACAT activity of M_p was already 100% greater than that of M_s . Long periods of feeding were not used in the

present study because previous work using diets supplemented with sunflower seed oil or coconut oil showed that the maximum change in hepatic ACAT activity occurred within 20 days.¹

Additional experiments were carried out in which cholesterol was added to the menhaden oil and cocoa butter diets so that the total cholesterol content in both cases was 1%. After 11 days of feeding, the rats were sacrificed and their livers were removed for analysis. These results are shown in Table 2. A considerable increase in liver microsomal ACAT activity occurred as a result of adding cholesterol to each of the diets. However, the activity was 59% higher when the diet contained menhaden oil as opposed to cocoa butter.

Microsomes obtained from the livers of rats fed the menhaden oil and cocoa butter diets were enriched with cholesterol *in vitro* by incubation with liposomes containing dipalmitoyl phosphatidylcholine and cholesterol. By this procedure it was possible to increase the cholesterol content of the microsomes almost twofold. As seen in Figure 1, the ACAT activity of both M_p and M_s increased linearly as the cholesterol content was raised. At each microsomal cholesterol content, the ACAT activity of M_p was greater than that of M_s . The regression lines for ACAT activity as a function of the cholesterol content of M_p and M_s were essentially parallel as judged by covariance analysis and estimate of common slope *F* ratio.^{21, 22}

Other Microsomal Enzymes

The activity of palmitoyl CoA hydrolase was measured in the hepatic microsomal preparations obtained from the four dietary groups. As shown in Table 2, this enzymatic activity was not significantly different in M_p as compared with M_s . When cholesterol was added to the diets, however, the activity of palmitoyl CoA hydrolase was approximately twice as high as when the rats were fed the same diet containing no added cholesterol.

The activities of three other microsomal enzymes also were compared in M_p and M_s . There was no

Table 2. Enzyme Activities and Lipid Contents of Liver Microsomes

Diet	No. of livers	ACAT activity (pmol/mg protein/min)	Palmitoyl CoA hydrolase activity (μ mol/mg protein/min)	Cholesterol (μ g/mg protein)	Phospholipids (μ g/mg protein)	Cholesterol to phospholipid ratio (mol/mol)
Menhaden	5	65.4 ± 4.4	843 ± 90	20 ± 1	375 ± 20	0.11
Cocoa butter	5	$30.1 \pm 0.6^*$	754 ± 30	22 ± 2	348 ± 24	0.13
Menhaden/1% cholesterol	6	245 ± 26.2	1580 ± 18	40 ± 3	417 ± 10	0.19
Cocoa butter/1% cholesterol	6	$154 \pm 4.2^\dagger$	1552 ± 37	34 ± 4	379 ± 25	0.18

Values are given as means \pm SE.

**p* < 0.001 compared to menhaden group.

†*p* < 0.01 compared to menhaden/1% cholesterol group.

significant difference in NADPH cytochrome c reductase activity, 59 ± 6.5 nmol/min/mg protein in M_p as compared with 72 ± 9.3 in M_s ($n=5$). Glucose-6-phosphatase activity was somewhat higher in M_s , 611 ± 67 nmol/min/mg protein as compared with 450 ± 26 in M_p ($n=5$). Conversely, NADPH-dependent lipid peroxidation was much higher in M_p , 2.16 ± 0.15 nmol/min/mg protein as compared with 0.21 ± 0.04 in M_s ($n=5$).

Microsomal Lipid Composition

The unesterified cholesterol and phospholipid contents of the microsomal preparations are listed in Table 2. There were no significant differences between M_p and M_s . In both cases, however, the cholesterol content was larger and the ratio of cholesterol to phospholipid higher when the diets were supplemented with cholesterol.

The fatty acid composition of the hepatic microsomes from the rats fed the menhaden oil and cocoa butter diets containing no added cholesterol for 11 days is given in Table 3. With regard to phospholipid fatty acid classes, M_p contained about one-half as much monounsaturated and 40% more polyunsaturated fatty acid as compared with M_s . The content of several fatty acids differed appreciably between M_p and M_s . The phospholipids of M_s contained 80% more oleic acid than the M_p phospholipids. The difference in n-3 polyunsaturated fatty acid content was especially pronounced, the n-3 polyunsaturates accounting for 36% of the phospholipid fatty acids in M_p but only 7% in M_s .

