Biliary Cholesterol and Bile Acid Excretion Do Not Increase in Hamsters Fed Cereal-Based Diets Containing Cholesterol

Guowen Cai and Timothy P. Carr

The major compensatory responses to increased cholesterol consumption are decreased cholesterol synthesis and increased cholesterol excretion through the bile either as free cholesterol or bile acids. The objective of this study was to test the hypothesis that biliary cholesterol excretion is increased in hamsters fed low levels of cholesterol reflecting normal human intake. The hypothesis was based on observations that hamsters generally resist changes in bile acid synthesis when fed large amounts of cholesterol; therefore, increased biliary cholesterol excretion represents a potentially significant pathway for elimination of excess cholesterol in this species. Hamsters were fed modified NIH-07 cereal-based diets containing 0.02%, 0.03%, and 0.05% cholesterol (0.04, 0.06, and 0.10 mg cholesterol/kcal, respectively). The primary response to increasing amounts of dietary cholesterol was downregulation of whole-body cholesterol synthesis, reduced from 3.93 ± 0.14 μmol · d^{-1} · 100 g^{-1} body weight in hamsters fed 0.02% cholesterol to 0.52 ± 0.14 μmol · d^{-1} · 100 g^{-1} in the 0.05% cholesterol group. Biliary cholesterol excretion was also slightly reduced in hamsters fed 0.05% cholesterol, whereas bile acid excretion was not altered by dietary cholesterol. Despite a pronounced downregulation of whole-body cholesterol synthesis, liver and plasma cholesterol concentrations increased in hamsters fed 0.05% cholesterol. The data indicate that increased biliary cholesterol excretion is not a major compensatory route of cholesterol excretion in hamsters consuming cholesterol. Furthermore, cholesterol added to the diet at 0.05% appears to be the approximate threshold at which compensatory mechanisms can prevent increases in liver and plasma cholesterol in male Syrian hamsters. Consequently, this species may be an appropriate animal model for "hyperresponding" individuals in the human population.
Copyright © 1999 by W.B. Saunders Company

INCREASING EVIDENCE indicates that dietary cholesterol plays a limited role in hypercholesterolemia and coronary heart disease (CHD). Two recent meta-analyses[1,2] indicate that dietary cholesterol per se has little effect on the plasma cholesterol concentration when considered on a population basis. McNamara[3] calculated that, on average, a 100-mg/d change in cholesterol intake will change the plasma total cholesterol level by 2.5 mg/dL. However, the individual response to dietary cholesterol is highly variable. About one third of the population exhibit greater changes in plasma cholesterol levels with concurrent changes in dietary cholesterol.[4,5] Because increased plasma cholesterol is a primary risk factor for CHD, understanding the mechanisms that regulate an individual's cholesterol response to the diet is important for developing appropriate and effective treatments to reduce CHD risk.

The liver plays a central role in mediating sterol movement through the body in response to dietary cholesterol. The primary pathways by which the liver eliminates excess cholesterol are conversion of cholesterol to bile acids and direct secretion into bile. The addition of cholesterol to experimental diets can also result in decreased cholesterol synthesis and low-density lipoprotein (LDL) receptor activity. In cholesterol-fed rats, the major compensatory mechanisms are decreased cholesterol synthesis and increased bile acid synthesis and excretion.[6,7] LDL receptor activity and plasma cholesterol levels are generally unaffected by dietary cholesterol in rats because of their large capacity to compensate by increasing bile acid excretion and decreasing cholesterol synthesis. Hamsters, on the other hand, have a limited capacity to increase bile acid excretion in response to dietary cholesterol,[7,8] and cholesterol synthesis can be maximally suppressed with relatively low amounts of dietary cholesterol.[7,9,10] Consequently, hamsters fed large amounts of cholesterol accumulate cholesterol in the liver and decrease LDL receptor activity, resulting in increased plasma cholesterol.[9,11-15] In this way, the hamster could serve as a model of "hyperresponding" humans, in which downregulation of cholesterol synthesis and increased bile acid output are unable to completely prevent increases in plasma cholesterol, as previously noted in some human subjects consuming cholesterol.[16,17]

In view of the excessive amounts of cholesterol typically fed to laboratory animals, this study was conducted in hamsters fed low levels of cholesterol that more closely reflect Western human diets. Our goal was to examine compensatory mechanisms to dietary cholesterol within a range where cholesterol synthesis was not fully suppressed. Because bile acid excretion is unlikely to increase in hamsters, we tested the hypothesis that biliary cholesterol excretion increases in hamsters fed low amounts of cholesterol.

MATERIALS AND METHODS

Animals and Diets

Male Syrian hamsters (Charles River, Wilmington, MA) weighing 40 to 50 g were individually housed in polycarbonate cages with a bedding of wood chips according to the Institutional Animal Care and Use Committee requirements at the University of Nebraska. All animals were housed in the same room maintained at 25°C with a 12-hour light-dark cycle for the duration of the 15-week experiment.

