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Publisher's version / Version de l'éditeur:

<https://doi.org/10.1016/j.lab.2006.03.009>

Translational Research, 148, 1, pp. 37-45, 2006-07

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Effects of chenodeoxycholic acid and deoxycholic acid on cholesterol absorption and metabolism in humans

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Quantitative and qualitative differences in intraluminal bile acids may affect cholesterol absorption and metabolism. To test this hypothesis, 2 cross-over outpatient studies were conducted in adults with apo-A IV 1/1 or apo-E 3/3 genotypes. Study 1 included 11 subjects 24 to 37 years of age, taking 15 mg/kg/day chenodeoxycholic acid (CDCA) or no bile acid for 20 days while being fed a controlled diet. Study 2 included 9 adults 25 to 38 years of age, taking 15 mg/kg/day deoxycholic acid (DCA) or no bile acid, following the same experimental design and procedures as study 1. CDCA had no effect on plasma lipid concentrations, whereas DCA decreased ($P < 0.05$) plasma high-density lipoprotein (HDL)-cholesterol and tended to decrease ($P = 0.15$) low-density lipoprotein (LDL)-cholesterol. CDCA treatment enriched ($P < 0.0001$) bile with CDCA and increased cholesterol concentration in micelles, whereas meal-stimulated bile acid concentrations were decreased. DCA treatment enriched ($P < 0.0001$) bile with DCA and tended to increase intraluminal cholesterol solubilized in micelles ($P = 0.06$). No changes were found in cholesterol absorption, free cholesterol fractional synthetic rate (FSR), or 3-hydroxy-3 methylglutaryl (HMG) CoA reductase and LDL receptor messenger ribonucleic acid (mRNA) levels after CDCA treatment. DCA supplementation tended to decrease cholesterol absorption and reciprocally increase FSR and HMG CoA reductase and LDL receptor mRNA levels. Results of these 2 studies suggest that the solubilization of cholesterol in the intestinal micelles is not a rate-limiting step for its absorption. (Translational Research 2006;148:37–45)

Abbreviations: ANOVA = analysis of variance; apo = apolipoprotein; BID = twice a day; CA = cholic acid; CDCA = chenodeoxycholic acid; CAC = cholesterol absorption coefficient; CRC = Clinical Research Center; DCA = deoxycholic acid; DNA = deoxyribonucleic acid; FSR = free cholesterol fractional synthetic rate; HDL = high-density lipoprotein; HDL-C = HDL-cholesterol; HMG = 3-hydroxy-3 methylglutaryl; IRMS = isotope ratio mass spectrometry; LDL = low-density lipoprotein; LDL-C = LDL-cholesterol; mRNA = messenger RNA; PCR = polymerase chain reaction; RBC = red blood cell; RNA = ribonucleic acid; TC = total cholesterol; TG = triglyceride; UDCA = ursodeoxycholic acid

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Supported by National Institutes of Health Awards P01DK-54504 and HD-39419 and National Center for Research Resources Grant #M01RR8084.

Submitted for publication July 29, 2005; revision submitted December 28, 2005; accepted for publication March 22, 2006.

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1931-5244/\$ – see front matter

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doi:10.1016/j.lab.2006.03.009

Before absorption into enterocyte, cholesterol traverses the unstirred water layer and reaches the brush border.^{1,2} This process is carried out by cholesterol carriers, predominantly micelles. The formation of micelles and cholesterol incorporation into micelles are, therefore, considered important for cholesterol absorption, although micellar solubilization may not be the rate-limiting step.²⁻⁵ As bile acids are essential for micelle formation, they are obligatory for cholesterol absorption.^{2,3,6} The extent to which bile acids influence cholesterol absorption may depend on the hydrophilic-hydrophobic properties of bile acids and other coexisting lipids, including phospholipids, free fatty acids, and monoglycerides.^{7,8} The effect of biliary enrichment with the primary and secondary bile acids and UDCA on cholesterol absorption remains unclear because of previous variable results.^{5,7,9,10} The impact of enrichment of the bile pool with individual bile acids on cholesterol distribution between micelles and vesicles in the human intestinal lumen is largely unresolved. Recent development of methodology required for reproducible sample collection and analysis has allowed the authors to carefully look at the influences of individual bile acids on cholesterol solubilization in micelles and vesicles and, accordingly, on cholesterol absorption.¹¹

