Dietary Fatty Acids Regulate Acyl-CoA:Cholesterol Acyltransferase and Cytosolic Cholesteryl Ester Hydrolase in Hamsters1,2

Ji-Young Lee and Timothy P. Carr3

Department of Nutrition and Health Sciences, University of Nebraska, Lincoln, NE 68583

ABSTRACT To investigate the effects of dietary fatty acids on acyl-CoA:cholesterol acyltransferase (ACAT) and cytosolic cholesteryl ester hydrolase (cCEH), male Syrian hamsters (F1B hybrid) were fed a modified version of the NIH-07 open formula, cereal-based rodent diet enriched with one of the following 4 dietary fatty acids: palmitic acid (16:0), trans fatty acids (18:1t), oleic acid (18:1c), or linoleic acid (18:2). Hamsters fed 16:0 and 18:1t had significantly higher plasma non-HDL cholesterol concentrations compared with those fed 18:1c and 18:2. However, differences in plasma apolipoprotein (apo)B100 concentration, hepatic cCEH mRNA abundance, and hepatic ACAT activity between 16:0- and 18:1t-fed hamsters suggest that the hypercholesterolemic effects are achieved by different mechanisms. Specifically, an increase in ACAT activity by 16:0 may induce enrichment of cholesteryl esters in apoB100-containing particles, whereas 18:1t may increase the number of the particles. Hepatic cholesteryl esters accumulated in the 18:1c- and 18:2-fed groups with no differences in hepatic ACAT activity and cCEH mRNA abundance among hamsters fed unsaturated fatty acids (i.e., 18:1t, 18:1c, and 18:2). Considering the lack of change in free cholesterol concentration and increased cholesteryl esters in the liver, the hypocholesterolemic effect of 18:1c and 18:2 compared with 18:1t may be attributed to decreased production of apoB100-containing particles. ACAT-1 was expressed in all the tissues examined; in contrast, ACAT-2 was highly expressed in the liver and small intestine. Hepatic ACAT activity was disproportionate to the levels of ACAT-1 and ACAT-2 mRNA and protein, indicating post-transcriptional regulation of ACAT by dietary fatty acids. The data suggest that cholesterolemic effects of individual dietary fatty acids can be achieved through their independent modulation of pathways regulating assembly and secretion of apoB100-containing particles. J. Nutr. 134: 3239–3244, 2004.

KEY WORDS: • trans fatty acids • cholesteryl • acyl-CoA:cholesterol acyltransferase • cholesteryl ester hydrolase • hamsters

The liver plays a major role in maintaining whole-body cholesterol homeostasis, i.e., it is the major site for elimination of cholesterol from the body via bile, either through conversion of cholesterol into bile acids or direct biliary cholesterol secretion. The liver also produces VLDL, and it is a major catabolic site for LDL through the LDL receptor–mediated pathway (1,2). Hepatic free cholesterol concentration was suggested to be a signal to trigger the transcriptional regulatory pathway (1,2). Hepatic free cholesterol concentration was shown to increase as hepatic cholesteryl ester synthesis is induced (4). In this way, mechanisms that influence hepatic free cholesterol and cholesteryl ester levels in the liver are important for maintaining body cholesterol homeostasis.

Acyl-CoA:cholesterol acyltransferase (ACAT) is an integral enzyme present in the rough endoplasmic reticulum (ER) that catalyzes the formation of cholesteryl esters from cholesteryl and fatty acyl coenzyme A (5,6). Two isoforms of ACAT (named ACAT-1 and ACAT-2) were identified to date in several species including humans (7,8), nonhuman primates (9), and mice (10,11). ACAT-1 is ubiquitously expressed with its active site oriented toward the cytosol. The main function of ACAT-1 is to prevent the excess accumulation of free cholesterol within cell membranes. In contrast, ACAT-2 is expressed mainly in the liver and intestine with its active site in the lumen of the ER, suggesting that ACAT-2 may play a primary role in hepatic lipoprotein synthesis and secretion (4,12) and cholesterol absorption in the small intestine (6,13). Although the structure and general function of the ACAT enzymes are well defined, the factors that regulate ACAT activity are still unclear. Cholesterol availability (14,15), allosteric regulation (16,17), and post-transcriptional regulation (18,19) were suggested to regulate ACAT.