All hamsters were fed a modified version of the NIH-07 open-formula cereal-based rodent diet.[18,19] An open-formula cereal-based diet was chosen over a purified diet for this study to better mimic human diets while maintaining the control needed to manipulate specific constituents. The diets were prepared by Dyets (Bethlehem, PA) according to our specifications (Table 1). All diets were identical except

From the Department of Nutritional Science and Dietetics, University of Nebraska, Lincoln, NE.

Submitted June 10, 1998; accepted August 20, 1998.

Supported by the US Department of Agriculture National Research Initiative Competitive Grant No. 9601088 and the Nebraska Agriculture Research Division (Journal Series No. 12261).

Address reprint requests to Timothy P. Carr, PhD, Nutritional Science and Dietetics, University of Nebraska, 316 Ruth Leverton Hall, Lincoln, NE 68583.

Copyright © 1999 by W.B. Saunders Company

0026-0495/99/4803-0023$10.00/0

Table 1. Modified NIH-07 Open-Formula Diet Composition (g/Kg)

Ingredient	Original NIH-07 Diet	Modified NIH-07 Diets 0.02%C	0.03%C	0.05%C
Dried skim milk	50	50	50	50
Fish meal	100	100	100	100
Soybean meal	120	120	120	120
Alfalfa meal	40	40	40	40
Cornmeal	30	30	30	30
Ground #2 yellow shelled corn	235	185	185	185
Ground hard winter wheat	230	180	180	180
Wheat middlings	100	75	75	75
Dried brewer's yeast	20	20	20	20
Dried molasses	15	15	15	15
Fat blend*	25	150	150	150
Crystalline cholesterol	—	—	0.1	0.3
Salt mix	30	30	30	30
Vitamin mix	5	5	5	5

NOTE. The original NIH-07 diet was modified to contain 15% fat by weight and additional cholesterol. To compensate for the increase in fat, the proportion of ground shelled corn, ground hard winter wheat, and wheat middlings was reduced. The percent cholesterol in the modified diets was 0.02%, 0.03%, and 0.05%. Cholesterol in the 0.02%C diet was contributed by dried skim milk and fish meal; crystalline cholesterol was added to the 0.03%C and 0.05%C diets.

*Fat blend contained 100 parts refined palm oil, 12 parts shea butter, and 2 parts sunflower oil. The resulting fatty acid composition was 0.8% lauric, 1.2% myristic, 41.8% palmitic, 8.7% stearic, 37.8% oleic, 8.8% linoleic, 0.5% linolenic, and 0.4% other fatty acids. The polyunsaturated/saturated ratio of the fat blend was 0.22.

for the concentration of cholesterol. The original NIH-07 diet required soybean oil to be added at 2.5% by weight of diet. For this study, the amount of fat was increased to 15% by weight using a specific blend of 100 parts refined palm oil, 12 parts shea butter, and 2 parts sunflower oil. Refined palm oil was purchased from Welch, Holme & Clark (Newark, NJ) and shea butter from ABITEC (Janesville, WI), and sunflower oil was purchased at a local grocery store. The cholesterol content of the modified diets was verified by gas chromatography using a DB-1 capillary column (J & W Scientific, Folsom, CA). The energy content of each diet was 4.75 kcal/g (19.8 kJ/g).

Experimental Design

Forty-eight hamsters were randomly divided into three groups (n = 16) and given free access to food and water. The animals were allowed to become accustomed to their environment for 3 days before the start of the experiment. Body weight and food intake were recorded weekly during the 15-week study to monitor the effectiveness and appropriateness of the modified NIH-07 diet in male Syrian hamsters (Fig 1). The mean body weight appeared to stabilize at about week 9 in each treatment, with no further weight gain through week 15. Food intake decreased concomitantly with weight gain and maintenance of stable body weight. All animals in the study appeared healthy by daily visual inspection throughout the study, and no pathological indications were detected at necropsy. The modified NIH-07 diet used in this study appeared appropriate and satisfactory for promoting growth and health maintenance in male Syrian hamsters.

The mean stable body weight of each animal was calculated from weekly values collected at weeks 10 to 15. The mean body weight for the 0.02% cholesterol (0.02% C), 0.03%C, and 0.05%C groups were 148, 151, and 147 g, respectively. The mean daily food intake for each animal was calculated from weekly determinations during weeks 1 to 15. The mean daily food intake for the 0.02%C, 0.03%C, and 0.05%C groups was 8.81, 8.81, and 8.77 g/d, respectively. No significant differences in body weight or food intake were detected among treatment groups.

Feces were collected for 7 days during week 9 to determine fecal bile acid and neutral steroid excretion. The mean daily fecal output for the 0.02%C, 0.03%C, and 0.05%C groups was 1.42, 1.35, and 1.46 g/d, respectively. Cholesterol absorption efficiency was measured during week 10 of the study. The animals were killed in random order on 3 consecutive days during week 16. Food was removed 24 hours before termination, and the animals were given an overdose of ketamine hydrochloride (~25 mg/100 g body weight). The abdomen and thorax were opened by incision, and blood was collected by cardiac puncture. The liver was removed and its weight recorded. Details of each procedure are described in the following sections.

