Effect of ursodeoxycholic acid on cholesterol absorption and metabolism in humans

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Abstract Qualitative and quantitative changes in intraluminal bile acid composition may alter cholesterol absorption and synthesis and LDL receptor expression. In a randomized crossover design outpatient study, 12 adults aged 24–36 years took 15 mg/kg/day ursodeoxycholic acid (UDCA) or no bile acid supplement (control) for 20 days while being fed a controlled diet (AHA Step II). A liquid meal of defined composition was then given and luminal samples collected. Cholesterol absorption and cholesterol fractional synthetic rate (FSR) were assessed by stable isotopic methods. With UDCA treatment, bile was enriched significantly ($P < 0.0001$) to $40.6 \pm 2.6\%$ (mean $\pm$ SEM) compared with $2.2 \pm 2.6\%$ for controls. Regardless, plasma total, HDL, and LDL cholesterol were unchanged with UDCA treatment. Intraluminal cholesterol solubilized in the aqueous phase during the entire collection was decreased ($P = 0.012$) in UDCA-treated subjects (101.0 ± 7.2 mg/ml/120 min) compared with controls (132.5 ± 7.2 mg/ml/120 min.). Percent micellar cholesterol was increased in UDCA-treated versus controls after meal ingestion. No changes were found in cholesterol absorption, FSR, or LDL receptor mRNA with UDCA treatment compared with controls.—Thus, despite marked enrichment in luminal bile with UDCA and decreased cholesterol solubilization, no differences in cholesterol absorption or metabolism are found when diet and genetic differences in absorption are carefully controlled.—Woollett, L. A., D. D. Buckley, L. Yao, P. J. H. Jones, N. A. Granholm, E. A. Tolley, and J. E. Heubi. **Effect of ursodeoxycholic acid on cholesterol absorption and metabolism in humans.** *J. Lipid Res.* 2003. **44:** 935–942.

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Cholesterol metabolism plays a pivotal role in the development of atherosclerosis. In mammals, relatively constant plasma cholesterol concentrations are regulated by several mechanisms, including intestinal cholesterol absorption, rates of cholesterol synthesis, LDL receptor activity, secretion of cholesterol into bile, and conversion of cholesterol into bile acids. In most nonhuman primates and humans, plasma cholesterol concentrations respond variably to challenges with dietary cholesterol, which has stimulated further investigations into the mechanisms of plasma cholesterol concentration regulation. Though it may have a tremendous impact upon plasma cholesterol levels, cholesterol absorption is the most understudied of the factors that potentially regulate circulating cholesterol levels, as the average North American woman and man consume 240 mg to 360 mg cholesterol per day, respectively, mostly as free cholesterol (1, 2). Thus, a significant difference in the amount of cholesterol entering the body can occur based upon the extent of cholesterol absorbed by the intestine.

The extent of cholesterol absorbed by the intestine is governed by intraluminal and mucosal events. The major intraluminal events essential to intestinal fat and sterol absorption include digestion and solubilization of lipolytic products. For the small proportion of dietary cholesterol present in esterified forms (8–19%) (2), pancreatic cholesterol esterase hydrolyzes the esters, producing free cholesterol and fatty acid. Dietary and biliary cholesterol is emulsified with triglycerides and other undigested lipids in oily droplets. Intraluminal fat digestion is affected by several lipolytic enzymes, thereby generating a multilamellar crystalline phase. The liquid crystalline phase, comprised of vesicles, provides substrates for micelles. The micelles and/or monomeric cholesterol crosses the unstirred

Abbreviations: CRC, Clinical Research Center; FSR, fractional synthetic rate; IRMS, isotope ratio mass spectrometry; RBC, red blood cell; UDCA, ursodeoxycholic acid.

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water layer followed by movement of monomeric cholesterol across the brush border membrane either through passive diffusion or by a carrier-mediated mechanism (3–5). Thus, the ability to form micelles, as occurs in vitro with bile acids of different compositions, could have a dramatic effect on cholesterol absorption.

It has been suggested for years that the amount of exogenous cholesterol that enters the body plays a major role in the formation of hypercholesterolemia. For example, dietary cholesterol directly correlates with plasma total and LDL cholesterol concentrations and can impact upon the development of atherosclerosis (6). Additionally, the variations that occur in plasma cholesterol levels within various populations have been suggested to occur at the level of cholesterol absorption (7–10). Most convincing, however, is that when cholesterol absorption is directly inhibited, plasma cholesterol concentrations decrease (11).

The purpose of the current investigation was to examine the role of intraluminal bile acid composition on intraluminal micelle formation, and ultimately cholesterol absorption and synthesis and circulating levels. To accomplish this goal, adults were fed diets with or without supplementation with ursodeoxycholic acid (UDCA), a bile acid whose micelle formation is less efficient than the primary bile acids in vitro (12). We hypothesized that quantitative and qualitative alterations in intraluminal bile acid composition would influence micelle formation and, therefore, the efficiency of cholesterol absorption, which in turn would vary inversely with cholesterol fractional synthetic rate (FSR), HMG-CoA reductase, and LDL-receptor mRNA levels.

MATERIALS AND METHODS

Subjects

Subjects were healthy adult Caucasian males and females, ages 24–36, recruited by advertisement and screened for any evidence of cardiovascular, pulmonary, renal, or gastrointestinal, hepatobiliary disease, or soy allergy. Subjects were excluded with diabetes mellitus, chronic usage of any medication, including oral contraceptives, and plasma total and LDL cholesterol exceeding 200 mg/dl and 120 mg/dl, respectively. Subjects were screened for apolipoprotein (apo) A-IV and apoE genotypes. Only subjects with apoA-IV 1/1 and apoE 3/3 genotypes were included. Females of childbearing age could not be pregnant, had to be using barrier contraception, and could not be planning pregnancy during the course of this study. The Institutional Review Boards of the University of Cincinnati and the Children's Hospital Medical Center approved the study.