The intestinal absorption of cholesterol is closely associated with its concentration in the plasma.^{12,13} However, net changes of plasma cholesterol concentration rely on the kinetic metabolism of cholesterol because an alteration in cholesterol absorption generally causes inverse changes in cholesterol synthesis and LDL receptor-mediated cholesterol clearance.¹⁴ Recently, Woollett et al¹⁵ assessed the effects of CA (49) and UDCA supplementation on cholesterol distribution in micelles versus vesicles and, consequently, on cholesterol absorption. CA enrichment of bile increased cholesterol absorption, which was partially mediated by increasing micellar cholesterol incorporation; UDCA enrichment had no effect on cholesterol absorption and metabolism, although a decreased cholesterol distribution in micelles was observed. In both studies, cholesterol synthesis and mRNA expression of LDL receptor and HMG CoA reductase were not affected by either CA or UDCA. The effects of other bile acids such as CDCA and DCA on cholesterol absorption and metabolism remain poorly characterized. The objectives of this study were to examine the effects of dietary supplementation with CDCA and DCA on circulating cholesterol levels, intraluminal bile acid concentration and composition, cholesterol distribution between micelles and vesicles, cholesterol absorption and synthesis, and monocyte LDL receptor and HMG CoA reductase mRNA levels in healthy subjects.

MATERIALS AND METHODS

Subjects and study design

Study 1. Subjects were healthy adults (5 women and 6 men) of any race, ranging from 24 to 37 years of age, recruited by advertisement, and screened for any evidence of cardiovascular, pulmonary, renal, gastrointestinal, hepatobiliary disease, soy allergy, and none-apolipoprotein (apo) A-IV 1/1 and none-apo E 3/3 genotypes.^{4,15} The work was carried out in accordance with the Declaration of Helsinki regarding human subjects. Approval of the research protocol was obtained from the Institutional Review Board of the Cincinnati Children's Hospital Medical Center before subject enrollment.

Subjects who met screening criteria were given a complete verbal explanation of the study and signed a consent form describing the study. Diets were prepared and provided by the CRC staff as previously described.¹⁵ Using a crossover design, subjects who participated in this study had completed previous arms including control, UDCA, or CA and received CDCA (15 mg/kg/day in a BID dosing schedule) for this study. Uneaten food and bile acid pills were returned. Subjects were weighed each week in light clothing without shoes to ensure maintenance of basal weight.

On day 0, subjects came to the CRC after an overnight fast and had blood drawn for the plasma concentration of TC, LDL-C, HDL-C, and TG. After 2 weeks on the diet (day 14), subjects returned to the CRC after a 16-h fast. Plasma was obtained for lipid analysis. A nasoduodenal tube was placed, and duodenal drainage was collected by siphonage for 15 min. Subjects then ingested a standardized meal containing 123-mg cholesterol, and duodenal drainage was collected in 15-min intervals for 90 min and then one 30-min interval.^{11,16} Samples were processed and saved as described previously.¹¹ On days 16 through 19, cholesterol absorption was measured using the dual-stable isotopic method.^{4,15} On days 19 and 20, cholesterol synthesis was assessed by the deuterium incorporation method.^{4,15} After completing either the control or CDCA supplementation period, subjects were given a minimum 4-week washout period and then participated in the alternative arm of the study, which was performed as described for the first phase of the study.

Study 2. Study 2 included 11 subjects who completed study 1. According to a cross-over design, subjects received nothing (control) first and DCA (15 mg/kg/day in a BID dosing schedule) second. The experiment was carried out following the same subject-screening criteria, experimental design, sample collection, and analytical procedures as study 1. Overall, 2 subjects dropped out during the study and 9 subjects (4 women and 5 men of any race and 25 to 38 years of age) completed study 2.

Analytical methods

Plasma lipid profiles. Plasma TC, LDL-C, HDL-C, and TG concentrations were measured using methods validated by the Center for Disease Control, Lipid Research Clinics.^{4,15}

Duodenal aspirates. Lipid composition in duodenal aspirates was analyzed as described elsewhere.^{11,16} Samples were separated into the oil, aqueous, and pelleted subphases by ultracentrifugation.¹⁶ Samples were extracted, and phospho-

Table I. Effects of chenodeoxycholic and deoxycholic acids on the peak total bile acids and composition