Cholesterol Absorption Efficiency

Cholesterol absorption efficiency was measured by simultaneous oral administration of [^{3}H]-β-sitostanol and [^{14}C]-cholesterol as previously described.[20] β-Sitostanol has been shown to be essentially nonabsorbed in the intestinal tract of hamsters,[21] thus serving as a reference compound for cholesterol absorption. Radiolabeled sterols were purchased from American Radiolabeled Chemicals (St Louis, MO). On 2 consecutive days, hamsters received 50 μL vegetable oil containing approximately 2 μCi [^{3}H]-β-sitostanol and 1 μCi [^{14}C]-cholesterol. Animals were provided food immediately after the vegetable oil so that the dose was mixed with their usual diet. Feces were collected 2, 4, and 6 days following the initial dose. Fecal samples from each 2-day collection were saponified, and the lipids were extracted into hexane. The hexane was transferred to 7-mL scintillation vials and the samples were placed under an UV lamp (short wavelength 254 nm) for 3 to 5 days to eliminate any residual color. This "bleaching" step was necessary, as previous experience indicated that the yellow-green color of fecal lipid extracts may cause quenching during scintillation counting. After decolorization, any remaining hexane was evaporated and 5

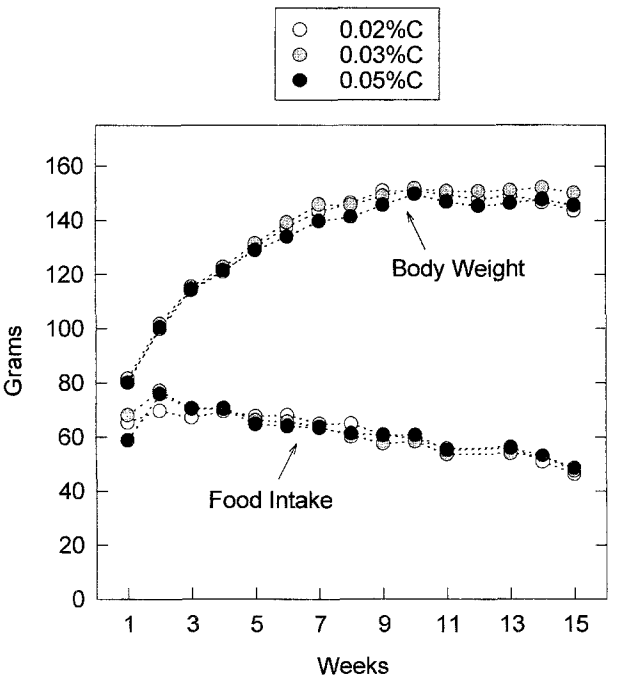

Fig 1. Weekly body weight and food intake of hamsters fed cereal-based diets containing 0.02%, 0.03%, and 0.05% cholesterol (C). Animals were allowed free access to food and water. No significant differences were detected among the treatment groups throughout the 15-week study.

mL Bio-Safe II scintillation cocktail (Research Products International, Mt Prospect, IL) was added. Samples were counted on a Packard (Meriden, CT) Tri-Carb Scintillation Counter using a dual-channel method for simultaneous counting of [^{3}H] and [^{14}C]. Cholesterol absorption efficiency was calculated as a percentage from the ratio of the two radiolabels in the dose and feces using the following equation: percent cholesterol absorption = [(^{14}C/^{3}H in dose − ^{14}C/^{3}H in feces)/ ^{14}C/^{3}H in dose] × 100.

Absorption efficiency was calculated as the average of each 2-day fecal collection.

Plasma Cholesterol

Blood was collected by cardiac puncture using 10-mL syringes containing 10 mg EDTA as anticoagulant. Red blood cells were removed by centrifuging the blood at 1,000 × g for 30 minutes at 4°C. Approximately 2 to 4 mL plasma was recovered from each hamster. Aprotinin (1 μg/mL) and phenylmethylsulfonyl fluoride (80 μg/mL) were added to the plasma as preservatives. The plasma total cholesterol concentration was determined enzymatically (Boehringer Mannheim, Indianapolis, IN) using the microtiter plate method of Carr et al.[22]

Liver Lipids

The liver was perfused with saline through the portal vein before excision to eliminate contamination by plasma lipids. The livers were blotted, weighed, and immediately frozen at −70°C. Approximately 1.5 g frozen liver was minced and transferred to a tube on the analytical balance. Liver lipids were extracted into chloroform:methanol (2:1 vol/vol) according to the method of Folch et al.[23] Total cholesterol, free cholesterol, and triglycerides were quantified enzymatically using the procedure of Carr et al.[22] Total cholesterol and triglyceride reagents were purchased from Boehringer Mannheim, and the free cholesterol reagent was purchased from Wako Chemicals USA, Inc. (Richmond, VA). Liver esterified cholesterol was determined as total cholesterol minus free cholesterol. Liver cholesteryl esters were calculated as the esterified cholesterol × 1.67 to account for the increased molecular mass contributed by the fatty acids. Liver phospholipid was determined by quantifying inorganic phosphorus using the method of Morrison.[24]