In contrast to the function of ACAT, cholesteryl ester hydrolase (CEH) converts cholesteryl esters to free cholesterol when cellular free cholesterol levels are depressed. CEH and...
ACAT thus participate in cyclic reactions that are necessary to maintain cellular free cholesterol at a relatively constant level. Among several CEH enzymes present in cells, neutral cytosolic CEH (cCEH) is the key enzyme required for releasing free cholesterol from intracellular cholesteryl ester storage and is thus involved in a tight regulation of the cellular free cholesterol pool (20,21). Responding to cellular free cholesterol levels, cCEH is regulated similarly to 3-hydroxy-3-methyl-glutaryl-CoA reductase, the rate-limiting enzyme in cholesterol synthesis, and oppositely to ACAT (22). The regulation of cCEH in the liver is mainly at the transcriptional level (22,23). Because ACAT and cCEH act together in a cyclic and opposite manner, these enzymes are likely to be coordinately regulated.

Dietary fatty acids influence several aspects of cholesterol metabolism including cholesterol absorption, bile acid synthesis, biliary cholesterol secretion, hepatic VLDL synthesis, and oppositely to ACAT (22). The regulation of cCEH in the liver is mainly at the transcriptional level (22,23). Because ACAT and cCEH act together in a cyclic and opposite manner, these enzymes are likely to be coordinately regulated.

In the present study, we investigated the extent to which dietary fatty acids influence cholesterol esterification and cholesterol ester hydrolysis in the liver to help explain the hypo- or hypercholesterolemic effect of individual dietary fatty acids. We focused our attention on 4 fatty acids commonly found in the U.S. food supply, i.e., palmitic acid (16:0), or hypercholesterolemic effect of individual dietary fatty acids. Dietary fatty acids influence cholesterol esterification and cholesterol metabolism including cholesterol absorption, bile acid synthesis, biliary cholesterol secretion, hepatic VLDL synthesis, and oppositely to ACAT (22). The regulation of cCEH in the liver is mainly at the transcriptional level (22,23). Because ACAT and cCEH act together in a cyclic and opposite manner, these enzymes are likely to be coordinately regulated.

Plasma lipids and apolipoprotein B_{100}. Lipids were extracted from liver into chloroform:methanol (2:1, v:v) by the method of Folch et al. (33). Enzymatic analysis was performed to measure hepatic free cholesterol (Wako Chemicals), total cholesterol, and triacylglycerol (Roche Diagnostics) (32). Phospholipid concentration in the liver was also determined enzymatically (Kit #990-54009, Wako Chemicals).

RT-PCR. Total RNA was isolated from 10 different hamster tissues including liver, kidney, lung, heart, small intestine, aorta, spleen, testis, adipose, and adrenal using TRIzol reagent (Life Technologies) according to the manufacturer's protocol. First-strand cDNA was prepared using AMV RT (Fisher BioReagents) primed with random primers according to manufacturer's instruction. PCR was performed using Taq DNA polymerase (Takara). For hamster ACAT-1, a 767-base pair (bp) fragment was amplified using forward (5'-AAT CCT GAG CAA GAT GAA GCC CAG-3') and reverse (5'-ACT CTC GCA GCA TTC TCT CTG-3') primers. An 813-bp hamster ACAT-2 fragment was amplified using forward (5'-CTG GCC ATC GAC TTC ATT GAT GAG-3') and reverse (5'-TAG CTG TAC AGC CAG TCA TGG ACCA-3') primers. Amplification was conducted of a 378-bp β-actin fragment as an internal control using forward (5'-TCT GGC ACC ACA CCT TCT AC-3') and reverse (5'-CAC GCA CAA TAT CCC TTC TCT-3') primers. A 449-bp cytosolic CEH cDNA fragment was also amplified using forward (5'-TTT AAC AAT CCT CCT CTA CC-3') and reverse (5'-ACC CAC TAC CAA TCC ACC TC-3') primers. Anti-sense RNA probes were subsequently prepared by in vitro transcription of each cDNA using the Riboprobe system (Promega). The 307-, 400-, 543-, and 290-base in vitro transcription products were produced for ACAT-1, ACAT-2, cytosolic CEH, and β-actin, respectively. Probes were stored at −70°C until they were used.