Study design

After determining that they fit screening criteria (see above) and a complete verbal explanation of the study, subjects signed a consent form describing the study. To be eligible to enroll, subjects had to score at least 75% on a test that determined their understanding of the study. The Clinical Research Center (CRC) research dietitian reviewed a 3 day diet diary, and nutrient analysis was calculated for total calories, fat (saturated, unsaturated), and cholesterol. Based upon caloric intake from the diet diary, initial weight, and activity levels, the CRC dietitian estimated to-

tal calories to maintain steady weight, and diet menus were made on a 3 day rotating schedule. The diets were designed to contain 220 mg cholesterol/day with 30% calories as fat (15% monosaturated) with a P-S ratio of 0.5. Subjects consumed diets prepared and provided by the CRC staff. After Day 0, subjects came to the CRC daily or every third day to pick up prepared diets that had been frozen after preparation. Uneaten food and bile acid pills were returned. Each week while on the study, subjects were weighed in light clothing without shoes using a Detecto® digital balance to ensure maintenance of basal weight. On Day 0, subjects came to the CRC after an overnight fast and had blood drawn for plasma total, HDL, and LDL cholesterol, and triglyceride concentrations, and monocyte HMG-CoA reductase and LDL-receptor mRNA levels. Using a randomized crossover design, subjects received either UDCA (15 mg/kg/day in a BID dosing schedule) or nothing (control period) first. Assignment of bile acid or no supplement was determined using a random number table.

After 2 weeks on the diet (Day 14), study subjects were seen after a 16 h fast at the CRC. Plasma was obtained for total, LDL, and HDL cholesterol, and triglyceride concentrations, and monocytes for mRNA levels (HMG-CoA reductase and LDL receptor). A nasoduodenal tube was placed with fluoroscopic guidance with the tube tip placed at the ligament of Trietz. Duodenal drainage was collected by siphonage for 15 min. Subjects then ingested a previously described standardized meal (13–15). Duodenal drainage was collected in 15 min intervals for 90 min and then one 30 min interval. After completion of collection periods, 15 ml aliquots were saved for each time period for analysis of cholesterol and phospholipid. The remainder of the drainage was returned to the subject and the tube removed. Samples were processed as described previously (15).

Two days later (Day 16), subjects returned to the CRC. At that time, oral and intravenous doses of stably labeled cholesterol were given and blood collected at baseline and at +6, +24, +48, +72, and +96 h. On Day 19, cholesterol FSR was assessed. After an overnight fast, baseline blood was drawn for red blood cell (RBC) cholesterol isotope. Subjects were given oral deuterated water, and the next day (Day 20), blood was obtained at the same time as the isotope administration on Day 19.

After completing the first dietary period, subjects participated in the alternative arm of the study after a washout period of at least 1 month. Thereafter, the same sequence of events was performed as described for the first phase of the study.

Analytical methods

Plasma lipid profiles. Plasma total cholesterol, LDL cholesterol, HDL-cholesterol, and triglyceride concentrations were measured using methods validated by the National Institutes of Health Lipid Research Clinics.

Duodenal aspirates. After collection of samples from the nasoduodenal intubation/meal study, lipase and bacterial inhibitors [diisoprophylfluorophosphate, diethyl (*p*-nitrophenyl) phosphate, acetophenone, phenylboronic acid, sodium azide, and chloramphenicol] were added to the retained aliquots (13). Analysis of the lipid composition was performed using previously described methods (13–15). Samples were separated into the oil, subphase (aqueous), and pelleted subphases by ultracentrifugation. Samples were extracted by the method of Folch et al. (16), and phospholipids were measured using the chemical assay (17). Cholesterol was extracted in petroleum ether and measured by gas-liquid chromatography (GLC) using stigmastanol as an internal standard, and bile acids were quantitated by HPLC using 5β-cholanic acid-7α, 12α-diol as an internal standard (18, 19).

The intermicellar bile acid concentration was determined

(15) and used to make a buffer to separate the micelles and non-micellar particles, including vesicles, by size exclusion chromatography. Each sample had its own specific buffer. Particles were separated on a sizing column comprised of Sepharose® 4B. Cholesterol was measured in each fraction using either an enzymatic assay (Roche Diagnostics Corp., Indianapolis, IN) or by GLC. Micelles were defined as particles that eluted as particles the same diameter as HDL, and nonmicellar particles were larger.

Cholesterol absorption. ISOTOPE ADMINISTRATION AND PREPARATION. Cholesterol absorption was performed by the method of Bosner et al. as originally proposed by Zilversmit (20, 21). Fifteen milligrams of intravenous cholesterol $(25,26,26,26,27,27,27-D_7)$ (Cambridge Isotopes, Andover, MA) was dissolved in 20% Intralipid® and administered over 30 min into running saline infusion. Simultaneously, each subject was given 75 mg cholesterol-$^{13}C_2$ (Mass Trace, Woburn, MA) orally that was administered dissolved in corn oil at 15 mg/ml and added to an English muffin. The muffin was consumed with a breakfast of 240 ml orange juice, one slice of dry toast, 50 g corn flakes, and 240 ml of whole milk. Blood samples at 0, +6, +24, +48, +72, and +96 h were spun, and plasma stored at $-70°C$ until analyzed.

ANALYTICAL METHOD. Free cholesterol extracted from RBCs was used to determine ^{13}C cholesterol and D_7 cholesterol enrichments. The ^{13}C enrichments of free cholesterol were measured by differential isotope ratio mass spectrometry (IRMS) using an automated dual inlet system (SIRA 12, Isomass, Cheshire, United Kingdom). The D_7 enrichments of free cholesterol were measured by differential IRMS using a manually operated dual inlet system with electrical H^{3+} compensation (VG Isomass 903D). The average ^{13}C and D_7 enrichments of 48 h and 72 h RBC free cholesterol relative to baseline (t = 0) samples were used to calculate the cholesterol absorption coefficient using the ratio of orally ingested ^{13}C cholesterol to intravenously administered D_7 cholesterol as described by Bosner et al. (20).