Liver cholesteryl ester fatty acid composition was determined by first separating liver cholesteryl esters from other liver lipids using thin-layer chromatography (TLC). Cholesteryl esters were extracted from the TLC plates using chloroform:methanol (2:1 vol/vol), and fatty acid methyl esters were prepared according to the method of Metcalfe et al.[25] Cholesteryl ester fatty acid distribution was determined by gas chromatography using a 0.25-mm × 100-m CP-Sil-88 capillary column (Chrompack, Raritan, NJ) under the following conditions: initial temperature 180°C for 20 minutes, increased to 250°C at 5°C/min, injector temperature 270°C, flame ionization detector (FID) temperature 300°C, helium carrier gas, and split ratio of 50:1.

Fecal Bile Acids

Approximately 100 mg of ground, nonradioactive feces were placed in tubes and 0.7 mL deionized water was added, followed by 10 mL chloroform:methanol (2:1 vol/vol). After 30 minutes, 2 mL 0.88% KCl was added and mixed, and the sample was centrifuged at 1,000 × *g* for 15 minutes to separate the phases. The upper phase containing bile acids was quantitatively transferred to a clean tube, and the lower phase was washed once by adding fresh upper phase (chloroform:methanol:water 3:48:47). The sample was gently mixed and centrifuged as before. The upper phase from the wash step was added to the upper phase from the first extraction. An aliquot of upper phase was transferred to a 1-cm diameter round cuvette, and the solvent was evaporated at 50°C under a stream of nitrogen.

Total bile acids in the cuvette were quantified by first dissolving the bile acids in 0.1 mL methanol. Exactly 3.5 mL incubation buffer was added to the samples and mixed. The incubation buffer contained 0.2 mg β-NAD (Sigma Chemical, St Louis, MO) per 1 mL 0.05-mmol/L CAPS buffer (pH 10.8) and was prepared immediately prior to use. The background absorbance of the samples was determined at 340 nm. The reaction was initiated by adding 0.4 mL 3α-hydroxysteroid dehydrogenase (0.75 U/mL 0.01-mol/L phosphate buffer, pH 7.2). Hydroxysteroid dehydrogenase was purchased from Sigma Chemical. The samples were incubated at 37°C for 30 minutes. Absorbance was read again at 340 nm to determine the concentration of NADH. The concentration of total bile acids was calculated by the difference of the two absorbance readings (accounting for the dilution of 0.4 mL enzyme solution) compared against a calibration curve using cholic acid standards in methanol.

Fecal Neutral Steroids

Approximately 50 mg ground feces were extracted into methanol: chloroform (2:1 vol/vol) containing 10 μg/mL 5α-cholestane as an internal standard. The lower-phase solvent was evaporated, and the samples were saponified in 2 mL 1N methanolic KOH for 1 hour at 50°C. After addition of 2 mL deionized water, the nonsaponifiable lipids were extracted into 5 mL hexane. The hexane was evaporated under nitrogen, and the sterols were derivatized by adding 100 μL pyridine, followed by 50 μL Sylon BTZ (Supelco, Bellefonte, PA). The samples were allowed to stand for 30 minutes at room temperature, and the reaction was stopped by placing the samples on ice. Fecal neutral steroids were quantified by gas chromatography using a 0.25-mm × 15-m DB-1 capillary column (J & W Scientific) under the following conditions: initial temperature 190°C for 1 minute, increased to 220°C at 3°C/min, injector temperature 270°C, FID temperature 300°C, helium carrier gas, and split ratio of 50:1.

Statistical Analyses

After confirming that the data were normally distributed, one-way ANOVA was used to compare experimental endpoints of the three treatment groups. Statistical differences among mean values were consider significant at a P level less than .05 as determined by the Tukey multiple-comparison procedure. Associations between variables were determined by Pearson product-moment correlation analysis. All statistical analyses were performed on a personal computer using SigmaStat (SPSS, Chicago, IL).

RESULTS

The influence of low cholesterol intake on the liver weight, liver cholesterol, and plasma cholesterol concentration is presented in Table 2. Increasing the cholesterol content of the diet from 0.02% to 0.03% did not significantly increase the cholesterol concentration of the liver or plasma. However, increasing dietary cholesterol from 0.03% to 0.05% resulted in increased ($P < .05$) cholesterol in both the liver and plasma. There was a

Table 2. Liver Weight, Liver Cholesterol Concentration, and Plasma Cholesterol Concentration in Hamsters Fed Cholesterol-Containing Diets

Group	Liver Weight (g)	Liver Cholesterol (mg/g)	Plasma Cholesterol (mg/dl)
0.02%C	4.14 ± 0.15	3.05 ± 0.08[a]	119 ± 4[a]
0.03%C	4.45 ± 0.18	3.31 ± 0.09[a]	120 ± 4[a]
0.05%C	4.77 ± 0.21	4.18 ± 0.36[b]	158 ± 5[b]

NOTE. Values are the mean ± SEM (n = 16 for each treatment). Means within the same column with different superscripts are significantly different ($P < .05$) by 1-way ANOVA and the Tukey multiple-comparison test. Liver and plasma samples were collected during week 16 of the study.

trend for increasing liver weight with increasing dietary cholesterol, but it was not statistically significant ($P = .057$).