	Study 1 (n = 11)					Study 2 (n = 9)			
	TBA (mM)	CA (%)	CDCA (%)	DCA (%)		TBA (mM)	CA (%)	CDCA (%)	DCA (%)
Control	88.3 ± 6.5*	43.0 ± 5.3*	27.1 ± 5.3†	27.7 ± 5.5*	Control	89.5 ± 10.7	42.4 ± 3.3*	26.7 ± 2.4*	28.2 ± 5.6†
CDCA	37.1 ± 6.5†	15.7 ± 5.3†	78.3 ± 5.3*	5.5 ± 5.5†	DCA	96.7 ± 10.7	5.1 ± 3.3†	11.4 ± 2.4†	82.2 ± 5.6*

Notes: Data are means ± SEM. Values with different superscripts in each column are significantly different ($P < 0.05$). Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; TBA, total bile acids.

lipids, cholesterol, and bile acids were measured.^{17–20} The intermicellar bile acid concentration was determined and used to make a buffer to separate the micelles and non-micellar particles, including vesicles, by size exclusion chromatography.^{4,11,15,20,21} Cholesterol was measured in each fraction using either an enzymatic assay (Roche Diagnostics Corp., Indianapolis, Ind) or by gas liquid chromatography using stigmastanol as an internal standard.^{4,15}

Cholesterol absorption. Measurement of cholesterol absorption was performed as described.¹⁵ After blood sampling in the morning of day 16, 15-mg [25,26,26,26,27,27,27-D₇]cholesterol (Cambridge Isotopes, Andover, Mass) dissolved in 20% Intralipid was administered intravenous over 30 min into running saline infusion. Each subject was given an oral dose of 75-mg [3,4-¹³C₂]cholesterol (Mass Trace, Woburn, Mass) simultaneously with intravenous cholesterol by dissolving in corn oil at 15 mg/mL and added to an English muffin. Blood samples were collected at 24, 48, and 72 h. RBCs and plasma were separated and stored at –70°C until analyzed. Enrichment of ¹³C and D in the free cholesterol fraction of RBCs was determined using differential IRMS (Isomass, Cheshire, UK). The average ¹³C and D enrichments in 48- and 72-h RBC-free cholesterol relative to baseline (t = 0) samples were used to calculate the CAC.²²

Cholesterol fractional synthesis rate. Cholesterol synthesis rate was measured over a 24-hr period using the method described by Jones et al.²³ On day 19, blood was obtained for baseline body water and erythrocyte cholesterol deuterium enrichment. The subject was then orally dosed with 0.7-g D₂O per kg estimated body water and asked to consume water containing 0.7-g D₂O per kg. The next day, a blood sample for postloading deuterium excess enrichment was obtained. Deuterium enrichments in RBC-free cholesterol and plasma water measured by IRMS were used to calculate FSR.²³

Mononuclear leukocyte LDL receptor and HMG CoA reductase mRNA. Isolymp (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, Tex). Reverse transcription was performed by a standard method to yield cDNA products, and the copy number of LDL receptor and HMG CoA reductase was quantified as previously described.¹⁵

Apolipoprotein genotypes. DNA from peripheral blood was isolated according to instructions in the High Pure PCR Template Preparation Kit (Boehringer Mannheim, Indianap-

olis, Ind). Apolipoprotein E genotype was determined as described by Hixson and Vernier.²⁴ Apolipoprotein A-IV genotype was determined as described by Hixson and Powers.²⁵

Data analysis. As no treatment sequence and carry-over effects were detected, these effects were pooled with the residual error. The percentages of cholesterol solubilized in micelles and vesicles were determined at the various time points using a 3-way mixed model ANOVA with subjects as the random block effect. ANOVA fixed effects were treatment (CDCA and no treatment for study1; DCA and no treatment for study 2) and time of treatment. For all other outcome measurements, the statistical model was a 2-way mixed model ANOVA with subject as the random block effect and treatment as the fixed effect. Area-under-the-curve values were calculated for intraluminal lipid composition using the trapezoidal method.^{26,27} Pearson correlation coefficients were used 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 pre-planned at alpha of 0.05.

RESULTS

During the 3-week periods of each study, subjects maintained their baseline weights and had similar average energy intakes in both study periods. In each study, total dietary cholesterol, saturated fat, polyunsaturated fat, and monounsaturated fat were similar during control and bile acid supplementation periods (data not shown). Upon questioning and food record, subjects were compliant with the diet with few diet violations. No subjects experienced diarrhea or cramps while on bile acid therapy and no sedation-related complications occurred in study 1. Overall, 2 subjects in study 2 dropped out on day 13 because of diarrhea and abdominal cramping, respectively.

