# Effect of dietary n–3 fatty acids on HMG-CoA reductase and ACAT activities in liver and intestine of the rabbit

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**Abstract** The regulation of hepatic and intestinal 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and acyl-CoA:cholesterol acyltransferase (ACAT) activities by dietary fish oil was examined in the rabbit. Rabbits were fed 10% menhaden oil or menhaden oil plus 1% cholesterol for 14 days. They were compared with animals fed a control diet or one enriched with long-chain saturated fats consisting of 10% cocoa butter oil or cocoa butter oil plus 1% cholesterol. Plasma cholesterol was increased in rabbits fed the fish oil and the two cholesterol-containing diets. In the liver, ACAT activity was increased and HMG-CoA reductase activity was decreased in rabbits ingesting the fish oil. The same was true for animals ingesting both cholesterol-containing diets. In the intestine, ACAT activity was not affected by the ingestion of the fish oil compared to control rabbits; however, it was significantly higher in animals fed the fish oil compared to animals ingesting the cocoa butter. HMG-CoA reductase activity was decreased in the distal two-thirds of the intestine in animals fed the menhaden oil compared to activities observed in controls. In animals ingesting the cholesterol diets, intestinal reductase was significantly decreased, whereas intestinal ACAT activity was increased in rabbits ingesting the cocoa butter and cholesterol diet when compared to their controls. Lipid analysis of hepatic and intestinal microsomes demonstrated an enrichment of n-3 polyunsaturated fatty acids in membranes from rabbits ingesting the menhaden oil. Hepatic microsomal cholesterol was decreased in rabbits fed the fish oil whereas, in microsomes from livers and intestines of animals ingesting the cholesterol diets, membrane cholesterol was increased. We conclude that changes in membrane fatty acid saturation induced by dietary manipulation will result in the regulation of HMG-CoA reductase and ACAT activities in the liver and intestine. The regulation of these two key enzymes in cholesterol metabolism is most likely related to the degree of fat saturation and not necessarily to the specific class of polyunsaturates within the membrane. Dietary fat saturation regulates the metabolic steps necessary to monitor the amount of unesterified cholesterol within hepatocytes and enterocytes. — **Field, F. J., E. J. Albright, and S. N. Mathur.** Effect of dietary n-3 fatty acids on HMG-CoA reductase and ACAT activities in liver and intestine of the rabbit. *J. Lipid Res.* 1987. **28:** 50–58.

**Supplementary key words** cholesterol • unsaturated fatty acids • saturated fatty acids • fish oil

The activities of membrane-bound enzymes can be regulated by changes in membrane fatty acid and cholesterol content (1–5). Previous investigations have shown that cholesterol metabolism in cells can be regulated by altering dietary fat and/or cholesterol. This usually results in the modification of microsomal lipid composition which in turn regulates the activity of enzymes involved in cholesterol metabolism (6–14). Acyl-coenzyme A:cholesterol acyltransferase (ACAT) and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase are two microsomal enzymes that control the rates of intracellular cholesterol esterification and synthesis, respectively. These two enzymes, which regulate the amount of unesterified cholesterol within a cell, are responsive to changes in microsomal lipid composition (10). It has been suggested that the reciprocal regulation of these enzymes is responsible for providing the cell with the necessary amount of free cholesterol for membrane structuring, bile acid synthesis, lipoprotein formation, and other cellular functions (15–18).

In an earlier study, we demonstrated that microsomal ACAT activity in intestines of rabbits ingesting different dietary fats with or without cholesterol was regulated by membrane fatty acid saturation and cholesterol content. Although the amount of cholesterol present in the membrane was felt to be the major regulator of ACAT activity, an increase in the unsaturated fatty acid content of the microsomal membrane also resulted in an increase in ACAT activity independently of the sterol content (6, 8). A similar observation was made in rat liver (7). The regu-

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; ACAT, acyl-CoA:cholesterol acyltransferase; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

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lation of HMG-CoA reductase activity in rat liver microsomes was also demonstrated by altering microsomal fatty acid saturation by dietary manipulation (9–11). The effect of dietary fatty acid saturation on HMG-CoA reductase activity in the intestine is controversial (12, 14).