Table 3 represents whole-body cholesterol flux in hamsters fed the low-cholesterol diets. The data have been normalized to 100 g body weight. Fecal bile acid and neutral steroid excretion represent the primary sources of cholesterol output from the body. The 0.05%C group exhibited greater ($P < .05$) neutral steroid output compared with the 0.02%C group, whereas bile acid output was not significantly affected by dietary cholesterol. Daily cholesterol intake was calculated based on mean food intake and then subtracted from total cholesterol output (bile acids + neutral steroids) to estimate whole-body cholesterol synthesis. Increasing the amount of cholesterol in the diet caused a significant reduction in whole-body cholesterol synthesis.

To further examine cholesterol movement through the body, cholesterol absorption efficiency was determined. Varying the amount of dietary cholesterol within the range of 0.02% to 0.05% in this study did not significantly alter absorption efficiency (Table 4). However, the mass of dietary cholesterol absorbed and not absorbed (ie, excreted in feces) significantly increased as the amount of cholesterol in the diet increased. These data were calculated from the cholesterol absorption efficiency and total cholesterol intake measured in each animal. Conversely, the excretion rate of cholesterol derived from bile was significantly lower in the 0.05%C group compared with the other groups.

Many of the compensatory mechanisms that are sensitive to dietary cholesterol are regulated through changes in liver cholesterol. Figure 2 illustrates the relationship between the mass of dietary cholesterol absorbed per day and the liver cholesterol content ($r = .996$, $P = .056$). Within the range of cholesterol consumed in the present study, increased cholesterol absorption resulted in cholesterol accumulation in the liver. Figure 3 further illustrates cholesterol excretion and whole-body synthesis as a function of liver cholesterol content. The most sensitive response to increasing liver cholesterol was a downregulation of cholesterol synthesis ($r = .996$, $P = .055$). Biliary cholesterol excretion also decreased in a linear fashion with increasing liver cholesterol content ($r = .999$, $P = .021$), while bile acid excretion was essentially unchanged.

Table 3. Daily Cholesterol Output and Input (μmol · d^{-1} · 100 g^{-1}) in Hamsters Fed Cholesterol-Containing Diets

	Cholesterol Output		Cholesterol Input	
Group	Fecal Bile Acids	Fecal Neutral Steroids	Dietary Cholesterol	Whole-Body Synthesis*
0.02%C	2.00 ± 0.05	5.32 ± 0.13[a]	3.40 ± 0.04[a]	3.93 ± 0.14[a]
0.03%C	2.01 ± 0.12	5.69 ± 0.21[a,b]	4.85 ± 0.07[b]	2.85 ± 0.26[b]
0.05%C	2.30 ± 0.10	6.23 ± 0.14[b]	8.06 ± 0.11[c]	0.52 ± 0.14[c]

NOTE. Values are the mean ± SEM (n = 16 for each treatment). Means within the same column with different superscripts are significantly different ($P < .05$) by 1-way ANOVA and the Tukey multiple-comparison test. Fecal samples were collected during week 9 of the study.

*Determined as the sum of daily steroid output (bile acids + neutral steroids) minus daily cholesterol intake.

Table 4. Daily Cholesterol Absorption and Excretion (μmol · d^{-1} · 100 g^{-1}) in Hamsters Fed Cholesterol-Containing Diets

Group	Cholesterol Absorption Efficiency (%)	Dietary Cholesterol Absorbed* (μmol · d^{-1} · 100 g^{-1})	Dietary Cholesterol Excreted† (μmol · d^{-1} · 100 g^{-1})	Biliary Cholesterol Excreted‡ (μmol · d^{-1} · 100 g^{-1})
0.02%C	57.0 ± 1.2	1.94 ± 0.05[a]	1.46 ± 0.04[a]	3.86 ± 0.12[a]
0.03%C	57.4 ± 1.5	2.78 ± 0.08[b]	2.07 ± 0.09[b]	3.63 ± 0.24[a]
0.05%C	56.7 ± 0.9	4.57 ± 0.10[c]	3.49 ± 0.08[c]	2.74 ± 0.14[b]

NOTE. Values are the mean ± SEM (n = 16 for each treatment). Means within the same column with different superscripts are significantly different ($P < .05$) using 1-way ANOVA and the Tukey multiple-comparison test. Cholesterol absorption efficiency was determined during week 10 of the study.

*Calculated by multiplying cholesterol absorption efficiency and total cholesterol intake.

†Calculated by subtracting dietary cholesterol absorbed from total cholesterol intake.

‡Calculated by subtracting dietary cholesterol excreted from fecal total neutral steroids excreted.

