Dietary Stearic Acid Alters Gallbladder Bile Acid Composition in Hamsters Fed Cereal-Based Diets

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ABSTRACT Dietary stearic acid (18:0) lowers plasma and liver cholesterol concentration by reducing intestinal cholesterol absorption. We tested the hypothesis that dietary 18:0 reduces cholesterol absorption by altering hepatic bile acid synthesis and gallbladder bile acid composition. Male Syrian hamsters were fed modified NIH-07 open formula diets that differed in one of the following fatty acids: 18:0, palmitic acid (16:0), trans fatty acids (18:1t), oleic acid (18:1c) or linoleic acid (18:2). After 18 wk, gallbladders were removed and bile acid composition determined by HPLC. The distribution of primary bile acids (mol/100 mol) was unaffected by treatment. In contrast, dietary 18:0 significantly reduced the proportion of hydrophobic secondary bile acids, resulting in a lower hydrophobicity index of the bile. These data suggest that reduced cholesterol absorption by dietary 18:0 is due, at least in part, to reduced cholesterol solubility. The data further suggest that 18:0 may have altered the microflora populations that synthesize secondary bile acids. Although cholesterol 7α-hydroxylase (CYP7A1) activity was significantly higher in hamsters fed 18:0 compared with 16:0, this finding is most likely due to increased fecal bile acid output in the 18:0 group rather than transcriptional regulation of CYP7A1 by 18:0 or specific bile acids. J. Nutr. 132: 3119–3122, 2002.

KEY WORDS: • stearic acid • bile acids • cholesterol absorption • microflora • hamsters

Stearic acid (18:0) is unique among the saturated fatty acids commonly found in the food supply because, unlike palmitic, myristic and lauric acid, it does not raise plasma cholesterol when consumed (1–3). Studies in rats (4–6) and hamsters (7,8) have indicated that dietary 18:0 also reduces liver cholesterol concentration. The hypocholesterolemic effect of dietary 18:0 is related to a reduction in intestinal cholesterol absorption (9–12) and increased cholesterol excretion from the body (4,7,13). Nevertheless, it is still unclear exactly how 18:0 alters cholesterol absorption.

Our present hypothesis is that dietary 18:0 reduces cholesterol absorption by altering hepatic bile acid synthesis and gallbladder bile acid composition. Because intestinal absorption of cholesterol requires secretion of bile acids and formation of mixed micelles, the efficiency of cholesterol solubilization, and therefore absorption, depends in part on the relative hydrophobicity of the bile acids present in bile. Using relative hydrophobicity values established for individual bile acids (14), a “hydrophobicity index” was developed to describe the overall hydrophilic-hydrophobic balance of a bile acid mixture (15). Studies in rats (6) and hamsters (8) have shown that dietary 18:0 alters fecal bile acid composition, suggesting that 18:0 could reduce intestinal cholesterol absorption by decreasing the proportion of hydrophobic bile acids secreted into the small intestine.

This work is an extension of a previous study in which we reported significantly reduced cholesterol absorption and increased cholesterol excretion in hamsters fed 18:0–enriched diets (16). A unique aspect of the study design is that hamsters were fed modified NIH-07 open formula diets that differed in only one single fatty acid, i.e., 18:0, palmitic acid (16:0), trans fatty acids (18:1t), oleic acid (18:1c) or linoleic acid (18:2). This design eliminated the confounding variable of multiple fatty acid difference among treatments and allowed us to isolate the metabolic effects of the specific fatty acids.

MATERIALS AND METHODS

Animals and diets. Male Syrian hamsters (~30 d old; Charles River, Wilmington, MA) weighing ~70 g were housed individually in polycarbonate cages with sawdust bedding. Hamsters were kept in an environmentally controlled room at 25°C with a 12-h light:dark cycle. They were fed a modified version of the NIH-07 open formula, cereal-based rodent diet (17,18). Each diet provided (g/100 g) 17 total fat, 24 protein, 40 carbohydrate, 4 crude fiber and 0.05 cholesterol. Details of diet formulation and composition are published elsewhere (16). Syrian hamsters were used in this study because of their well-documented similarities to human cholesterol and bile acid metabolism (19). All experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of Nebraska.