Cholesterol biosynthesis. ISOTOPE ADMINISTRATION AND SAMPLE COLLECTION. Endogenous cholesterol synthesis determination based on deuterium incorporation was first described and applied in humans by Rittenberg and Schoenheimer (22), and later modified by Wong and Hachey (23) and Jones et al. (24, 25). This method is based on the fact that the rate of incorporation of the stable isotope deuterium oxide into RBC membrane cholesterol serves as an index of hepatic cholesterol synthesis rates (26, 27). The testing period was carried over 2 days. On study Day 19, the subject was weighed, and 15 ml blood (replicate samples of 3 g of RBCs and plasma) obtained for baseline body water and erythrocyte cholesterol deuterium enrichment. The subject was then dosed with 0.7 g D_2O per kg estimated body water [0.6 (% body water) × kg] by mouth, measured and administered with a disposable syringe. Between RBC samples, subjects were asked to consume water containing 0.7 g D_2O/kg. On day 20, a blood sample (15 ml) for postloading deuterium excess enrichment was obtained. Samples were drawn at the same time as administration on Study Day 19. Samples were numbered to "blind" the laboratory technicians. By performing this study on Days 19 and 20, interference from the administration-deuterated cholesterol on Day 16 was minimized as determined by baseline RBC enrichment on Day 19.

ANALYTICAL METHOD. Cholesterol biosynthesis was determined as the rate of incorporation of deuterium from body water into RBC membrane free cholesterol over the period between 72 h and 96 h at the end of each feeding period. Deuterated water equilibrates quickly between intracellular and extracellular water pools and permits direct determination of cholesterol formation rates (28, 29). Deuterium enrichment was measured in both RBC-free cholesterol and plasma water. To de-

termine plasma cholesterol deuterium enrichment, total RBC lipids were extracted and isolated using the same procedure described above.

Cholesterol fractional synthesis rate (FSR) was taken to represent the RBC-free cholesterol deuterium enrichment values relative to the corresponding plasma water sample enrichment after correcting for the free cholesterol pool. The FSR represents that fraction of the cholesterol pool that is synthesized in 24 h and was calculated as previously described (30).

MONONUCLEAR LEUKOCYTE LDL RECEPTOR AND HMG-COA REDUCTASE MRNA. Isolymph® (Gallard-Schlesinger Industries, Inc., Carle Place, NY) was used to isolate mononuclear cells from 10 ml of peripheral blood. Total RNA was isolated from mononuclear cells according to instructions provided with RNA Stat-60 (Tel-Test, Friendswood, TX).

Reverse transcription was performed by standard methods to yield a cDNA product. A competitive polymerase chain reaction was used to titrate 100 ng aliquots of cDNA with 10-fold increments from 10^2 copies to 10^6 copies of an internal standard (pAW109 RNA) that was included within each reaction tube (31). This standard uses the same primers as used for the cellular mRNA; therefore, no primer efficiency differences occur. A reaction product was generated that differs detectably in size from that generated by cellular mRNA (LDL receptor: 258 bp cellular mRNA, 301 bp internal standard; HMG-CoAR: 247 cellular mRNA, 303 bp internal standard). PCR products were separated on a 10% acrylamide gel, and stained with SYBR Green (Molecular Probes, Eugene, OR), and signal intensity was quantified with a PhosphorImager (Molecular Dynamics, Amersham Biosciences, Sunnyvale, CA). The point of equivalence between the signal for cellular mRNA and the signal for internal standard was determined from the *x*-axis intercept for the line equation of the graph of the intensity ratio for log (standard/cellular mRNA) against log (copy number standard) (32). When identical amounts of the polymerase chain reaction products were produced by both the internal standard and the native message, it was inferred that the amount of native message corresponded to the known concentration of the external template. Control tubes included tubes without template RNA and without reverse transcriptase.

The primer sequences used were human LDL receptor: 5′ CAA TGT CTC ACC AAG CTC TG 3′ and 5′ TCT GTC TCG AGG GGT AGC TG 3′ and human HMG-CoAR: 5′ TAC CAT GTC AGG GGT ACG TC 3′ and 5′ CAA GCC TAG AGA CAT AAT CAT C 3′.

APOLIPOPROTEIN GENOTYPES. DNA from peripheral blood was isolated according to instructions in the High Pure PCR Template Preparation Kit (Boehringer Mannheim, Indianapolis, IN). ApoE genotype was determined as described by Hixon and Vernier (33). ApoA-IV genotype was determined as described by Hixson and Powers (34).

Data analysis. DATA ENTRY AND MANAGEMENT. Automated data entry software was used to define data recognition fields on previously designed forms. The study coordinator checked the form for errors and legibility. Completed forms were sent to the biostatistician (Elizabeth A. Tolley) for data entry. Databases were transferred to the mainframe, stored on disk, and maintained in the computer center at the University of Tennessee, Memphis; these files were backed up daily.

HYPOTHESIS TESTING. Quantitative and qualitative alterations in intraluminal bile acid composition influence the efficiency of cholesterol absorption. To test this hypothesis, we used the following analytical approaches. Neither the main effect of order of randomization or the interaction of order with treatment (UDCA or no treatment) had a statistically significant effect on any outcome measurement. Therefore, these effects were

pooled with the residual error. For the percentages of micelles and vesicles determined at the various times, the statistical model was a three-way mixed model ANOVA with subject as the random block effect. Two fixed effects were included in the model treatment (UDCA or no treatment) and time of treatment with UDCA or no treatment. Because of missing values, the interactions of subject with the fixed effects could not be fit and were assumed to be zero. For all other outcome measurements, the statistical model was a two-way mixed model ANOVA with subject as the random block effect and treatment (UDCA supplementation or no treatment) as the fixed effect. In the context of ANOVA with only two treatments, this difference is equivalent to that obtained from paired data. AUC calculations were made for lipid composition within the lumen for analyses using the trapezoidal method (35–37). Finally, for each subject, changes were obtained by subtracting untreated values from UDCA-treated values. Pearson product moment correlation coefficients were estimated to examine relationships between changes in selected outcomes. All results are expressed as mean ± SEM obtained from ANOVA using the root mean square error to estimate the pooled standard error. All tests were preplanned at α of 0.05.