Recent studies investigating the effects of various dietary fish oils on plasma lipid levels have demonstrated beneficial lowering responses (review, 19). Since fish oils contain highly unsaturated n-3 fatty acids compared to n-6 polyunsaturated fatty acids found in most vegetable and plant oils, we felt it would be important to investigate the potential regulation of cholesterol esterification and cholesterol synthesis in animals fed these oils. Since liver and intestine are the major organs of cholesterol metabolism, accounting for approximately 90% of the body's cholesterol production and 65–70% of the total catabolism of circulating low density lipoproteins (20, 21), the present investigation was initiated to study the effects of dietary fish oil on the activities of HMG-CoA reductase and ACAT in liver and intestine of rabbits. The results clearly demonstrate that microsomes prepared from livers and intestines of rabbits fed the fish oil, menhaden oil, are significantly enriched in n-3 fatty acids. This increase in microsomal n-3 polyunsaturated fatty acids is associated with a decrease in the activity of HMG-CoA reductase and a reciprocal increase in ACAT activity in both liver and certain segments of the intestine in these animals. This suggests that hepatic and intestinal cholesterol metabolism is regulated by a diet rich in polyunsaturated fatty acids containing a high content of n-3 polyunsaturates.

## METHODS

### Animals

Male New Zealand white rabbits weighing 1.5–2.0 kg were fed normal Purina rabbit chow for 1 week prior to the start of the experimental diets. Lights were on from 0700 to 1900 hr. The rabbits were then divided into five dietary groups of six animals each. The following diets were administered ad libitum for 14 days: control (normal Purina rabbit chow); rabbit chow plus 10% menhaden oil; rabbit chow plus 10% cocoa butter oil; rabbit chow, 10% menhaden oil plus 1% cholesterol; rabbit chow, 10% cocoa butter oil plus 1% cholesterol. **Table 1** shows the fatty acid analysis of the menhaden and cocoa butter oils. There were significant differences in the amounts of saturated and monounsaturated fatty acids in the two oils. The striking difference, however, was the nearly sixfold increase in the amount of polyunsaturated fatty acids in the fish oil with most of it being n-3 fatty acids.

The menhaden oil also contained 250 mg of cholesterol per 100 ml of the oil. Cocoa butter oil had no detectable cholesterol. Since this amount of cholesterol could conceivably affect the results of the study, 250 mg of cholesterol was dissolved in each 100 ml of the cocoa butter oil. The control diet was prepared by dissolving 250 mg of cholesterol in diethyl ether and thoroughly mixing this with 1 kg of chow. The ether was evaporated prior to administering the diet to the rabbits.

TABLE 1. Fatty acid composition of menhaden and cocoa butter oils

| Fatty Acids | Menhaden Oil | Cocoa Butter |
|---|---|---|
| | % | |
| 14:0 | 9 | <1 |
| 16:0 | 29 | 23 |
| 18:0 | 6 | 37 |
| 18:1 (n-9) | 15 | 25 |
| 18:2 (n-6) | 1 | 3 |
| 18:3 (n-3) | 2 | – |
| 20:4 (n-6) | 1 | – |
| 20:5 (n-3) | 14 | 1 |
| 22:5 (n-3) | 2 | – |
| 22:6 (n-3) | 9 | – |
| Others | 12 | 11 |
| Saturated | 45 | 60 |
| Monounsaturated | 15 | 25 |
| Polyunsaturated | 28 | 5 |
| n-3 | 27 | 1 |

### Materials

[1-$^{14}$C]Oleoyl-coenzyme A, [1,2-$^{3}$H]cholesterol, [5-$^{3}$H]mevalonolactone, and 3-hydroxy-3-methyl[3-$^{14}$C]glutaryl-CoA were obtained from New England Nuclear (Boston, MA). Oleoyl-coenzyme A, cholesterol, trypsin inhibitor, glucose-6-phosphate dehydrogenase, glucose-6-phosphate, and nucleotide adenine diphosphate were from Sigma Chemical Co., St. Louis, MO. HMG-CoA was purchased from P-L Biochemicals, Inc. (Milwaukee, WI). Menhaden oil was obtained from Zapata Haynie Corporation (Reedville, VA). Cocoa butter was purchased from Ruger Chemical Company, Inc. (Irvington, NJ).