Experimental design. Hamsters (n = 64) were randomly divided into five groups. Each group contained 13 hamsters (the 18:0 group contained 12 hamsters). Hamsters were fed for 18 wk and had free access to their diets and water supply. Body weights were recorded biweekly and food intake was recorded weekly during the 18-wk study (16). The hamsters were killed in random order on four consecutive days during wk 18. Food was removed 24 h before killing, and the hamsters were killed with an overdose of ketamine hydrochloride (~250 mg/kg body). The abdomen and thorax were opened by incision and blood was collected by cardiac puncture. The liver was quickly perfused with 3 mL of saline via the hepatic portal vein to flush out residual blood. The gallbladder was removed and immediately frozen at ~70°C. The liver was removed, lightly blotted, weighed and immediately frozen.
**Plasma lipoprotein cholesterol.** Blood was collected by cardiac puncture using 10-mL syringes containing 10 mg EDTA as an anticoagulant. RBC were removed by centrifuging the blood at 1000 X g for 30 min at 4°C. Approximately 2–3 mL plasma was recovered from each hamster. Aprotinin (1 mg/L) and phenylmethylsulfonyl fluoride (80 mg/L) were added to the plasma to prevent proteolysis during storage. Plasma total cholesterol concentration was determined enzymatically (20). HDL cholesterol was determined enzymatically after removal of apolipoprotein B–containing lipoproteins by precipitation (21). Plasma cholesterol associated with VLDL and LDL was calculated by subtracting HDL cholesterol from total cholesterol. Previous results in our laboratory have shown that the VLDL + LDL cholesterol fraction contains primarily LDL (22).

**Liver cholesterol.** Frozen liver (~1.5 g) was minced and transferred to a glass tube on an analytical balance. Total lipids were extracted into chloroform/methanol (2:1, v/v) according to the method of Folch et al. (23). Liver lipids were solubilized in Triton X-100 and cholesterol concentration was quantitated enzymatically as previously described (20). Liver total cholesterol was measured using reagent no. 704036 from Roche Diagnostics (Indianapolis, IN) and liver free cholesterol was measured using reagent no. 274–47109 from Wako Chemicals (Richmond, VA). Liver esterified cholesterol was calculated by the difference between liver total and free cholesterol.

**Cholesterol 7α-hydroxylase activity.** Liver microsomes were made from frozen liver samples as previously described (24). Cholesterol 7α-hydroxylase (CYP7A1) activity was determined using 0.20 or 0.25 mg microsomal protein and [3H]-cholesterol, delivered in a liposome vehicle as described by Jelinek et al. (25). Liposomes were made by sonication to phosphatidylcholine and [3H]-cholesterol (molar ratio 8:1) in deionized water. Liposomes and microsomal protein were added to a reaction buffer contained 50 mmol/L Tris-base (pH 7.5), 20% glycerol, 0.06% Triton X-100, 1 mmol/L EDTA, 2 mmol/L dithiothreitol, 2 mmol/L NADP and 20 mmol/L glucose-6-phosphate. All reagents were purchased from Sigma Chemical (St. Louis, MO). The reaction mixture was preincubated at 37°C for 60 min. The reaction was initiated by adding glucose-6-phosphate dehydrogenase and was allowed to proceed at 37°C for 30 min. The reaction was stopped by quickly adding chloroform/methanol (2:1, v/v). Extracted lipids were spotted onto TLC plates and developed using a solvent system of ethyl acetate/toluene (3:1, v/v). The lipids were visualized by spraying plates with iodine vapor or X-ray film, and the spots corresponding to 7α-hydroxycholesterol (as judged by authentic standards) were scraped into a scintillation vial and counted.

**Bile acids.** Gallbladders were removed for individual bile acid analysis. Bile acids were extracted and partially purified following the method of Lockett and Gallaher (26), and reverse-phase HPLC was used to separate individual bile acids (27). Detection was achieved by use of a second column containing immobilized 3-α-hydroxysteroid dehydrogenase. A buffer containing NAD (0.1 mol/L Tris-HCl, pH 8.5, 2.7 mmol/L EDTA, 0.82 mmol/L dithiothreitol and 0.5 mmol/L NAD) was introduced by means of a tee between the first and second columns at a constant rate of 1 mL/min. NADH produced by the reaction of bile acids and NAD+ with the immobilized enzyme was detected fluorometrically. Peaks areas were calculated and bile acids quantified using detector response factors established with known standards. Bile acid distribution data were used to calculate the hydrophobicity index as described by Heuman (15). The hydrophobicity index of bile acids is a numeric representation of their hydrophilic-hydrophobic balance, which implies the ability of bile acids to solubilize less polar lipids such as cholesterol.

**Statistical analyses.** The effects of dietary fatty acid treatment were analyzed statistically using one-way ANOVA. Differences among treatment groups were assessed by the Student-Newman-Keuls multiple comparison procedure. Differences with P < 0.05 were considered significant. Statistical analyses were performed using SigmaStat (SPSS Science, Chicago, IL).

**RESULTS**

Hamsters fed 18:0 had significantly lower plasma VLDL + LDL cholesterol concentration than hamsters fed 16:0 (Table 1). Plasma HDL cholesterol concentration was not significantly affected by dietary treatment. Liver free cholesterol concentration ranged from 6.32 to 7.50 μmol/g and was highest (P < 0.05) in hamsters fed 18:1c and 18:2 compared with the other treatment groups. In contrast, liver esterified cholesterol concentration was much more variable, ranging from 0.52 μmol/g in the 18:0 group to 32.4 μmol/g in the 18:1c group (P < 0.05). Table 1 also shows CYP7A1 activity, which was significantly higher in hamsters fed 18:0 compared with hamsters fed 16:0.