SAMPLE SIZE CALCULATION. Previous studies of the impact of bile acid composition on cholesterol absorption indicated that the effect of bile acid composition on percent absorption ranged from 8–23%, with standard deviations at baseline and follow up ranging from 8–15% (38, 39). Unfortunately, none of the previous studies reported the standard deviations of the differences in percent absorption. These previous studies suggested that a 10% difference in absorption would be clinically meaningful. Based upon the plan to use two-tailed paired Student's *t*-tests, a sample size of 10 provided an effect size (α) of 0.05 and a power of 0.8

RESULTS

Twelve adults (six men and six women, all Caucasian) age 24–36 years completed the study. During the 3 week course, subjects maintained their baseline weight with a −0.2 kg change on the diet alone versus −0.2 kg change on diet plus UDCA. Subjects consumed an average of 2,647 calories while receiving placebo and 2,637 calories while receiving UDCA. Total cholesterol and fat, saturated fat, polyunsaturated fat, and monounsaturated fat consumption was similar during both study periods. On questioning, subjects were compliant with the diet with few diet violations. Compliance with taking UDCA was high with only two subjects returning six or two of the 300 mg pills. No subjects experienced diarrhea or cramps with bile acid therapy, and no sedation-related complications occurred. No drainage beyond baseline could be collected on one subject when she was receiving UDCA, so paired data for the liquid meal study is available for only 11 subjects. The remainder of data represents paired data from all 12 participants.

Administration of UDCA at a dose of 15 mg/kg/day in divided doses resulted in significant ($P < 0.0001$) enrichment of the biliary bile acids with UDCA. Peak intraluminal bile acid concentrations, measured in the 15–30 min sample, were 58.6 ± 9.5 mM in UDCA-treated compared with 86.6 ± 14.4 mM during the untreated period. With the meal alone, UDCA enrichment of bile in the +30 min

sample accounted for 2.2 ± 2.6% (not significantly different from 0) of luminal bile acids, while with UDCA treatment, UDCA accounted for 40.6 ± 2.6%. With treatment with UDCA, the bile acid composition of the primary and secondary bile acids was proportionately reduced. Without bile acid supplementation, cholic acid accounted for 41.9 ± 2.9% of biliary bile acids, while on UDCA it declined to 24.2 ± 2.9%. Similarly, without supplementation, deoxycholic acid accounted for 28.9 ± 2.9%, which declined to 10.9 ± 2.9% with UDCA treatment. Chenodeoxycholic acid enrichment remained relatively constant with 27.0 ± 1.7% without supplementation versus 24.2 ± 1.7% with UDCA treatment. No lithocholic acid in bile was identified with either regimen. Plasma total, HDL, and LDL cholesterol, and trigycleride concentrations did not change with UDCA treatment. After 14 days of diet alone, the plasma total cholesterol concentration was 152.8 ± 2.2 mg/dl, HDL cholesterol was 45.7 ± 1.1 mg/dl, LDL cholesterol was 93.8 ± 2.5 mg/dl, and plasma triglycerides were 73.7 ± 5.2 mg/dl. After 14 days of diet plus UDCA at a dose of 15 mg/kg/day, there was no change (all P = ns) in the plasma cholesterol (149.6 ± 2.2 mg/dl), HDL cholesterol (46.3 ± 1.1 mg/dl), plasma LDL cholesterol (89.75 ± 2.5 mg/dl), or plasma triglyceride (73.7 ± 5.2 mg/dl) concentrations.

In response to the meal, intraluminal cholesterol solubilized in the aqueous phase during the entire collection period was decreased significantly ($P = 0.012$) with UDCA treatment (101.0 ± 7.2 mg/ml/120 min) compared with diet alone (132.5 ± 7.2 mg/ml/120 min) (**Fig. 1**). There were no differences in the area under the curve for phospholipids. The percent micellar cholesterol was variable but similar during UDCA treatment plus diet compared with diet alone at the three time points measured. There was a trend for higher amounts of cholesterol in micelles in the UDCA-treated period. When all three collection periods were combined, the mean micellar cholesterol percentage was higher (69.8 ± 4.8%) and nonmicellar cholesterol lower (30.2 ± 4.8%) during the UDCA treatment period ($P = 0.0076$). A majority of the nonmicellar cholesterol was eluted in the void volume as vesicles, with a smaller percentage as intermediate-sized particles (**Fig. 2**).

Cholesterol absorption measured by the dual-isotope technique utilizing stable isotopes of cholesterol was unchanged with UDCA treatment (**Fig. 3**). With diet alone, cholesterol absorption was 60.4 ± 3.6% compared with 62.3 ± 3.6% when subjects were treated with diet plus UDCA. With no changes observed in cholesterol absorption, it was not surprising that cholesterol FSRs were unchanged by UDCA treatment (**Fig. 4**). The FSR on diet plus UDCA was 0.032 ± 0.005/day while with diet alone, the FSR was 0.029 ± 0.005/day. Complementary data for other aspects of cholesterol metabolism, LDL receptor mRNA, and HMG-CoA reductase mRNA were unchanged by UDCA treatment. LDL receptor mRNA was 2,853 ± 712 copies mRNA/100 ng RNA in UDCA plus diet-treated subjects compared with 3,013 ± 712 copies mRNA/100 ng RNA in subjects given diet alone. HMG-CoA reductase mRNA was 6,696 ± 1,609 copies mRNA/100 ng RNA
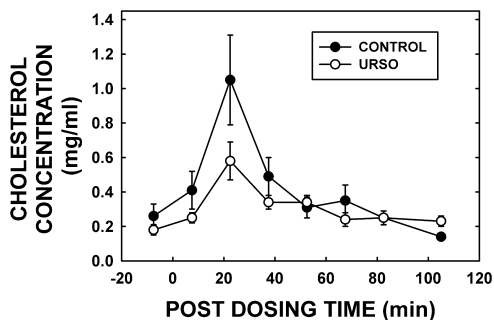
Fig. 1. The amount of cholesterol solubilized in the aqueous fraction (subphase) of the luminal samples of subjects after ingesting a standardized meal on no bile acid supplementation or ursodeoxycholic acid (UDCA) treatment. Cholesterol concentrations are shown for subjects consuming no bile acid supplements or UDCA. Data are presented as means ± SEM.

while receiving UDCA and diet, and 7,660 ± 1,609 copies mRNA/100 ng RNA on diet alone.