DISCUSSION

The present study was performed to determine the relative contribution of biliary cholesterol output as a compensatory mechanism in response to dietary cholesterol. Hamsters were fed cereal-based diets containing 0.02% to 0.05% cholesterol (0.04 to 0.10 mg/kcal). Increasing the amount of dietary cholesterol from 0.02% to 0.05% resulted in an accumulation of cholesterol in the liver and a decrease in whole-body cholesterol synthesis. When plotted against the liver cholesterol concentration, cholesterol synthesis decreased in a linear fashion and appeared to be the most sensitive metabolic response to small

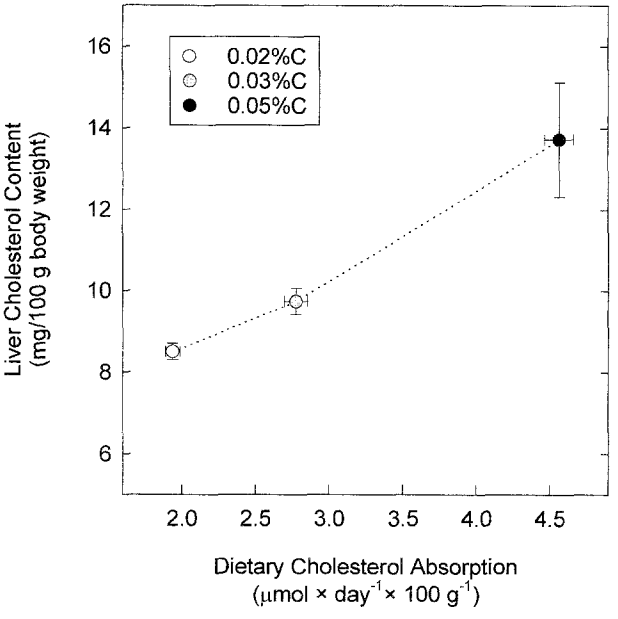

Fig 2. Relationship between dietary cholesterol mass absorption and liver cholesterol content in hamsters fed cereal-based diets containing 0.02%, 0.03%, and 0.05% cholesterol (C). Data are normalized to 100 g body weight. Cholesterol absorption was determined during week 10 of the study, and liver cholesterol content was measured in samples collected during week 16.

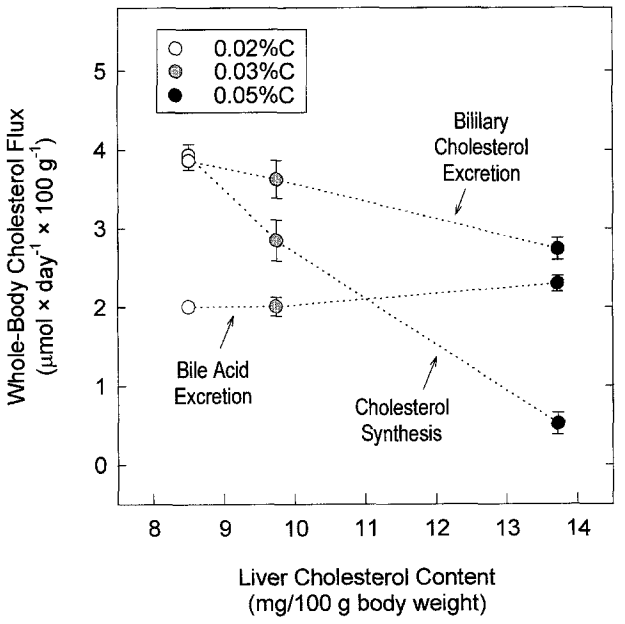

Fig 3. Cholesterol excretion and whole-body synthesis as a function of liver cholesterol content in hamsters fed cereal-based diets containing 0.02%, 0.03%, and 0.05% cholesterol (C). Data are normalized to 100 g body weight. Cholesterol flux measurements were made from fecal samples collected during week 9. Liver cholesterol content was measured in samples collected during week 16.

changes in dietary cholesterol. Biliary cholesterol excretion slightly decreased with cholesterol intake and was inversely related to the liver cholesterol concentration. Thus, the data suggest that biliary cholesterol excretion is not a quantitatively important pathway of cholesterol elimination in hamsters fed low levels of cholesterol.

The amount of dietary cholesterol fed to hamsters was chosen to reflect the normal human intake. The estimated daily cholesterol and energy intake of US adults provides approximately 0.1 mg cholesterol/kcal.[26,27] The three diets used in the present study contained 0.02%, 0.03%, and 0.05% cholesterol by weight, thus providing 0.04, 0.06, and 0.10 mg cholesterol/kcal, respectively. This amount translates into approximately 144, 216, and 360 mg/d for the average adult consuming 3,600 kcal/d.[26] The amount of cholesterol fed to the hamsters was calculated on an energy intake basis rather than a body weight basis because of the inverse relationship between body size and whole-body cholesterol turnover.[10] A further rationale for selecting dietary cholesterol levels of 0.05% (0.1 mg/kcal) or less is that cholesterol synthesis may be maximally suppressed in hamsters at a cholesterol intake at or above 0.05% of diet.[7,9,13] When cholesterol synthesis is fully suppressed by excess dietary cholesterol, the predictable outcome is accumulation of cholesterol in the liver and plasma. However, increases in liver and plasma cholesterol due to unrealistically high cholesterol intake may not be relevant with regard to clinical human outcomes. In the present study, cholesterol synthesis was essentially zero in three of 16 animals fed 0.05% cholesterol, while the group mean was 0.5 $\mu mol \cdot d^{-1} \cdot 100\ g^{-1}$ body weight. Under the current experimental conditions, 0.05% dietary cholesterol appeared to approach the threshold at which whole-body cholesterol synthesis was maximally suppressed.