### Preparation of microsomes

The rabbits were killed between 0800 and 1000 hr. The small intestine, from the pylorus to the cecum, and a 2-g wedge resection of the liver were excised for the preparation of microsomes. The intestine from each animal was divided into three equal segments and labeled proximal, middle, and distal. After the lumens of the intestinal segments were thoroughly rinsed with 60 to 100 ml of cold normal saline, the intestines were opened by cutting along the mesenteric side. Excess mucus was removed by a damp sponge and the mucosa was gently scraped with a glass slide. Approximately 1 g of liver was diced over ice, and both the diced liver and intestinal mucosa were homogenized in a buffered sucrose solution containing 0.1

M sucrose, 0.05 M KCl, 0.04 M $KH_2PO_4$, 0.03 M EDTA, pH 7.4, in a Potter-Elvehjem homogenizer. The whole homogenates were centrifuged for 20 min at 10,000 *g* and the resulting supernatants were centrifuged for 1 hr at 105,000 *g*. The pellets were resuspended in buffer and centrifuged again at 105,000 *g* for 1 hr.

### Enzyme assays

ACAT activity was determined as previously described (6), with the specific activity of oleoyl-CoA being 17,830 dpm/nmol. The activity of HMG-CoA reductase was measured as described (22) with the specific activity of HMG-CoA being 20,740 dpm/nmol.

### Lipoprotein preparation

Just prior to killing the rabbits, plasma was obtained from the ear vein of each animal for the preparation of lipoproteins. VLDL, LDL, and HDL were separated according to the protocol of the Lipid Research Clinics (23).

### Chemical analysis

Fatty acid analyses of the dietary oils and microsomes were performed by gas-liquid chromatography as previously described (8). Free and total cholesterol were also determined in the oils and microsomes by gas-liquid chromatography using cholestane as an internal standard (24). Microsomal phospholipids were measured according to the method of Chalvardjian and Rudnicki (25). Total plasma cholesterol and lipoprotein cholesterol levels were measured with an AutoAnalyzer II as described by the Lipid Research Clinics (23). Protein was determined according to the method of Lowry et al. (26) using bovine serum albumin as a standard.

### Statistical analysis

A one-way analysis of variance model was used to test for significance (8).

## RESULTS

### Plasma lipoprotein cholesterol levels

**Table 2** shows the plasma cholesterol response to the different dietary regimens. Rabbits that were fed the menhaden oil for 2 weeks had a significant increase in their plasma cholesterol levels compared to animals fed the control diet. This difference was secondary to a twofold increase in the quantity of LDL observed in the plasma of rabbits fed the fish oil. Although cocoa butter tended to increase plasma cholesterol as well, these concentrations were not significantly different from plasma levels in control or menhaden oil groups. The cholesterol-containing diets caused significant increases in plasma cholesterol levels. Both VLDL and LDL fractions were markedly elevated in these animals. HDL cholesterol was significantly increased in rabbits fed the cocoa butter and cholesterol diet but not in animals fed the menhaden oil and cholesterol diet. Substantially higher plasma cholesterol concentrations, especially in VLDL and HDL, were observed in rabbits fed the cocoa butter and cholesterol diet compared to the rabbits fed the fish oil and cholesterol.

### Microsomal ACAT activity

In rabbits fed the menhaden oil, hepatic ACAT activity was significantly increased compared to the activity observed in the livers of animals fed control chow or cocoa butter (**Table 3**). The addition of cholesterol to the diets resulted in an increase in hepatic ACAT activities above those observed in livers of rabbits fed the oils alone. However, in animals fed the fish oil plus cholesterol diet, hepatic ACAT activity was substantially greater than the activity observed in livers of rabbits fed the cholesterol and cocoa butter diet.

The effect of the diets on ACAT activities within the intestine was not as striking. The menhaden oil diet did not

TABLE 2. Effect of diets on plasma lipoprotein cholesterol levels[a]

| Diet | Whole Plasma | VLDL | LDL | HDL |
|---|---|---|---|---|
| Control (6)[b] | 64 ± 5 | 9 ± 2 | 26 ± 3 | 28 ± 4 |
| Menhaden oil (6) | 106 ± 14[c] | 8 ± 3 | 62 ± 14[c] | 33 ± 3 |
| Cocoa butter (6) | 79 ± 7 | 10 ± 2 | 29 ± 7 | 37 ± 5 |
| Menhaden oil + cholesterol (6) | 1321 ± 90[d] | 368 ± 136[d] | 933 ± 129[d] | 34 ± 5 |
| Cocoa butter + cholesterol (6) | 2253 ± 166[e,f] | 1371 ± 160[e,f] | 842 ± 45[e] | 59 ± 5[e,f] |

[a] Values are mean ± SEM; mg/dl.
[b] Number of animals.
[c] $P < 0.02$ vs. control.
[d] $P < 0.001$ vs. menhaden oil.
[e] $P < 0.001$ vs. cocoa butter.
[f] $P < 0.02$ vs. menhaden oil + cholesterol.