Correlations were examined comparing relationships between changes in various measures with treatment with UDCA. Specifically, no significant correlations were found between the change in cholesterol absorption in individual subjects and subphase area under the curve cholesterol concentration, percent micellar, or vesicular choles-
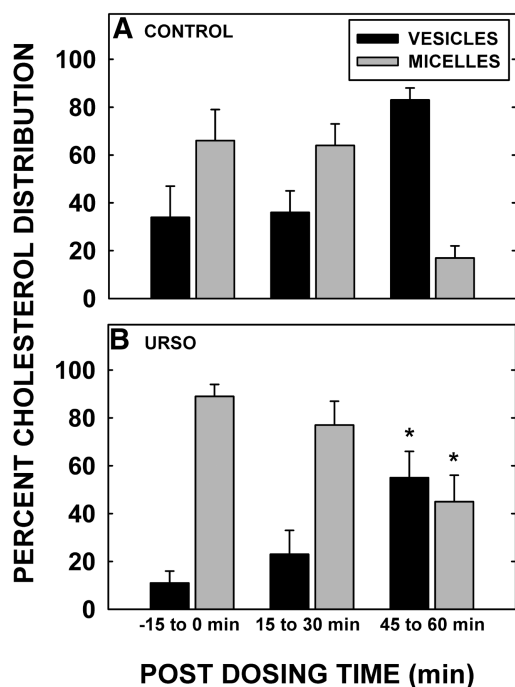

Fig. 2. The distribution of cholesterol between micelles and nonmicellar particles, including vesicles, of subjects on no bile acid supplementation or UDCA treatment. Luminal samples were collected as described in Materials and Methods, and separated based on size. The amount of cholesterol in each fraction was measured enzymatically or by gas-liquid chromatography. Cholesterol concentrations in fractions are shown for subjects consuming no bile acid supplements (A) or UDCA (B). Asterisks represent values significantly different from control values at the same time point. Data are presented as means ± SEM.
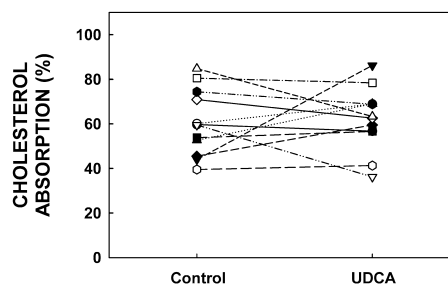

Fig. 3. The percent of cholesterol absorption is shown for each subject fed AHA II diets while receiving UDCA or no supplement. Subjects underwent an overnight fast and then consumed a solid breakfast containing stable isotope, and concurrently received intravenous isotope. Samples were collected and analyzed as described in Materials and Methods.

terol FSR, LDL-receptor mRNA, or HMG-CoA reductase mRNA. A negative correlation was found between the change in FSR and change in LDL receptor mRNA with UDCA treatment ($r = -.708$, $P < 0.01$). Surprisingly, there was a negative correlation between the change in mRNA HMG-CoA reductase and FSR (-0.577, $P < 0.05$).

DISCUSSION

The overall goal of this investigation was to determine the effect of differences in bile acid composition on intraluminal cholesterol and lipolytic product solubilization, intestinal cholesterol absorption, hepatic cholesterol synthesis, and LDL receptor numbers. This represents the first in a series of experiments designed to determine the effects of supplementation with UDCA, cholic acid, chenodeoxycholic acid, and deoxycholic acid on cholesterol absorption and metabolism in healthy adults.

Effect of UDCA on cholesterol absorption

Solubilization of the lipolytic products of fat digestion, cholesterol, and fat-soluble vitamins by bile salts is essential for lipid absorption because all are minimally soluble in aqueous systems and are dependent upon the detergent properties of bile acids for dispersion in the intralu-
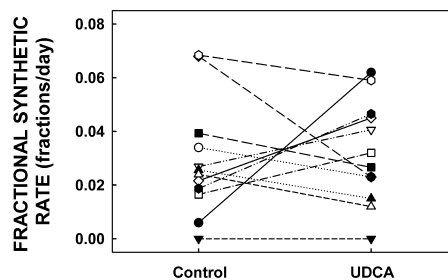

Fig. 4. The fractional synthetic rates for each subject fed AHA II diets for 19 days supplemented with UDCA or no bile acids. Subjects underwent an overnight fast and then consumed deuterated water. Samples were collected and analyzed as described in Materials and Methods.

minal environment (40–42). The efficiency of sterol absorption appears to be related to the saturation of the bile salt micelle. When intraluminal bile salt concentrations fall below the critical micellar concentration, cholesterol absorption is impaired.

The effects of qualitative differences in bile acid composition have been extensively investigated. Most of these past studies have examined bile salts in vitro or in studies with uncontrolled diets. Quantitative and qualitative differences in dietary fat composition or cholesterol content may alter responses to luminal bile acid content and lead to results that are difficult to interpret (43). Variable effects have been observed, and no single study has carefully evaluated the effect of a group of bile acids on cholesterol absorption with a well-controlled design. Differences in results, including the high variability of absorption rates, may be related to experimental design (perfusion vs. fecal collections), diet (including fat and cholesterol content), and analytical methods.