The range of values for whole-body cholesterol synthesis determined by sterol balance in the present study are consistent with reported values using the tritiated water method of Dietschy et al.[10] Using the tritiated water method, whole-body synthesis in Syrian hamsters consuming very low amounts of cholesterol and triacylglycerol was reported to be approximately 7 $\mu mol \cdot d^{-1} \cdot 100\ g^{-1}$ body weight.[10] In the present study, whole-body cholesterol synthesis in hamsters fed 0.02% cholesterol and 15% triacylglycerol was about 4 $\mu mol \cdot d^{-1} \cdot 100\ g^{-1}$ hamster, suggesting partial suppression of synthesis at the lowest level of dietary cholesterol.

Contrary to our prediction, biliary cholesterol excretion slightly decreased with increasing cholesterol intake. Unlike the highly regulated pathways involving cholesterol and bile acid synthesis, movement of free cholesterol into the bile is poorly understood. While biliary cholesterol originates from existing and newly synthesized cholesterol, there is a preference for newly synthesized cholesterol to be secreted into bile.[28] The newly synthesized cholesterol appears dependent on sterol carrier protein-2 for rapid transport into bile.[29] It is likely that the decreased biliary cholesterol excretion observed in our study reflects decreased availability of newly synthesized cholesterol. Because cholesterol feeding reduced whole-body synthesis, the data support the proposition that newly synthesized cholesterol is preferentially secreted into bile.

Bile acid excretion and, presumably, synthesis was not significantly altered in hamsters consuming the low amounts of cholesterol used in this study. Turley et al[8] recently reported in hamsters that the addition of 0.12% cholesterol to cereal-based diets modestly increased bile acid excretion about 38%. Further increasing the cholesterol content of the diet to 1.0% increased liver cholesterol fivefold, but bile acid excretion was not further increased.[8] Similar results were reported by Imaizumi et al[12] and Cohen et al,[14] in which increasing the cholesterol content of the diet to 0.2% did not increase bile acid excretion. Horton et al[7] recently suggested that the inability of the hamster to increase bile acid synthesis with increased dietary cholesterol intake is due to an inherently low basal synthetic rate. The consistency of the data supports this conclusion.

Increasing dietary cholesterol from 0.02% to 0.03% did not significantly alter the liver cholesterol concentration, bile acid excretion, biliary cholesterol excretion, or plasma total cholesterol concentration, whereas whole-body cholesterol synthesis was significantly decreased. This finding suggests that within the normal range of human consumption, small changes in dietary cholesterol can be compensated for by changes in cholesterol synthesis, thus preventing increases in plasma cholesterol. However, further increasing dietary cholesterol to 0.05% resulted in significantly higher liver and plasma cholesterol concentrations despite a large reduction in whole-body cholesterol synthesis. Consumption of cholesterol at the highest level used in this study (0.1 mg/kcal) represents the estimated average adult cholesterol intake in the United States.[26,27] These results suggest that cholesterol consumption at the normal

levels within the US population could influence the plasma cholesterol concentration in some people (ie, hyperresponders) by exceeding the capacity to compensate by decreasing cholesterol synthesis and increasing bile acid excretion. An inability to increase bile acid output in response to dietary cholesterol has been reported in humans[16,17,30] and nonhuman primates,[31] thus supporting the use of the hamster as a relevant model of sterol metabolism.