TABLE 3. Effect of diets on ACAT activity in liver and intestine

| Diet | Liver | Intestine | | |
|---|---|---|---|---|
| | | Proximal | Middle | Distal |
| | *pmol/mg per min* | | | |
| Control (6)[a] | 70 ± 8 | 205 ± 19 | 226 ± 41 | 220 ± 28 |
| Menhaden oil (6) | 181 ± 33[b] | 250 ± 42 | 301 ± 34 | 210 ± 32 |
| Cocoa butter (6) | 94 ± 12 | 121 ± 13[e] | 214 ± 18[f] | 161 ± 35[g] |
| Menhaden oil + cholesterol (6) | 298 ± 26[c] | 363 ± 65 | 494 ± 87 | 389 ± 82 |
| Cocoa butter + cholesterol (6) | 151 ± 16[d] | 342 ± 74 | 461 ± 49 | 344 ± 57 |

[a] Number of animals; values are mean ± SEM.
[b] $P < 0.05$ vs. control and cocoa butter.
[c] $P < 0.02$ vs. menhaden oil.
[d] $P < 0.05$ vs. cocoa butter and menhaden oil + cholesterol.
[e] $P < 0.02$ vs. control, menhaden oil, and cocoa butter + cholesterol.
[f] $P < 0.05$ vs. menhaden oil and cocoa butter + cholesterol.
[g] $P < 0.025$ vs. cocoa butter + cholesterol.

significantly alter intestinal ACAT activities when compared to controls. However, ACAT activities in the proximal and middle intestine of animals fed the menhaden oil were significantly higher than the activities observed in these segments prepared from rabbits fed the cocoa butter. In contrast to the stimulation of intestinal ACAT activity that was observed by adding cholesterol to the cocoa butter diet, the addition of cholesterol to the menhaden oil did not result in an additional stimulation of ACAT activity.

### Microsomal HMG-CoA reductase activity

The effects of the diets on HMG-CoA reductase activities are represented in **Table 4.** Hepatic HMG-CoA reductase activity was significantly decreased by dietary menhaden oil compared to the activities that were observed in livers from control rabbits and rabbits fed cocoa butter. Both cholesterol diets caused substantial reductions in hepatic reductase activities in these animals.

In the distal two-thirds of the intestine, dietary menhaden oil resulted in a significant decrease in HMG-CoA reductase activity. Except for an increase in reductase activity in the proximal intestinal segment of rabbits fed cocoa butter, there were no significant differences in reductase activities observed in intestines of animals fed the fish oil compared to cocoa butter. As was observed in the livers of rabbits fed cholesterol, HMG-CoA reductase activities were significantly decreased in intestines of rabbits fed cholesterol.

### Microsomal cholesterol and phospholipid content

Microsomes prepared from livers and intestines of animals on the dietary regimens were analyzed for cholesterol and phospholipid content. Hepatic microsomal total cholesterol was significantly decreased in rabbits fed the menhaden oil (**Table 5**) compared to microsomes obtained from animals fed the control diet. Both cholesterol diets caused pronounced increases in hepatic microsomal cholesterol content compared to microsomes prepared from rabbits fed menhaden and cocoa butter alone. These

TABLE 4. Effect of diets on HMG-CoA reductase activity in liver and intestine

| Diet | Liver | Intestine | | |
|---|---|---|---|---|
| | | Proximal | Middle | Distal |
| | *pmol/mg per min* | | | |
| Control (6)[a] | 299 ± 99 | 1065 ± 137 | 1364 ± 275 | 1013 ± 264 |
| Menhaden oil (6) | 69 ± 45[b] | 974 ± 147[d] | 660 ± 158[e] | 459 ± 67[e] |
| Cocoa butter (6) | 384 ± 108 | 1888 ± 402 | 671 ± 166[f] | 677 ± 327 |
| Menhaden oil + cholesterol (6) | 22 ± 5 | 209 ± 26 | 262 ± 44 | 231 ± 35 |
| Cocoa butter + cholesterol (6) | 30 ± 6[c] | 398 ± 40[c] | 406 ± 29[c] | 335 ± 35[c] |

[a] Number of animals; values are mean ± SEM.
[b] $P < 0.05$ vs. control, cocoa butter.
[c] $P < 0.001$ vs. cocoa butter.
[d] $P < 0.05$ vs. cocoa butter and menhaden oil + cholesterol.
[e] $P < 0.05$ vs. control and menhaden oil + cholesterol.
[f] $P < 0.05$ vs. control.