Administration of CDCA at a dose of 15 mg/kg/day in divided doses resulted in significant ($P < 0.0001$) enrichment of the biliary bile acids with CDCA, whereas the composition of CA ($P = 0.0046$) and DCA ($P = 0.02$) was decreased (Table I, Fig 1). Peak intraluminal total bile acid concentrations, observed in the 15- to 30-min samples, decreased ($P < 0.0001$) in

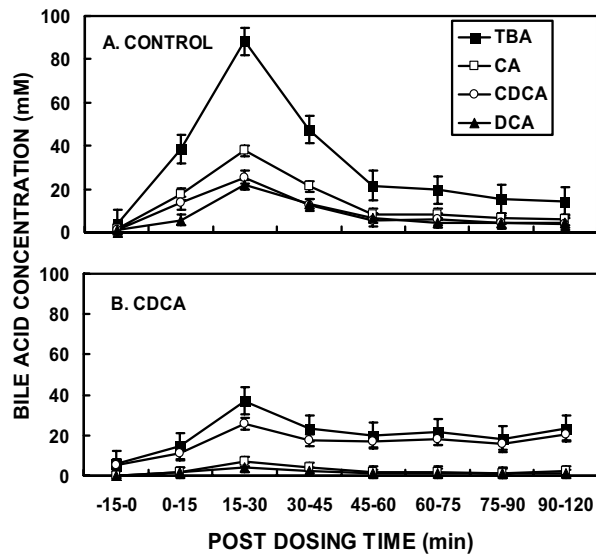


Fig 1. Bile acid concentration in the intestinal lumen of subjects fed a low-fat/cholesterol diet while receiving CDCA or no bile acid supplement. Luminal contents were obtained after an overnight fast with administration of a standardized meal. Bile acid concentrations are shown for subjects consuming no bile acid supplements (A) or CDCA (B). Data are presented as means \pm SEM (n = 11).

the CDCA treated period compared with the control period. Similarly, DCA supplementation resulted in a significant ($P < 0.0001$) enrichment of DCA in biliary bile acids, whereas CA ($P < 0.0001$) and CDCA ($P = 0.002$) were decreased (Table I, Fig 2). DCA treatment did not change the peak intraluminal total bile acid concentrations. Only trace amounts or no UDCA was detected in both studies.

When administered a standardized meal, no differences existed in the area-under-the-curve of intraluminal aqueous phase cholesterol concentration during the entire collection period after CDCA treatment. The values were $105.6 \pm 16.1 \text{ mg/mL} \times 120 \text{ min}$ for CDCA vs $118.7 \pm 16.1 \text{ mg/mL} \times 120 \text{ min}$ for the control period. DCA supplementation tended to increase cholesterol solubilization in the aqueous phase, which were $159.3 \pm 15.4 \text{ mg/mL} \times 120 \text{ min}$ for DCA vs $125.4 \pm 14.4 \text{ mg/mL} \times 120 \text{ min}$ for the control period ($P = 0.15$) (Fig 3). When selected post-meal luminal samples were fractionated into micelles and vesicles, the percent micellar cholesterol was similar during CDCA and control treatments in the -15- to 0- and 15- to 30-min post-meal samples. More cholesterol ($P = 0.03$) was detected in micelles of the 45- to 60-min post-meal samples after CDCA treatment. Administration DCA tended to increase percent micellar cholesterol in the -15- to 0-min ($P = 0.06$) and 15- to 30-min ($P = 0.10$) samples. In the samples collected after the meal, the area-under-the-curve of phospho-