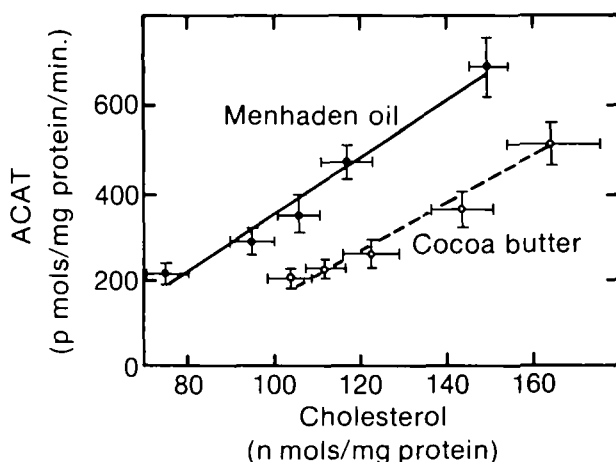


Figure 1. Effect of microsomal cholesterol enrichment on ACAT activity. The microsomes were enriched with cholesterol by incubation with liposomes containing cholesterol and dipalmitoyl phosphatidylcholine (2:1 mol/mol). Each point is the mean value obtained from 10 separate microsomal preparations. The vertical bars represent standard errors for ACAT activity; horizontal bars indicate standard errors for the microsomal cholesterol content. Based on covariance analysis, there is no difference in the slope of the two regression lines.

Table 3. Fatty Acid Composition of Liver Microsomal Phospholipids

Phospholipid fatty acids	Menhaden (%)	Cocoa butter (%)
Classes		
Saturated	37.2 ± 6.3	42.3 ± 4.4
Monoenoic	8.9 ± 0.8	15.4 ± 1.7
Polyenoic	51.4 ± 7.3	35.9 ± 5.3
Individual acids		
16:0	8.7 ± 2.0	9.7 ± 3.2
18:0	28.4 ± 4.8	32.3 ± 2.4
18:1 n-9	8.4 ± 0.8	$15.1 \pm 1.6^*$
18:2 n-6	2.5 ± 0.4	3.5 ± 0.5
18:3 n-3	0.5 ± 0.1	0.3 ± 0.1
20:4 n-6	11.1 ± 1.9	$17.9 \pm 2.2^\dagger$
20:5 n-3	7.6 ± 1.3	$0.2 \pm 0.1^\ddagger$
22:5 n-3	4.5 ± 0.2	$0.5 \pm 0.3^\ddagger$
22:6 n-3	23.9 ± 4.3	$6.3 \pm 2.0^*$

Each value is a mean \pm SE of five separate microsomal preparations. Diets that the rats were fed are as indicated in Table 1.

The values do not add up to 100% because some of the fatty acids were not identified. The fatty acids are abbreviated as number of carbon atoms:number of double bonds. The n-x notation indicates the number of carbon atoms that the first double bond is removed from the methyl terminus.

* $p < 0.01$ compared to menhaden group.

$^\dagger p < 0.05$ compared to menhaden group.

$^\ddagger p < 0.001$ compared to menhaden group.

Plasma Lipid Composition

Table 4 shows the plasma lipid composition of rats fed the menhaden oil and cocoa butter diets for 11 days. The cholesterol concentration was similar in the two groups of animals, but the triglyceride concentration was considerably higher in rats fed the cocoa butter diet.

Growth and Liver Size

All the animals appeared healthy throughout the study. At the start of the feeding period, the rats placed on the menhaden oil and cocoa butter diets weighed 74 ± 2 g. After 11 days, the weights were 107 ± 8 g for rats fed the menhaden oil diet and 121 ± 5 g for rats fed the cocoa butter diet ($p > 0.1$). The liver weights were 5.5 ± 0.5 and 6.4 ± 0.3 g for the two dietary groups after 11 days ($p > 0.1$). The rats placed on the cholesterol supplemented diets had a

Table 4. Plasma Lipid Composition

Lipid	Menhaden (mg/dl)	Cocoa butter (mg/dl)
Cholesterol	70.2 ± 8.3	84.0 ± 4.9
Triglycerides	62.5 ± 3.6	$164.0 \pm 21.4^*$

Rats were fed the respective diets (as indicated in Table 1) for 11 days. Each value is a mean \pm SE of plasma samples from six separate animals.

* $p < 0.01$ compared to menhaden group.

starting weight of 114 ± 3 g. After 11 days, the weights were 175 ± 8 g for rats fed menhaden with 1% cholesterol and 155 ± 8 g for rats fed cocoa butter with 1% cholesterol ($p > 0.1$). Liver weights for the two cholesterol-supplemented dietary groups were 12.6 ± 0.43 and 8.5 ± 0.69 g ($p < 0.001$).