TABLE 5. Effect of diets on microsomal cholesterol and phospholipid content in liver

| Diet | Total Cholesterol | Phospholipid | Free Cholesterol: Phospholipid Ratio |
|---|---|---|---|
| | *μg/mg* | *nmol/mg* | *nmol:nmol* |
| Control (6)[a] | 22 ± 1 | 724 ± 10 | 0.078 ± 0.01 |
| Menhaden oil (6) | 16 ± 1[b] | 829 ± 23[c] | 0.052 ± 0.01 |
| Cocoa butter (6) | 21 ± 2 | 804 ± 16 | 0.067 ± 0.01 |
| Menhaden oil + cholesterol (6) | 41 ± 3[c] | 693 ± 29 | 0.123 ± 0.01[e] |
| Cocoa butter + cholesterol (6) | 49 ± 5[d] | 754 ± 10 | 0.126 ± 0.01[d] |

[a] Number of animals; values are mean ± SEM.
[b] $P < 0.02$ vs. control.
[c] $P < 0.001$ vs. menhaden oil.
[d] $P < 0.001$ vs. cocoa butter.
[e] $P < 0.005$ vs. control and menhaden oil + cholesterol.

findings were also reflected in the free cholesterol to phospholipid ratios in these microsomal preparations.

Cholesterol and phospholipid contents of microsomes prepared from the middle segment of intestines of rabbits fed control, menhaden oil, and cocoa butter were not different (**Table 6**). Intestinal microsomal cholesterol and the free cholesterol-to-phospholipid ratio were increased only in rabbits fed the cholesterol-containing diets.

### Microsomal fatty acid composition

**Table 7** shows the fatty acid composition of the microsomes prepared from the livers of these animals. Compared to microsomes prepared from rabbits fed control and cocoa butter, microsomes from rabbits fed menhaden oil were strikingly enriched in n-3 fatty acids. These membranes also contained more palmitate and less oleic acid than microsomes from control and cocoa butter-fed rabbits. **Table 8** demonstrates similar observations made in the intestine except that palmitate was not increased in microsomes obtained from intestines of rabbits fed the menhaden oil. The accumulation of n-3 fatty acids in microsomes from intestines or livers of animals ingesting the fish oil was independent of the presence of cholesterol in the diet.

## DISCUSSION

It is of interest that, in the rabbit, the ingestion of menhaden oil resulted in an increase in plasma cholesterol concentration above levels observed in rabbits ingesting a control diet. This is in contrast to the effects of fish oil on plasma cholesterol levels observed in other animal models including rats (27), chickens, pigs, and humans (19). It is unlikely that this response of plasma cholesterol to the ingestion of fish oil is strictly related to the amount of n-3 fatty acids in the oil, as the ingestion of safflower oil, a plant oil enriched in n-6 polyunsaturated fatty acids also increases plasma cholesterol in the rabbit (8). It is not clear why, in this study, rabbits ingesting the cocoa butter and cholesterol diet had significantly higher plasma cholesterol concentrations than rabbits ingesting the menhaden oil and cholesterol diet. Perhaps the beneficial effects of the dietary fish oil on plasma cholesterol levels in this animal model are observed only when the oil is ingested with the sterol.