RESULTS

Weekly recorded body weight and food intake did not differ among the 4 groups throughout the 4-wk study period (data not shown). Hamster body weight indicated a mean growth rate of 6–7 g/wk in all groups.

Plasma total cholesterol concentrations were determined enzymatically using reagents from Roche Diagnostics. Plasma HDL cholesterol concentration was measured after apolipoprotein B (apoB_{100}) precipitation (Sigma Diagnostics). Plasma "non-HDL" cholesterol concentration was calculated by subtracting plasma HDL cholesterol from total cholesterol concentration. We showed previously that the non-HDL fraction contains >90% LDL cholesterol in hamsters (33). ApoB_{100} concentration in the plasma was determined immunoturbidimetrically (Sigma Diagnostics, St. Louis, MO).

Liver lipids. Lipids were extracted from liver into chloroform:methanol (2:1, v:v) by the method of Folch et al. (34). Enzymatic analysis was performed to measure hepatic free cholesterol (Wako Chemicals), total cholesterol, and triacylglycerol (Roche Diagnostics) (32). Phospholipid concentration in the liver was also determined enzymatically (Kit #990-54009, Wako Chemicals).

ACAT activity. Microsomes were prepared as previously described (35), and protein concentration was determined for each preparation using the method of Lowry et al. (36). ACAT activity was quantified using a constant amount of microsomal protein; in addition, excess free cholesterol was added to reaction mixtures to eliminate the effect of substrate availability on enzyme activity (35).

Statistical analysis. All results were expressed as means ± SEM. Dietary treatment effect was analyzed using one-way ANOVA, and pairwise comparisons were made by the Student-Newman-Keuls procedure using GraphPad Prism software. Differences were considered significant at P < 0.05.
because plasma HDL cholesterol concentrations did not differ among the 4 groups (Table 1). Plasma triacylglycerol and apoB<sub>100</sub> concentrations were significantly higher in 18:1t-fed hamsters than in the other groups.

Hepatic free cholesterol concentrations did not differ among the 4 groups, whereas a large accumulation of cholesterol ester occurred in the livers of hamsters fed 18:1c- and 18:2-enriched diets (Table 2). Dietary fatty acids did affect the hepatic phospholipid concentration. The hepatic triacylglycerol concentration in 18:1t-fed hamsters was significantly lower than that of the 18:2-fed group.

The greater plasma non-HDL cholesterol concentrations in the 16:0 and 18:1t groups may be due to the upregulation of hepatic ACAT activity by dietary fatty acids, consequently inducing the formation and secretion of VLDL. To investigate this possibility, the effects of dietary fatty acids on hepatic ACAT expression and activity were measured. RT-PCR analysis was conducted to identify the presence of ACAT-1 and ACAT-2 in hamster tissues (Fig. 1). ACAT-1 was expressed in all of the tissues examined including liver, kidney, lung, heart, aorta, spleen, small intestine, testis, adipose tissue, and adrenal. In contrast, ACAT-2 was expressed mainly in the liver and small intestine. The expression patterns of ACAT-1 and ACAT-2 in hamsters are consistent with those of monkeys (9) and mice (10). Hamsters fed 16:0 had significantly higher total hepatic ACAT activity than the other groups (Fig. 2). To further investigate the regulatory mechanism of ACAT activity by dietary fatty acids, the mRNA abundance of ACAT-1 and ACAT-2 was estimated by RNase protection assay. In spite of significant differences in hepatic ACAT activity, there were no significant differences in either ACAT-1 or ACAT-2 mRNA abundance among the groups (data not shown). Furthermore, ACAT-1 and ACAT-2 protein levels in the liver did not differ among the groups (data not shown). These findings suggest that ACATs are regulated post-translationally by dietary fatty acids.