REFERENCES

1. Clarke R, Frost C, Colins R, et al: Dietary lipids and blood cholesterol: Quantitative meta-analysis of metabolic ward studies. BMJ 314:112-117, 1997
2. Howell WH, McNamara DJ, Tosca MA, et al: Plasma lipid and lipoprotein responses to dietary fat and cholesterol: A meta-analysis. Am J Clin Nutr 65:1747-1764, 1997
3. McNamara DJ: Cholesterol intake and plasma cholesterol: An update. J Am Coll Nutr 16:530-634, 1997
4. Katan MB, Beynen AC, de Vries JHM, et al: Existence of consistent hypo- and hyperresponders to dietary cholesterol in man. Am J Epidemiol 123:221-234, 1986
5. McNamara DJ, Kolb R, Parker TS, et al: Heterogeneity of cholesterol homeostasis in man: Response to changes in dietary fat quality and cholesterol quantity. J Clin Invest 79:1729-1739, 1987
6. Russell DW, Setchell KDR: Bile acid biosynthesis. Biochemistry 31:4737-4749, 1992
7. Horton JD, Cuthbert JA, Spady DK: Regulation of hepatic 7α-hydroxylase expression and response to dietary cholesterol in the rat and hamster. J Biol Chem 270:5381-5387, 1995
8. Turley SD, Spady DK, Dietschy JM: Regulation of fecal bile acid excretion in male golden Syrian hamsters fed a cereal-based diet with and without added cholesterol. Hepatology 25:797-803, 1997
9. Spady DK, Dietschy JM: Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. J Clin Invest 81:300-309, 1988
10. Dietschy JM, Turley SD, Spady DK: Role of liver in the maintenance of cholesterol and low density lipoprotein homeostasis in different animal species, including humans. J Lipid Res 34:1637-1659, 1993
11. Spady DK, Turley SD, Dietschy JM: Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. J Lipid Res 26:465-472, 1985
12. Imaizumi K, Abe K, Kuroiwa C, et al: Fat containing stearic acid increases fecal neutral steroid excretion and catabolism of low density lipoproteins without affecting plasma cholesterol concentration in hamsters fed a cholesterol-containing diet. J Nutr 123:1693-1702, 1993
13. Dietschy JM, Woollett LA, Spady DK: The interaction of dietary cholesterol and specific fatty acids in the regulation of LDL receptor activity and plasma LDL-cholesterol concentrations. Ann NY Acad Sci 676:11-26, 1993
14. Cohen BI, Ayyad N, Mikami T, et al: Effects of dietary fat and fatty acids on sterol balance in hamsters. Lipids 29:503-508, 1994
15. Hassel CA, Mensing EA, Gallaher DD: Dietary stearic acid reduces plasma and hepatic cholesterol concentrations without increasing bile acid excretion in cholesterol fed hamsters. J Nutr 127:1148-1155, 1997
16. Nestel PJ, Poyser A: Changes in cholesterol synthesis and excretion when cholesterol intake is increased. Metabolism 25:1591-1599, 1976
17. Lin DS, Connor WE: The long term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption, and the sterol balance in man: The demonstration of feedback inhibition of cholesterol biosynthesis and increased bile acid excretion. J Lipid Res 21:1042-1052, 1980
18. Knapka JJ, Smith KP, Judge FJ: Effect of open and closed formula rations on the performance of three strains of laboratory mice. Lab Anim Sci 24:480-487, 1974
19. American Institute of Nutrition: Report of the American Institute of Nutrition ad hoc committee on standards for nutritional studies. J Nutr 107:1340-1348, 1977
20. Borgstrom B: Quantification aspects of the intestinal absorption and metabolism of cholesterol and β-sitosterol in the rat. J Lipid Res 9:473-481, 1968
21. Turley SD, Daggy BP, Dietschy JM: Psyllium augments the cholesterol-lowering action of cholestyramine in hamsters by enhancing sterol loss from the liver. Gastroenterology 107:444-452, 1994
22. Carr TP, Andersen CJ, Rudel LL: Enzymatic determination of triglyceride, free cholesterol, and total cholesterol in tissue lipid extracts. Clin Biochem 26:39-42, 1993
23. Folch J, Lees M, Sloane Stanley GH: A simple method for the isolation and purification of total lipids form animal tissues. J Biol Chem 224:497-509, 1957
24. Morrison WR: A fast, simple and reliable method for the micro-determination of phosphorus in biological materials. Anal Biochem 7:218-224, 1964
25. Metcalfe LD, Schmitz AA, Pelka JR: Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal Chem 38:514-515, 1966
26. Gerrior SA, Zizza C: Nutrient Content of the U.S. Food Supply, 1909-1990. Hyattsville, MD, US Department of Agriculture, Home Economics Research Report No. 52, 1994
27. McDowell MA, Briefel RR, Alaimo K, et al: Energy and Macronutrient Intakes of Persons Ages 2 Months and Over in the United States: Third National Health and Nutrition Examination Survey, Phase I, 1988-91. Advance Data From Vital and Health Statistics, No. 255. Hyattsville, MD, National Center for Health Statistics, 1994
28. Robins SJ, Fasulo JM, Collins MA, et al: Evidence for separate pathways of transport of newly synthesized and preformed cholesterol into bile. J Biol Chem 260:6511-6513, 1985
29. Puglielli L, Rigotti A, Amigo L, et al: Modulation of intrahepatic cholesterol trafficking: Evidence by in vivo antisense treatment for the involvement of sterol carrier protein-2 in newly synthesized cholesterol transport into rat bile. Biochem J 317:681-687, 1996
30. Maranhao RC, Quintao EC: Long term steroid metabolism balance studies in subjects on cholesterol-free and cholesterol-rich diets: Comparison between normal and hypercholesterolemic individuals. J Lipid Res 24:167-173, 1983
31. Rudel L, Deckelman C, Wilson M, et al: Dietary cholesterol and downregulation of cholesterol 7α-hydroxylase and cholesterol absorption in African green monkeys. J Clin Invest 93:2463-2472, 1994