The activity of ACAT in livers of rabbits ingesting the fish oil was significantly increased compared to activities observed in livers of animals fed control or cocoa butter diets. These results are in agreement with an earlier study

TABLE 6. Effect of diets on microsomal cholesterol and phospholipid content in mid-intestine

| Diet | Total Cholesterol | Phospholipid | Free Cholesterol: Phospholipid Ratio |
|---|---|---|---|
| | *μg/mg* | *nmol/mg* | *nmol:nmol* |
| Control (6)[a] | 24 ± 1 | 465 ± 22 | 0.136 ± 0.01 |
| Menhaden oil (6) | 25 ± 1 | 483 ± 17 | 0.138 ± 0.008 |
| Cocoa butter (6) | 23 ± 1 | 500 ± 20 | 0.121 ± 0.009 |
| Menhaden oil + cholesterol (6) | 41 ± 2[b] | 546 ± 29 | 0.169 ± 0.010[d] |
| Cocoa butter + cholesterol (6) | 43 ± 2[c] | 545 ± 18 | 0.161 ± 0.007[e] |

[a] Number of animals; values are mean ± SEM.
[b] $P < 0.001$ vs. menhaden oil.
[c] $P < 0.001$ vs. cocoa butter.
[d] $P < 0.02$ vs. menhaden oil.
[e] $P < 0.02$ vs. cocoa butter.

TABLE 7. Effect of diets on microsomal fatty acid content in liver

| Fatty Acids | Control | Menhaden Oil | Cocoa Butter | Menhaden Oil + Cholesterol | Cocoa Butter + Cholesterol |
|---|---|---|---|---|---|
| | | | % | | |
| 16:0 | 21 ± 1 | 27 ± 1 | 17 ± 1 | 29 ± 1 | 22 ± 1 |
| 18:0 | 19 ± 1 | 20 ± 1 | 23 ± 1 | 17 ± 1 | 20 ± 1 |
| 18:1 (n-9) | 15 ± 2 | 4 ± 1 | 19 ± 1 | 7 ± 1 | 19 ± 2 |
| 18:2 (n-6) | 31 ± 1 | 6 ± 1 | 30 ± 1 | 10 ± 1 | 32 ± 1 |
| 18:3 (n-3) | 1 ± 0.1 | | 1 ± 0.1 | | 1 ± 0.1 |
| 20:4 (n-6) | 6 ± 1 | 5 ± 1 | 5 ± 1 | 4 ± 1 | 5 ± 1 |
| 20:5 (n-3) | | 7 ± 1 | | 9 ± 1 | |
| 22:5 (n-3) | | 4 ± 1 | | 5 ± 1 | |
| 22:6 (n-3) | | 22 ± 1 | | 14 ± 1 | |
| Others | 5 ± 1 | 4 ± 1 | 3 ± 1 | 6 ± 1 | 4 ± 1 |
| Saturated | 40 ± 1 | 48 ± 1[a] | 41 ± 1 | 46 ± 1[a] | 42 ± 1 |
| Monounsaturated | 15 ± 2 | 4 ± 1[a] | 19 ± 1 | 7 ± 1[a] | 19 ± 2 |
| Polyunsaturated | 39 ± 2 | 45 ± 2[b] | 36 ± 1 | 41 ± 1[a] | 36 ± 1 |
| n-3 | 1 ± 0.1 | 33 ± 1 | 1 ± 0.1 | 28 ± 1 | 1 ± 0.1 |

[a] $P < 0.05$ vs control and cocoa butter or cocoa butter + cholesterol; mean ± SEM.
[b] $P < 0.05$ vs. cocoa butter.

investigating the effects of dietary fish oil and cocoa butter on hepatic ACAT activity in rats (28). In that study, however, no dietary control group was included and no adjustments were made for the amount of cholesterol present in the dietary fish oil. The present study, therefore, confirms and extends those observations in liver and intestine. A possible explanation as to why ACAT activity was higher in rabbits fed menhaden oil is that the change in membrane fatty acid composition may result in an increase in the amount of cholesterol available to the enzyme. Alternatively, a change in membrane fatty acid composition could regulate ACAT activity independently of its substrate. The potential of both mechanisms playing a role in ACAT regulation cannot be excluded and will be addressed later. It could also be argued that the increase in plasma cholesterol that was observed in rabbits ingesting the fish oil diet caused an increase in hepatic ACAT activity secondary to an increase in the metabolism of elevated LDL levels (29). This seems unlikely as hepatic microsomal cholesterol content was decreased in these animals. The reverse might be expected in a liver that is metabolizing more LDL. In addition, Johnson et al. (28) found an increase in hepatic ACAT activity in rats ingesting fish oil despite observing no change in plasma cholesterol levels.