We also measured mRNA abundance of cCEH to determine whether dietary fatty acids regulate its expression at the transcriptional level. Hamsters fed 16:0 had significantly lower hepatic cytosolic CEH mRNA abundance than the other groups (Fig. 3).

**TABLE 1**

<table>
<thead>
<tr>
<th>Diet</th>
<th>non-HDL Cholesterol</th>
<th>HDL cholesterol</th>
<th>Triacylglycerol</th>
<th>ApoB&lt;sub&gt;100&lt;/sub&gt;</th>
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<tr>
<td></td>
<td>mmol/L</td>
<td></td>
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<tr>
<td>16:0</td>
<td>2.87 ± 0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.64 ± 0.04</td>
<td>1.14 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>552 ± 61&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:1t</td>
<td>2.67 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.47 ± 0.19</td>
<td>1.85 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
<td>773 ± 122&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:1c</td>
<td>1.58 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.42 ± 0.06</td>
<td>0.99 ± 0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>372 ± 44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>18:2</td>
<td>1.60 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40 ± 0.14</td>
<td>0.53 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>370 ± 34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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</table>

<sup>1</sup> Values are means ± SEM, n = 7 or 6 (16:0 group). Means in a column with different superscripts are significantly different, P < 0.05.

<sup>2</sup> Molecular weight used for calculations: cholesterol = 387 g/mol; triacylglycerol = 885 g/mol.

**DISCUSSION**

The concentration of plasma cholesterol associated with non-HDL lipoproteins (primarily LDL) was significantly increased in hamsters fed 16:0 and 18:1t compared with 18:1c and 18:2. The hypercholesterolemic effect of dietary 16:0 is...
well established (37), whereas studies in humans and animals showed inconsistent results for dietary 18:1t (38–42). In this study, dietary 18:1t significantly increased plasma non-HDL cholesterol concentration to a similar extent as 16:0. Although both 16:0 and 18:1t are hypercholesterolemic, the mechanisms by which they increase plasma cholesterol concentration appear to be quite different. Plasma apoB100 concentration was significantly higher in 18:1t-fed hamsters than in those fed 16:0, suggesting that the apoB100-containing lipoproteins in the group fed 16:0 were enriched with cholesterol relative to apoB100. Indeed, the non-HDL cholesterol:apoB100 molar ratio was 5376 ± 402 in the group fed 16:0 group and 3483 ± 418 in those fed 18:1t. Therefore, the hypercholesterolemic effect of 16:0 was due, at least in part, to the presence of larger cholesterol-enriched non-HDL lipoproteins, whereas the hypercholesterolemic effect of 18:1t was due to the presence of more lipoprotein particles (indicated by apoB100 concentration). These data suggest that the hepatic production of apoB100-containing lipoproteins is regulated differentially by dietary 16:0 and 18:1t.

A major difference between 16:0 and 18:1t was their regulation of hepatic ACAT and cCEH. Hamsters fed 16:0 had significantly higher hepatic ACAT activity and lower cCEH mRNA abundance than those fed 18:1t, leading us to expect a greater accumulation of cholesteryl esters in the liver in hamsters fed 16:0 compared with those fed 18:1t. However, hepatic cholesteryl ester concentrations did not differ between these 2 groups. Accepting the hypothesis that ACAT-2, not ACAT-1, is responsible for VLDDL secretion (43), it is possible that 16:0 may increase ACAT-2 activity and consequently result in the incorporation of cholesteryl esters into VLDDL particles, which shifts cholesteryl esters from the liver to the circulation. Because of the differential regulation of ACAT and cCEH by 16:0 and 18:1t, we speculate that 16:0 may increase a pool of cholesteryl esters for VLDDL assembly by activating ACAT-2 and inhibiting cCEH, thus producing larger cholesteryl ester-enriched VLDDL particles. Alternatively, 18:1t may enhance the synthesis or stability of apoB100, increasing the number of particles. Further study with selective inhibition of ACAT-1 or ACAT-2 will help clarify this. Although the relation between LDL particle composition and atherogenicity remains unresolved (44), there is little doubt that dietary fatty acids can influence the composition of plasma LDL by regulating the lipid content of lipoproteins secreted by the liver (35,45,46).