Discussion

The hepatic microsomal ACAT activity of rats fed a diet supplemented with sunflower seed oil, which is rich in linoleic acid, has been observed to be about 75% greater than in rats fed a similar diet containing coconut oil, a highly saturated fat.¹ The results of this study extend this finding in several ways. First, they demonstrate that a similar response occurs when rats are fed a diet supplemented with menhaden oil, which is high in n-3 polyunsaturates. After the diet containing menhaden oil was consumed for 11 days, the hepatic microsomal ACAT activity (about 65 pmol/mg protein/min) was only slightly higher than that previously observed for the hepatic microsomes of rats fed a sunflower oil diet (between 50 and 54 pmol/mg protein/min).¹ The fact that both n-3 and n-6 polyunsaturates produce a similar effect is important with respect to possible mechanisms responsible for the enhancement in hepatic ACAT activity. Arachidonic acid accumulated in the liver microsomes of the rats fed the sunflower oil diet.¹ By contrast, the menhaden oil diet reduced the arachidonic acid content of the liver microsomes well below that contained in the microsomes isolated from the rats fed the saturated fat diet. Therefore, the mechanism of ACAT enhancement almost certainly is not specific for arachidonic acid or one of its metabolites.

Another important observation is that the cocoa butter diet, which is rich in 16- and 18-carbon-atom saturated fatty acids, produces a similar reduction in hepatic microsomal ACAT activity as a diet containing coconut oil, a medium-chain saturated fat.¹ The present results, together with the previous findings regarding sunflower seed oil and coconut oil,¹ indicate that the degree of saturation, rather than fatty acid chain length of the dietary fat, is the main factor that influences hepatic microsomal ACAT activity. This differs from a recent conclusion regarding the influence of dietary fat composition on rat liver hydroxymethylglutaryl coenzyme A reductase activity.¹¹ In the case of the reductase, the chain length of the dietary fat, rather than the degree of saturation, appears to be the major factor that affects the activity.

The menhaden oil that was added to the semisynthetic diets contained 0.06% cholesterol, whereas the cocoa butter contained no cholesterol. This small amount of dietary cholesterol could have contributed to the higher ACAT activity of M_p . However, the cholesterol content of the liver microsomes was not increased when the diet contained menhaden oil as opposed to cocoa butter. Since the ACAT assay

used washed microsomes, any effect involving cholesterol should have been mediated through a change in the microsomal cholesterol content. Furthermore, the higher ACAT activity of M_p as compared with M_s was maintained when both diets were supplemented so that they contained equal amounts of cholesterol or when M_p and M_s were enriched with cholesterol in vitro by incubation with liposomes. While these experiments clearly show that microsomal ACAT activity is regulated by cholesterol availability, they indicate that fatty acid saturation exerts an additional modulating effect. It is unlikely that the differences in ACAT activities are due to an artifact caused by the changes in liver weight. Only when the diets contained added cholesterol were the liver weight differences statistically significant. Yet, changes in ACAT activity occurred even when the diets contained no added cholesterol. Furthermore, the livers from the rats fed supplemental menhaden oil plus cholesterol, which weighed more, exhibited the higher ACAT activities. If liver weight was taken into consideration, the difference in total hepatic ACAT activity would be further magnified, not cancelled.

As compared with the cocoa butter diet, the menhaden oil diet produced a very large increase in the NADPH-dependent lipid peroxidation activity of the hepatic microsomes. A similar increase occurred with the sunflower seed oil diet.¹⁰ Therefore, this effect also is not specific for a given class of dietary polyunsaturates. The increase in both cases probably is due to the presence of larger amounts of microsomal polyunsaturated fatty acids that serve as the substrate for the peroxidation system.

When saturated fat is replaced in the human diet by polyunsaturated fat, the plasma cholesterol concentration decreases.²³⁻²⁶ The metabolic effects responsible for this reduction appear to be very complex.²⁶⁻³⁰ Based on the present findings, it is possible that changes in hepatic ACAT activity, by affecting cholesterol metabolism in the liver, could play some role in the plasma cholesterol response. For example, the liver has been shown to remove some of the low density lipoproteins (LDL) contained in the plasma.³¹ By increasing the esterification of the cholesterol released in the degradative process, an elevation in hepatic ACAT activity might indirectly facilitate LDL clearance and thereby lead to a reduction in the plasma cholesterol concentration. However, several factors make any correlation between the present results and the reduction in plasma cholesterol concentration that occurs when humans are fed polyunsaturated fat extremely tenuous. First, there are considerable differences between the rat and the human regarding cholesterol and lipoprotein metabolism.³² The rat has very little plasma LDL, probably because of the rapid clearance of chylomicron and very low density lipoprotein remnants by specific hepatic remnant receptors.³² In addition, cholesteryl esters accumulate in rat high density lipoproteins, rather than in LDL, because rat plasma

lacks a cholesteryl ester transfer protein.³² These basic differences suggest that plasma cholesterol concentration probably is not regulated in the same way in rats and humans. Second, there was no significant reduction in the plasma cholesterol concentration of the rats fed menhaden oil for 11 days, even though the hepatic ACAT activity was elevated. Since the plasma triglyceride concentration was already decreased at this time, an 11-day feeding period appears to be sufficient to produce plasma lipid changes. The failure to observe any plasma cholesterol reduction argues against any relationship between the ACAT response and the cholesterol-lowering effect of polyunsaturated fats.

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