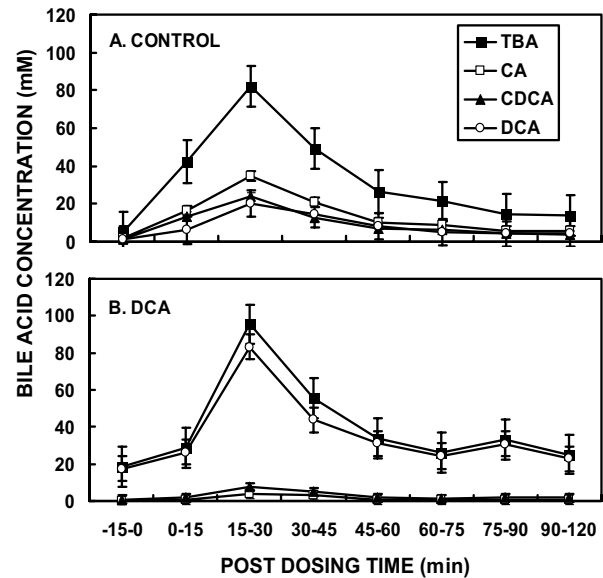


Fig 2. Bile acid concentration in the intestinal lumen of subjects fed a low-fat/cholesterol diet while receiving DCA or no bile acid supplement. Luminal contents were obtained after an overnight fast with administration of a standardized meal. Bile acid concentrations are shown for subjects consuming no bile acid supplements (A) or DCA (B). Data are presented as means \pm SEM (n = 9).

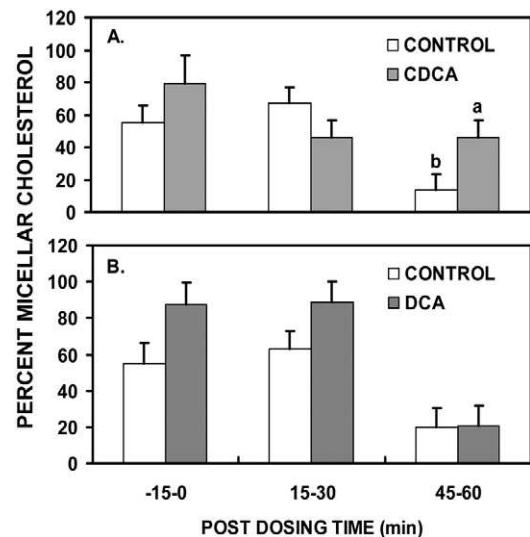


Fig 3. Percent micellar cholesterol in the luminal contents of subjects on CDCA treatment and no bile acid supplementation (A) or DCA and no bile supplementation (B). Superscripts represent significant difference between treatment and control ($P = 0.0097$). Data are presented as means \pm SEM (n = 11).

lipid concentrations was considerably increased after CDCA ($P = 0.009$, $1069.2 \pm 136.0 \text{ mg/mL} \times 120 \text{ min}$ for CDCA-treated periods vs $453.5 \pm 136.0 \text{ mg/mL} \times 120 \text{ min}$ for control periods) and DCA ($P = 0.0004$,

Table II. Effects of chenodeoxycholic and deoxycholic acids on lipid profiles, cholesterol absorption and synthesis, and gene expression of LDL receptor and HMG CoA reductase in healthy humans

	T-C (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)	CAC (%)	FSR (pools/day)	HMG CoA Reductase (mRNA copies/100-ng RNA)	LDL receptor (mRNA copies/100-ng RNA)
Study 1 (CDCA vs Control, n = 11)								
Control	147.9 ± 4.0	42.1 ± 1.8	90.4 ± 3.2	80.0 ± 10.1	60.5 ± 4.9	0.031 ± 0.008	5803 ± 1370	2049 ± 415
CDCA	149.1 ± 4.0	41.1 ± 1.8	92.8 ± 3.2	75.9 ± 10.1	55.4 ± 4.9	0.044 ± 0.008	7428 ± 1370	2270 ± 415
Study 2 (DCA vs Control, n = 9)								
Control	145.6 ± 5.9	43.9 ± 1.1*	87.6 ± 4.6	76.1 ± 12.2	60.0 ± 5.1	0.029 ± 0.007	5048 ± 2001	1802 ± 732
DCA	137.0 ± 5.9	40.1 ± 1.1†	77.3 ± 4.6	98.1 ± 12.2	48.2 ± 5.1	0.047 ± 0.007	11221 ± 2001	3985 ± 732

Notes: Data are means ± SEM. Values bearing different superscripts within each column under each study were significantly different ($P < 0.05$).
Abbreviations: CAC, cholesterol absorption coefficient; FSR, free cholesterol fractional synthetic rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T-C, total cholesterol; TG, triglyceride.