The present data also confirm the cholesterol-lowering properties of dietary 18:1c and 18:2 by decreasing plasma non-HDL cholesterol concentration compared with 16:0 and 18:1t. The significant accumulation of cholesteryl esters in the livers of 18:1c- and 18:2-fed hamsters should be noted. Other studies also reported that dietary 18:1c and 18:2 induce hepatic cholesteryl ester accumulation compared with SFA (45–48). Because unsaturated fatty acids are preferred fatty acid substrates for ACAT (49), it is possible that feeding 18:1c and 18:2 increases ACAT activity, consequently increasing the cholesteryl ester concentration in the liver. However, hepatic ACAT activity was lower in hamsters fed 18:1c and 18:2 compared with those fed 16:0. A more likely explanation is that increased hepatic cholesteryl ester concentration in hamsters fed 18:1c and 18:2 is due in part to the inhibition of VLDDL secretion by 18:1c and 18:2. Possible reductions in VLDDL secretion could be a mechanism responsible for the hypcholesterolemic effect in hamsters fed 18:1c and 18:2. LDL receptor knockout mice were studied in this regard because the concentration of non-HDL cholesterol should reflect VLDDL secretion in this model. Xie et al. (50) reported that 18:1c and 18:2 increased both hepatic cholesteryl ester concentration and plasma non-HDL cholesterol concentration in LDL receptor knockout mice, indicating that the hypcholesterolemic effect of 18:1c and 18:2 is due to increased LDL receptor-mediated catabolism of plasma LDL independent of regulation of VLDDL secretion by these fatty acids. Considering that LDL receptors are regulated mainly at the transcriptional level by SREBP in response to cellular cholesterol concentration (51,52), it does not seem the case in our study because hepatic free cholesterol concentrations did not differ among the 4 groups. However, we cannot rule out the following possibilities: 1) the transcriptional regulation of LDL receptors could be achieved by mechanisms independent of the cellular cholesterol regulatory pool; 2) dietary fatty acids could redistribute free cholesterol into a putative cholesterol regulatory pool within a cell without a change in total cellular free cholesterol concentration, thus affecting LDL receptor activity, (53); or 3) LDL could be taken up by the liver by non-LDL receptor-mediated pathways (54). These possibilities notwithstanding, our data suggest that the primary mechanism whereby dietary 18:1c and 18:2 decreased plasma cholesterol concentration was by reduced VLDDL secretion rather than increased LDL clearance.

The regulatory mechanisms of ACAT activity are not fully understood. Cholesterol availability may be one of the regulatory mechanisms for ACAT activity (14,15). However, our study did not support this possibility. Micosomal cholesterol concentrations, which can be an indication of cholesterol in the ER where ACAT-1 and ACAT-2 reside, did not differ among the 4 groups in spite of significantly higher ACAT activity in hamsters fed 16:0 than in the other groups. Therefore, mechanisms other than cholesterol availability may play a major role in the regulation of ACAT activity by dietary fatty acids. Although hepatic ACAT activity was significantly higher in 16:0-fed hamsters, significant differences were not found in either ACAT-1 or ACAT-2 mRNA and protein levels. These results suggest that ACAT activity is regulated at the post-transcriptional level as reported in several other studies (15,18,19).
In conclusion, different types of dietary fatty acids clearly have variable and independent effects on plasma cholesterol concentration. The hypocholesterolemic effect of dietary 16:0 was likely due to the enrichment of cholesteryl esters in apoB-containing particles secreted by the liver, whereas 18:1t increased the number of lipoprotein particles. The effects of 16:0 and 18:1t on hepatic lipoprotein assembly and/or secretion are achieved in part by the differential regulation of ACAT and cCEH. Conversely, the hypocholesterolemic effect of the unsaturated fatty acids, 18:1c and 18:2, is likely due to their inhibition of hepatic lipoprotein assembly and/or secretion. These data suggest that regulation of plasma cholesterol concentration by individual dietary fatty acids can be achieved through their independent mechanisms of assembly and secretion of apoB-containing particles.

**LITERATURE CITED**