Although the content of n-3 fatty acids was significantly increased in hepatic microsomes, suggesting that these fatty acids may be playing a role in the regulation of

TABLE 8. Effect of diets on microsomal fatty acid content in mid-intestine

| Fatty Acids | Control | Menhaden Oil | Cocoa Butter | Menhaden Oil + Cholesterol | Cocoa Butter + Cholesterol |
|---|---|---|---|---|---|
| | | | % | | |
| 16:0 | 18 ± 1 | 19 ± 1 | 16 ± 1 | 23 ± 2 | 19 ± 1 |
| 18:0 | 21 ± 1 | 23 ± 1 | 23 ± 1 | 19 ± 1 | 22 ± 1 |
| 18:1 (n-9) | 13 ± 1 | 9 ± 1 | 30 ± 1 | 10 ± 1 | 27 ± 1 |
| 18:2 (n-6) | 42 ± 1 | 12 ± 1 | 28 ± 1 | 14 ± 2 | 28 ± 1 |
| 18:3 (n-3) | 2 ± 1 | 0.3 ± 0.0 | 1 ± 0.0 | 0.3 ± 0.0 | 1 ± 0.1 |
| 20:4 (n-6) | 1 ± 0.1 | 4 ± 0.2 | 2 ± 1 | 3 ± 1 | 2 ± 0.1 |
| 20:5 (n-3) | | 22 ± 1 | | 16 ± 1 | |
| 22:5 (n-3) | 0.3 ± 0.1 | 1 ± 0.3 | | 1 ± 0.2 | |
| 22:6 (n-3) | 1.0 ± 0.2 | 8 ± 0.4 | | 6 ± 1 | |
| Others | 2 ± 1 | 1 ± 0.3 | 1 ± 0.2 | 9 ± 1 | 2 ± 1 |
| Saturated | 39 ± 1 | 42 ± 1 | 39 ± 1 | 42 ± 1 | 41 ± 1 |
| Monounsaturated | 13 ± 1 | 9 ± 1[a] | 30 ± 1 | 10 ± 1[a] | 27 ± 1 |
| Polyunsaturated | 46 ± 1 | 48 ± 1[a] | 30 ± 1 | 41 ± 2[a] | 31 ± 1 |
| n-3 | 3 ± 1 | 31 ± 1 | 1 ± 0.0 | 33 ± 1 | 1 ± 0.1 |

[a] $P < 0.05$ vs. cocoa butter or cocoa butter + cholesterol; mean ± SEM.

ACAT activity, the total amount of polyunsaturates was also increased in microsomes prepared from rabbits ingesting the menhaden oil. It cannot be assumed, therefore, that dietary fish oil per se regulates hepatic ACAT activity, as other dietary oils rich in n-6 polyunsaturates also stimulate ACAT activity (7, 8). The change in microsomal fatty acid saturation appeared to regulate the response of hepatic ACAT activity to an increase in microsomal cholesterol content. In hepatic microsomes prepared from rabbits fed cholesterol and cocoa butter, ACAT activity was significantly less than ACAT activity in microsomes prepared from rabbits ingesting cholesterol and menhaden oil, despite equivalent amounts of cholesterol present in the microsomes.

In contrast to what was observed in the liver, intestinal ACAT activity was not significantly affected by dietary fish oil compared to controls. There is a plausible explanation for this when the microsomal lipid composition in the intestines of these animals is examined. Except for an increase in the amount of n-3 fatty acids, the amounts of cholesterol, saturated fatty acids, and polyunsaturated fatty acids were similar in the membranes prepared from these rabbits. This also speaks against the likelihood that n-3 polyunsaturates themselves regulate ACAT activity. The differences observed in intestinal ACAT activities between animals ingesting menhaden oil and cocoa butter were reflected in the differences observed in their microsomal fatty acid compositions (Table 8), because their microsomal cholesterol contents (Table 6) were similar. The data suggest that intestinal ACAT activity is regulated by microsomal fatty acid saturation independently of cholesterol and the type of polyunsaturated fatty acid found within the membrane. Despite a significant increase in intestinal microsomal cholesterol content in rabbits ingesting the two cholesterol diets, ACAT activities were significantly increased only in intestines of animals fed the cocoa butter plus cholesterol diet compared to their controls fed the cocoa butter alone. The reasons for this are not clear; suffice it to say, intestinal ACAT differs from hepatic ACAT in its response to the ingestion of cholesterol and fish oil.