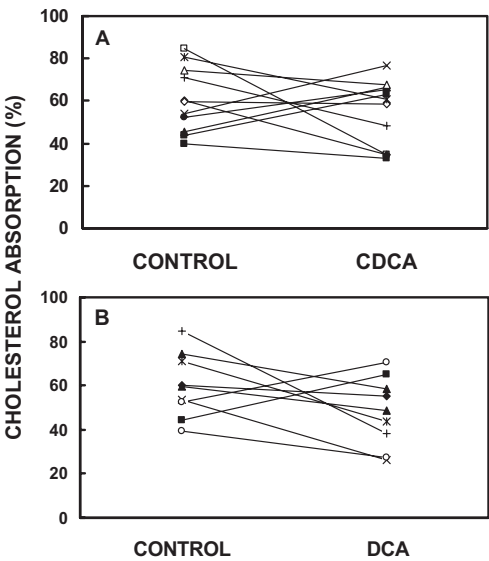


Fig 4. (A) The percent of cholesterol absorption is shown for the same subjects in Table I fed a control diet while receiving CDCA or no supplement. (B) The percent of cholesterol absorption is shown for the same subjects in Table I fed a control diet while receiving DCA or no supplement.

1255.6 ± 101.0 mg/mL × 120 min for DCA-treated periods vs 430.7 ± 101.0 mg/mL × 120 min for control periods) treatments. The concentrations of triglycerides, diacylglycerides, monoglycerides, and free fatty acids was not affected by CDCA treatment (data not shown) and were not measured in DCA study because no alterations had been shown in previous studies of UDCA,⁴ CA,¹⁵ or CDCA.

Cholesterol absorption and FSR were not different between CDCA-treated periods vs control periods (Table II, Fig 4, Fig 5). The expression of LDL receptor and HMG-CoA reductase mRNA was also unchanged after CDCA supplementation (Table II). Consistently, the plasma TC, HDL-C, LDL-C, and TG concentra-

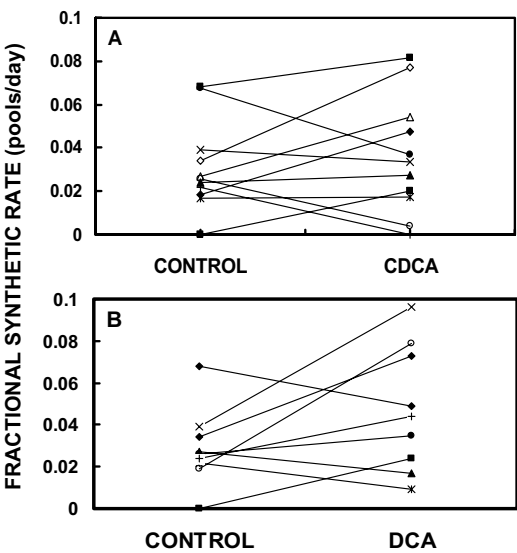


Fig 5. (A) Cholesterol FSR for the same subjects in Table I fed a control diet for 19 days supplemented with CDCA or no bile acids. (B) FSR for the same subjects in Table I fed a control diet for 19 days supplemented with DCA or no supplement.

tions were not changed by CDCA treatment. A trend occurred toward a reduction (19.7%, $P = 0.14$) in cholesterol absorption and an increase in FSR (62.1%, $P = 0.09$) with biliary DCA enrichment as compared with controls (Table II, Fig 4, Fig 5). DCA supplementation also tended to increase LDL receptor (121%, $P = 0.07$) and HMG CoA reductase (122%, $P = 0.06$) mRNA levels (Table II). DCA supplementation decreased ($P < 0.05$) plasma HDL-C and tended to decrease LDL-C ($P = 0.15$), while showing no effect on TC and TG concentrations.

Correlations were examined comparing relationships between changes in various measures with CDCA treatment in study 1 and with DCA treatment in study 2. Specifically, for both studies, no significant correlations

existed between the changes in cholesterol absorption and the changes in area-under-the-curve subphase cholesterol concentration, percent micellar or vesicular cholesterol, FSR, LDL receptor mRNA, or HMG CoA reductase mRNA. With CDCA treatment, the changes of the area-under-the-curve of subphase cholesterol concentration were negatively correlated with the changes in FSR ($r = -0.728$, $P = 0.01$) and HMG CoA reductase mRNA levels ($r = -0.897$, $P = 0.003$). Moreover, after CDCA supplementation, the changes in FSR were positively correlated ($r = 0.728$, $P = 0.04$) with the changes of HMG CoA reductase mRNA, which were correlated with the changes in LDL receptor mRNA ($r = 0.749$, $P = 0.03$). No correlations were detected in DCA treatment between the changes of area-under-the-curve of subphase cholesterol concentration and the changes of FSR or HMG CoA reductase mRNA, and between the changes of FSR and the changes of HMG CoA reductase mRNA or LDL receptor mRNA.