In the current study, we found no differences in cholesterol absorption using the dual-isotope method in a group of healthy adults who were carefully screened for underlying disease, and apoE and apoA-IV genotypes because of their potential effects on cholesterol absorption (6, 44–46) and plasma lipid concentrations after receiving 2 weeks of a carefully controlled, low-cholesterol diet. Previous studies examining sterol absorption with UDCA treatment have yielded conflicting results. Our findings are in agreement with those of LaRusso and Thistle, who found no change in cholesterol absorption (45.4% vs. 46.8%) in UDCA-treated adults with gallstones exposed to diets containing 250 mg cholesterol/day for 2 days (47), and with Salvioli et al., who found no change in cholesterol absorption in normal subjects given diets containing 500 mg cholesterol/day (48), even though these studies did not control for diet as well as did the present study. In contrast to our findings, Ponz de Leon et al. found that cholesterol absorption declined from 36.7% to 17.5% while subjects were on UDCA consuming a diet containing 500 mg cholesterol/day (38). Leiss et al. found reduced cholesterol absorption (29% baseline vs. 14% on UDCA) in subjects with gallstones without any dietary control (39). Hardison and Grundy found reductions in cholesterol absorption in six normal males receiving a high-cholesterol (500 mg/day) liquid feed containing 20% fat with a P-S ratio of 4.0. They found that absorption was 57% at baseline compared with 47% on UDCA and 42% on tauroursodeoxycholic acid (49). Lanzini and Northfield found reduced cholesterol absorption from 25% to 15% in six subjects with gallstones on no dietary control (50). The reasons for the discrepancies observed between the previous studies and ours may be related to differences in experimental design. The study of LaRusso and Thistle utilized the serum dual isotopic method to measure absorption, while Salvioli et al. used the sterol balance technique. The other conflicting results utilized perfusion and balance techniques. In addition, in the three studies in which diet was carefully controlled, subjects consumed large quantities of cholesterol relative to what was consumed by our subjects. The

effect of dietary cholesterol on the ability of UDCA to reduce plasma cholesterol concentrations is unknown. This selected population included both men and women, whereas most subjects in previous studies were men. This is particularly relevant since there may be gender differences in cholesterol absorption in humans, as previously discovered in mice (personal communication, P. Tso).

Effect of biliary UDCA enrichment on intraluminal digestion/solubilization

UDCA is recognized to form micelles less efficiently than the primary bile acids (12). It was anticipated that enrichment of the pool with UDCA would lead to reduced cholesterol absorption, secondary to poor cholesterol solubilization. As expected from the in vitro data, cholesterol solubilized in the aqueous phase of subjects fed UDCA was reduced significantly compared with the period without bile acid supplementation. Despite the reduced quantities of cholesterol in the subphase, there were no observed differences in cholesterol absorption. The percentage of cholesterol in micellar phase was actually higher after a liquid meal when subjects were treated with UDCA and diet versus with diet alone. It is possible that the greater micellar cholesterol observed in the UDCA treatment phase may have been offset by the reduced subphase cholesterol, thereby leading to no net effect of UDCA on intraluminal solubilization of dietary cholesterol and no direct impact on cholesterol absorption. In addition, phospholipid solubilization in the aqueous phase was similar during both treatment phases. In aggregate, these findings suggest that UDCA does not have a major impact upon intraluminal lipid digestive events when mixed with other bile acids and triglyceride-digested products, or in the experimental conditions that we used.

Effect of UDCA on cholesterol metabolism

Since there was no significant change in cholesterol absorption with UDCA treatment, it is not surprising that the FSR for cholesterol, or LDL-receptor and HMG-CoA reductase mRNA numbers, were unchanged. Although we did not directly measure hepatic LDL receptor and HMG-CoA reductase mRNA levels, previous studies have demonstrated an excellent correlation between the hepatic and monocyte values (51). Since one would anticipate that there would be a reciprocal relationship between FSR/HMG-CoA reductase mRNA levels and cholesterol absorption (52–54), it is reassuring to see that without changes in absorption, these other measurements did not change, thereby serving as an internal validation for each other. Interestingly, the absence of change in plasma cholesterol concentration might not be unexpected in normolipemic subjects, because the greatest UDCA-dependent reductions in plasma cholesterol concentrations occurred in patients with the highest plasma cholesterol concentrations (55, 56). Previous studies in normolipemic and hyperlipemic adults have yielded variable results. The greatest reduction has been shown in those with highest plasma cholesterol concentrations, with cholestatic patients having consistently significant reductions (55, 56).

Conclusion

Our studies have failed to demonstrate a major effect of biliary enrichment with UDCA on cholesterol absorption and synthesis, plasma lipids, and LDL receptor mRNA in a healthy population. Although intraluminal solubilization of cholesterol was reduced when bile was enriched with UDCA, its impact on cholesterol absorption and metabolism appears limited. Future studies are planned to assess the effects of UDCA on cholesterol solubilization and the importance of micellar and vesicular cholesterol on absorption in animal models using the lymph fistula technique. Additional human studies are planned to assess the effects of biliary enrichment with cholic, chenodeoxycholic, and deoxycholic acid on cholesterol absorption and metabolism. ⬛