As previously mentioned, changes in membrane fatty acid composition could result in changes in the availability of cholesterol to ACAT, thereby regulating its activity. This possibility was tested in liver and mid-intestinal microsomes. Exogenous cholesterol solubilized in Triton WR-1339 was incbuated with the microsomes prior to determining ACAT activities according to the method of Billheimer, Tavani, and Nes (30). Using this assay, ACAT activities in hepatic microsomes prepared from rabbits ingesting the menhaden oil remained significantly higher than activities in microsomes prepared from rabbits fed cocoa butter (278 ± 43 vs. 170 ± 19 pmol/mg per min, $P < 0.05$). Therefore, in hepatic microsomes obtained from these two groups of animals, differences in ACAT activities cannot be related to changes in cholesterol availability. However, the differences that were observed between ACAT activities in liver microsomes prepared from rabbits fed the control diet compared to the menhaden oil diet and the menhaden oil plus cholesterol diet compared to the cocoa butter plus cholesterol diet no longer existed when exogenous cholesterol was added to the microsomes. In the intestine, the relationships of ACAT activities between the dietary groups were not altered by adding exogenous cholesterol to the assays. ACAT activity in microsomes prepared from the mid-intestine of rabbits fed the menhaden oil diet remained significantly higher than ACAT activity in microsomes from rabbits fed the cocoa butter diet (470 ± 45 vs. 360 ± 18 pmol/mg per min, $P < 0.05$). The results suggest that ACAT activity is regulated by changes in membrane fatty acid composition even when cholesterol is not thought to be limiting. However, there are circumstances whereby changes in membrane fatty acid composition regulate "expressed" ACAT activity, which suggests that these membrane lipid changes affect the availability of cholesterol to the enzyme.

The regulation of hepatic and intestinal HMG-CoA reductase activities by the different dietary regimens was reciprocal of that observed for ACAT activities in these two organs. In the liver, the activity of HMG-CoA reductase was decreased in microsomes prepared from rabbits ingesting the fish oil diet and the two cholesterol diets. This is in agreement with an earlier study demonstrating that hepatic HMG-CoA reductase activity was less in rats fed a diet rich in polyunsaturated fatty acids compared to the activity observed in rats fed a diet rich in long-chain saturated fatty acids (9, 10). Since, in these previous studies, the unsaturated fatty acids in the diet were of the n-6 class, it suggests that hepatic reductase is regulated by the degree of fatty acid saturation and not by any differences in the class of polyunsaturates. In the intestine, the ingestion of menhaden oil decreased HMG-CoA reductase activity in the distal two-thirds of the gut compared to the activity in control rabbits. These observations differ somewhat from those of Bochenek and Rodgers (12) who showed no effect of dietary fat saturation on jejunal or ileal HMG-CoA reductase activity. In that study, however, 10% tripalmitin and 10% safflower oil were used for the saturated and polyunsaturated diets, respectively. The differences observed in the results between the two studies may be secondary to the qualitative (n-6 vs. n-3 fatty acids) or quantitative differences in the degree of fatty acid saturation that occurred within the membranes. Both cholesterol diets significantly inhibited reductase activities in all segments of the intestine and this is in agreement with an earlier study (14).

It is well established that the activity of HMG-CoA reductase is regulated by reversible phosphorylation (31). In the present study, microsomes were prepared in the ab-

sence of a phosphatase inhibitor. The measured activity of HMG-CoA reductase, therefore, represents total activity of the enzyme (32). It has been shown previously that, after only a few hours of dietary manipulation, the alteration that occurs in cholesterol synthesis is the result of a change in total HMG-CoA reductase protein rather than a change in the state of phosphorylation of the enzyme (32, 33). Therefore, in a dietary study that spans 14 days, the long-term regulation of reductase activity would most likely involve changes in the quantity of the enzyme. The regulation of HMG-CoA reductase activity by reversible phosphorylation was, therefore, not investigated in this study.

The data presented here clearly demonstrate that changes in membrane fatty acid saturation induced by dietary manipulation will result in the regulation of both hepatic and intestinal HMG-CoA reductase and ACAT activities. This effect appears to be independent of the class of long-chain polyunsaturates within the membrane. The reciprocal regulation of these two key enzymes in cholesterol metabolism by dietary unsaturated fat suggests that hepatocytes and enterocytes are tightly regulating their requirement for unesterified cholesterol during the processing of these fatty acids.

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