The distribution of primary bile acids (mol/100 mol) in gallbladder bile was not affected by dietary fatty acids (Table 2). However, the hydrophobic secondary bile acids (i.e., deoxycholic and lithocholic acid) were significantly lower in hamsters fed 18:0, including both the glycine and taurine conjugates. (Unlike the primary bile acids, secondary bile acids are not made in the liver, but are synthesized in the gastrointestinal tract by the resident microflora.) Glycoursodeoxycholic acid, which is relatively hydrophilic, was not affected by dietary fatty acids. Taking into account all of the bile acids measured, the overall hydrophobicity index was significantly lower in the 18:0 group compared with the other groups.

**DISCUSSION**

Studies have established that dietary 18:0 reduces cholesterol absorption (9–12,16). One explanation for this phenomenon is that dietary 18:0 reduces intestinal bile acid absorption, which is consistent with the lower plasma HDL cholesterol concentration found in the current study. The results are consistent with previous findings that dietary 18:0 reduces CYP7A1 activity and bile acid synthesis, which is consistent with the lower plasma HDL cholesterol concentration found in the current study.
enon is that 18:0 alters the bile acid profile such that choles-
terol solubility in the small intestine is reduced. The present
study indicates that the composition of gallbladder bile acids
was signi-
ificantly altered by dietary 18:0, resulting in a de-
creased proportion of hydrophobic secondary bile acids.
Because cholesterol is hydrophobic, a reduction in overall hydro-
phobicity of bile may lower cholesterol solubility and thus
decrease cholesterol absorption.
In a previous study of these same hamsters, we reported a
significant reduction in cholesterol absorption with a concur-
rent increase in cholesterol excretion in the 18:0 group (16).
These observations led us to speculate that dietary 18:0 dis-
rupts micelle formation either by altering bile acid metabolism or
by physically interfering with micelle formation in the small
intestine. Regarding the latter possibility, Cohen and Carey
(28) reported that micelle “stability” and cholesterol solubility
were enhanced by the presence of unsaturated fatty acids in
micelle phospholipids, whereas the presence of 18:0 or 16:0
“destabilized” the micelle. This possibility, however, is likely of
minor significance because neither 16:0 nor 18:1 increased
cholesterol absorption (16), despite having similar physical
dimensions to 18:0. Therefore, it seems more likely that di-
ter 
ary 18:0 affects cholesterol absorption by altering bile acid metab-
olism.
Dietary 18:0 significantly decreased the proportion of sec-
ondary bile acids in the gallbladder. Similarly, Hassel et al. (8)
reported a lower percentage of secondary bile acids in feces of
hamsters fed 18:0 compared with hamsters fed diets high in
16:0, indicating that dietary 18:0 decreases the proportion of
secondary bile acids in the enterohepatic circulation. Because
18:0 fed to hamsters is absorbed less efficiently than other
dietary fatty acids (13,16,29), its abundance in the intestinal
tract could have affected the micellar populations that nor-
ma-
ally synthesize secondary bile acids. Studies in mice (30) and
rats (31) have shown that dietary fatty acids influence the
growth and localization of intestinal bacteria. The specific role
of 18:0 on micellar populations and secondary bile acid
synthesis has not been reported in hamsters or humans.
Because the secondary bile acids in hamsters are largely
hydrophobic, their diminished presence may have decreased
their ability to solubilize cholesterol. Schmidt and Gallaher
(32) reported that intestinal cholesterol solubility decreased
proportionally with increasing dietary 18:0. Further studies
should help delineate the role of hydrophobic bile acids in
cholesterol solubilization and absorption.
Hydrophobic bile acids have also been shown in rat hepato-
cytes to decrease mRNA levels for CYP7A1, whereas hy-
drophilic bile acids have no effect (33). Increased activity of
CYP7A1 in hamsters fed 18:0 would be consistent with fewer
hydrophilic bile acids returning to the liver in the enteroh-
patic circulation, although the role of hydrophilic bile acids in
hamster liver has not been reported. It seems more likely
that CYP7A1 activity in the 18:0 group increased in response
to a reduction of total bile acid returning to the liver rather
than transcriptional regulation of CYP7A1 by hydrophobic
bile acids because we reported previously that fecal total bile
acid excretion was increased in hamsters fed 18:0 (16).
The results of this study suggest that the hypocholester-
olicemic action of dietary 18:0 is mediated through its inhibitory
effect on secondary bile acid synthesis in the small intestine,
which, in turn, reduces cholesterol solubility and absorption.
Although the effect of 18:0 on cholesterol absorption has been
known for many years, the present study is the first to implicate
intestinal microflora and the production of secondary bile
acids as a possible mechanism of action. Future research should
focus on the microflora populations involved and the extent to
which dietary 18:0 alters the physical and chemical environ-
ment within the gastrointestinal tract.

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