REFERENCES

1. Dietschy, J. M., S. D. Turley, and D. K. Spady. 1993. 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.
2. Grundy, S. M. 1983. Absorption and metabolism of dietary cholesterol. *Annu. Rev. Nutr.* **3:** 71–96.
3. Hauser, H., J. H. Dyer, A. Nandy, M. A. Vega, M. Werder, E. Bieliauskaite, F. E. Weber, S. Compassi, A. Gemperli, D. Boffelli, E. Wehrli, G. Schulthess, and M. C. Phillips. 1998. Identification of a receptor mediating absorption of dietary cholesterol in the intestine. *Biochemistry.* **37:** 17843–17850.
4. Hernandez, M., J. Montenegro, M. Steiner, D. Kim, C. Sparrow, P. A. Detmers, S. D. Wright, and Y-S. Chao. 2000. Intestinal absorption of cholesterol is mediated by a saturable inhibitable transporter. *Biochim. Biophys. Acta.* **1486:** 232–242.
5. Yu, L., J. Li-Hawkins, R. E. Hammer, K. E. Berge, J. D. Horton, J. C. Cohen, H. H. Hobbs. 2002. Overexpression of ABCG5 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J. Clin. Invest.* **110:** 671–680.
6. Gylling, H., and T. A. Miettinen. 1992. Cholesterol absorption and synthesis related to low density lipoprotein metabolism during varying cholesterol intake in men and different apoE phenotypes. *J. Lipid Res.* **33:** 1361–1371.
7. Katan, M. B., A. C. Beynen, J. H. M. DeVries, and A. Nobels. 1986. Existence of consistent hypo- and hyper responders to dietary cholesterol in man. *Am. J. Epidemiol.* **123:** 221–234.
8. Katan, M. B., and A. C. Beynen. 1987. Characteristics of human hypo- and hyper responders to dietary cholesterol. *Am. J. Epidemiol.* **125:** 387–399.
9. Kesaniemi, Y. A., and T. A. Miettinen. 1987. Cholesterol absorption efficiency regulates plasma cholesterol level in the Finnish population. *Eur. J. Clin. Invest.* **17:** 391–395.
10. Safonova, I. G., D. D. Sviridov, A. Roytman, F. M. Rytikov, V. V. Dolgov, J-L. Nano, P. Rampal, and V. S. Repin. 1993. Cholesterol uptake in the human intestine. Hypo- and hyper responsiveness. *Biochim. Biophys. Acta.* **1166:** 313–316.
11. Harwood, H. J., Jr., C. E. Chandler, L. D. Pellarin, F. W. Bangerter, R. W. Wilkins, C. A. Long, P. G. Cosgrove, M. R. Malinow, C. A. Marzetta, J. L. Pettini, Y. E. Savoy, and J. T. Mayne. 1993. Pharmacologic consequences of cholesterol absorption inhibition: alteration in cholesterol metabolism and reduction in plasma cholesterol concentration induced by the synthetic saponin β-tigogenin cellobioside (CP-88818; tiqueside). *J. Lipid Res.* **34:** 377–395.
12. Roda, A., A. F. Hofmann, and K. J. Mysels. 1983. The influence of bile salt structure on self-association in aqueous solutions. *J. Biol. Chem.* **258:** 6362–6370.
13. Hernell, O., J. E. Staggers, and M. C. Carey. 1990. Physical-chemical behavior of dietary and biliary lipid during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry.* **29:** 2041–2056.
14. Armand, M., P. Borel, B. Pasquier, C. Dubois, M. Senft, M. Andre, J. Peyrot, J. Salducci, and D. Lairon. 1994. Characterization of emulsions and lipolysis of dietary lipids in the human stomach. *Am. J. Phyisol.* **266:** G372–G381.
15. Yao, L., J. E. Heubi, D. D. Buckley, H. Fierra, K. D. R. Setchell, N. A. Granholm, P. Tso, D. Y. Hui, and L. A. Woollett. 2002. Separation of micelles and vesicles within lumenal aspirates from healthy humans: Solublization of cholesterol after a meal. *J. Lipid Res.* **43:** 654–660.
16. Folch, J., M. Lees, and G. H. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
17. Sokoloff, L., and G. H. Rothblat. 1974. Sterol to phospholipid molar ratios of L cells with qualitative and quantitative variations of cellular sterol. *Proc. Soc. Exp. Biol. Med.* **146:** 1166–1172.
18. Roda, A., C. Cerre, P. Simoni, C. Polimeni, C. Vaccari, and A. Pistillo. 1992. Determination of free and amidated bile acids by high-performance liquid chromatography with evaporative light-scattering mass detection. *J. Lipid Res.* **33:** 1393–1402.
19. Turley, S. D., M. W. Herndon, and J. M. Dietschy. 1994. Reevaluation and application of the dual-isotope plasma ratio method for the measurement of intestinal cholesterol absorption in the hamster. *J. Lipid Res.* **35:** 328–339.
20. Bosner, M. S., R. E. Ostlund, Jr., O. Osofisan, J. Grosklos, C. Friotschle, and L. G. Lange. 1993. Assessment of percent cholesterol absorption in humans with stable isotopes. *J. Lipid Res.* **34:** 1047–1053.
21. Zilversmit, D. B. 1972. A single blood sample dual isotope method for the measurement of cholesterol absorption in rats. *Proc. Soc. Exp. Biol. Med.* **140:** 862–865.
22. Rittenberg, D., and R. Schoenheimer. 1937. Deuterium as an indicator in the study of intermediary metabolism. XI. Further studies on the biological uptake of deuterium into organic substances, with special reference to fat and cholesterol formation. *J. Biol. Chem.* **121:** 235–253.
23. Wong, W. W., and D. L. Hachey. 1991. Measurement of in vivo cholesterol synthesis from ^{2}H$_2$O: a rapid procedure for the isolation, combustion, and isotopic assay of erythrocyte cholesterol. *J. Lipid Res.* **32:** 1049–1056.
24. Jones, P. J. 1990. Use of deuterated water for measurement of short-term cholesterol synthesis in humans. *Can. J. Physiol. Pharmacol.* **68:** 955–959.
25. Jones, P., C. A. Leitch, Z. C. Li, and W. E. Connor. 1993. Human cholesterol synthesis measurement using deuterated water: Theoretical and procedural considerations. *Arterioscler. Thromb.* **13:** 247–253.
26. Dietschy, J. M., and D. K. Spady. 1984. Measurement of rates of cholesterol synthesis using tritiated water. *J. Lipid Res.* **25:** 1469–1476.
27. Jones, P. J. H., L. M. Ausman, D. H. Croll, J. Y. Feng, E. A. Schaefer, and A. H. Lichtenstein. 1998. Validation of deuterium incorporation against sterol balance for measurement of human cholesterol biosynthesis. *J. Lipid Res.* **39:** 1111–1117.
28. London, I., and H. Schwarz. 1953. Erythrocyte metabolism. The metabolic behavior of the cholesterol of human erythrocytes. *J. Clin. Invest.* **32:** 1248–1252.
29. Jeske, D. J., and J. M. Dietschy. 1980. Regulation of rates of cholesterol synthesis in vivo in the liver and carcass of the rat measured using ^{3}H water. *J. Lipid Res.* **21:** 364–376.
30. Jones, P. J. H., C. A. Leitch, Z-C. Li, and W. E. Connor. 1993. Human cholesterol synthesis measurement using deuterated water: theoretical and procedural consideration. *Arterioscler. Thromb.* **13:** 247–253.

31. Wang, A. M., M. V. Doyle, and D. F. Mark. 1989. Quantitation of mRNA by the polymerase chain reaction. *Proc. Natl. Acad. Sci. USA.* **86:** 9719–9721.

32. Freeman, W. M., S. J. Walker, and K. E. Vrana. 1999. Quantitative RT–PCR: Pitfalls and potential. *Biotechniques.* **26:** 112–125.

33. Hixon, J. E., and D. T. Vernier. 1990. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with *Hha* I. *J. Lipid Res.* **31:** 545–548.

34. Hixon, J. E., and P. K. Powers. 1991. Restriction isotyping of human apolipoprotein A-IV: Rapid typing of known isoforms and detection of a new isoform that deletes a conserved repeat. *J. Lipid Res.* **32:** 1529–1535.

35. Sokal, R. R., and F. J. Rohlf. 1981. Comparisons among means: planned comparisons. *In* Biometry. 2nd edition. W. H. Freeman and Company, New York. 232–242.

36. Kirk, R. E. 1982. Split-plot factorial design: design with group-treatment confounding. *In* Experimental Design. 2nd Edition. Wadsworth Inc., Belmont, CA. 489–569.

37. Cohen, J. 1977. Statistical Power Analysis for the Behavior Sciences. Revised edition. Academic Press, New York.

38. Ponz De Leon, M., N. Carulli, P. Loria, R. Iori, and F. Zironi. 1980. Cholesterol absorption during bile acid feeding. *Gastroenterology.* **78:** 214–219.

39. Leiss, O., K. vonBergmann, U. Streicher, and H. Strotkoetter. 1984. Effect of three different dihydroxy bile acids on intestinal cholesterol absorption in normal volunteers. *Gastroenterology.* **87:** 144–149.

40. Siperstein, M. D., I. L. Chaikoff, and W. O. Reinhardt. 1952. ^{14}C-cholesterol. V. Obligatory function of bile in intestinal absorption of cholesterol. *J. Biol. Chem.* **198:** 111–114.

41. Swell, L., E. C. Trout, Jr., J. R. Hopper, H. Field, Jr., and C. R. Treadwell. 1958. Specific function of bile salts in cholesterol absorption. *Proc. Soc. Exp. Biol. Med.* **98:** 174–176.

42. Westergaard, H., and J. M. Dietschy. 1976. The mechanism whereby bile acid micelles increase the rate of fatty acid and cholesterol uptake into the intestinal mucosal cell. *J. Clin. Invest.* **58:** 97–108.

43. Shioda, R., and L. W. Kinsell. 1966. Dietary regulation of cholesterol metabolism. *Lancet.* **2:** 604–607.

44. Miettinen, T. A., H. Gylling, H. Vanhanen, and A. Ollus. 1992. Cholesterol absorption, elimination, and synthesis related to LDL kinetics during varying fat intake in men with different apoprotein E phenotypes. *Arterioscler. Thromb.* **12:** 1044–1052.

45. McCombs, R. J., D. E. Marcadis, J. Ellis, and R. B. Weinberg. 1994. Attenuated hypercholesterolemic response to a high-cholesterol diet in subjects heterozygous for the apolipoprotein A-IV-2 allele. *N. Engl. J. Med.* **331:** 706–710.

46. Mata, P., J. M. Ordovas, J. Lopze-Miranda, A. H. Lichtenstein, B. Clevidence, J. T. Judd, and E. J. Schaefer. 1994. Apo A-IV phenotype affects diet-induced plasma LDL cholesterol lowering. *Arterioscler. Thromb.* **14:** 884–891.

47. LaRusso, N. F., and J. L. Thistle. 1983. Effect of litholytic bile acids on cholesterol absorption in gallstone patients. *Gastroenterology.* **84:** 265–271.

48. Salvioli, G., R. Lugli, and J. M. Pradelli. 1985. Cholesterol absorption and sterol balance in normal subjects receiving dietary fiber or ursodeoxycholic acid. *Dig. Dis. Sci.* **30:** 301–307.

49. Hardison, W. G. M., and S. M. Grundy. 1984. Effect of ursodeoxycholate and its taurine conjugate on bile acid synthesis and cholesterol absorption. *Gastroenterology.* **87:** 130–135.

50. Lanzini, A., and T. C. Northfield. 1988. Effect of ursodeoxycholic acid on biliary lipid coupling and on cholesterol absorption during fasting and eating in subjects with cholesterol gallstones. *Gastroenterology.* **95:** 408–416.

51. Powell, E. E., and P. A. Kroon. 1994. Low density lipoprotein receptor and 3-hydroxy-3-methylglutaryl Coenzyme A reductase gene expression in human mononuclear leukocytes is regulated coordinately and parallels gene expression in human liver. *J. Clin. Invest.* **93:** 2168–2174.

52. Jones, P. J. H., A. S. Pappu, L. Hatcher, Z-C. Li, D. R. Illingworth, and W. E. Connor. 1996. Dietary cholesterol feeding suppresses human cholesterol synthesis measured by deuterium incorporation and urinary mevalonic acid levels. *Arterioscler. Thromb. Vasc. Biol.* **16:** 1222–1228.

53. Applebaum-Bowden, D., S. M. Haffner, E. Hartsock, K. H. Luk, J. J. Albers, and W. R. Hazzard. 1984. Down-regulation of the low-density lipoprotein receptor by dietary cholesterol. *Am. J. Clin. Nutr.* **39:** 360–367.

54. Gordon, T., M. Fisher, N. Ernst, and B. M. Rifkind. 1982. Relation of diet to LDL cholesterol, VLDL cholesterol, and plasma total cholesterol and triglycerides in white adults. *Arteriosclerosis.* **2:** 502–512.

55. Bachrach, W. H., and A. F. Hofmann. 1982. Ursodeoxycholic acid in the treatment of cholesterol cholelithiasis, Part I. *Dig. Dis. Sci.* **27:** 737–761.

56. Poupon, R. E., K. Ougerram, Y. Chretien, C. Verneau, E. Eschwege, T. Magot, and R. Poupon. 1993. Cholesterol lowering effect of ursodeoxycholic acid in patients with primary biliary cirrhosis. *Hepatology.* **17:** 577–582.