DISCUSSION

The results of this study have demonstrated no significant change in cholesterol absorption or synthesis after CDCA supplementation. A trend occurred toward reduced cholesterol absorption with DCA treatment. As CDCA and DCA are more hydrophobic than CA, enhanced cholesterol solubilization within the luminal aqueous environment would have been anticipated with an increase in micellar incorporation of cholesterol.⁷ As such, increased cholesterol absorption would also have been expected. Instead, no change in the amount of cholesterol solubilized in the luminal aqueous phase was found with either bile acid supplement and no consistent increase in micellar cholesterol was observed after CDCA treatment. Significant reductions in total intraluminal bile acids may, in part, explain why no consistent increase occurred in micellar incorporation of cholesterol in CDCA-treated subjects compared with controls. Paradoxically, a trend of reduction in cholesterol absorption was observed with DCA supplementation even though the aqueous cholesterol solubilization and micellar cholesterol tended to increase and total bile acid concentrations were similar to the controls. The authors' findings suggest that enrichment of the luminal environment with hydrophobic bile acids is not sufficient to enhance cholesterol solubilization and absorption and that other factors must play important roles in this process. Both CDCA supplementation and DCA supplementation increased intraluminal subphase phospholipids, and it is unclear what impact this increase had on cholesterol solubilization. As no impact of CDCA enrichment in bile on cholesterol absorption existed, it is not surprising that the other measures of

cholesterol metabolism, including synthesis (measured directly by FSR and indirectly by monocyte HMG CoA reductase mRNA), and plasma lipids were minimally affected.

Results of the current study confirmed the observation that CDCA supplementation enriched bile significantly with CDCA.^{5,10} Reductions in the peak total bile acid concentration collected after a meal stimulus after CDCA administration might be a result of the decreases in bile acid secretion as observed in a previous study.²⁸ Bile acid secretion was not measured in this study. Alternatively, CDCA enrichment may have altered gall bladder contractility in response to a meal stimulus because CDCA has been shown to inhibit gall bladder muscle cell contraction *in vitro* induced by agonists CCK-8, ACh, and KCl.^{29,30} Future studies will address the impact of CDCA supplementation on cholecystokinin secretion and gall bladder contraction.^{30,31} In agreement with previous studies, DCA supplementation resulted in a considerable increase in intraluminal DCA, whereas peak total bile acid concentrations were not significantly altered after a meal stimulus.³² The current results are partially consistent with a previous study that showed predominant enrichment of DCA in bile and a slight increase of daily bile acid secretion after DCA treatment in normal subjects.³³

Differences in intraluminal phospholipid concentration cannot be explained by the dietary phospholipids because the meal composition for the control and CDCA or DCA study periods was identical. The increased phospholipid concentrations in the intraluminal subphase in CDCA- or DCA-treated subjects may be the result of increased hepatic secretion of phospholipids through increasing coupling of phospholipids with bile acids.³⁴ It has been reported that phospholipids such as phosphatidylcholine inhibit cholesterol absorption.^{35,36} However, the impact of the increased luminal phospholipids could have been attenuated because bile phospholipids are digested in the lumen to lysophospholipids, which are not known to inhibit cholesterol absorption.^{35,36} As the phospholipids and lysophospholipids were not measured separately in the current studies, it is not possible to elucidate if the increased phospholipids in the luminal aqueous phase could have altered cholesterol absorption.

As CDCA is known to decrease biliary cholesterol secretion in normal subjects and patients with gallstones by reducing the proportion of cholesterol relative to the solubilizing lipids-bile acids and lecithin in bile, unchanged fractional cholesterol absorption with CDCA enrichment would indicate that total cholesterol absorption is reduced.^{34,37,38} This notion was not confirmed by this study because CDCA had no effect on the serum cholesterol, which is in accordance with

Table III. Summarized results of effects of bile acid supplementation

BA	Peak TBA (mM)	% cholesterol in micelles (15–30 min)	ABS (%)	FSR (pools/day)
Control	89.5 ± 5.9†	62.8 ± 4.0*†	0.600 ± 0.048†‡	0.029 ± 0.006*†
UDCA	67.9 ± 3.8†	79.8 ± 3.8*	0.614 ± 0.051*†	0.030 ± 0.006*
CA	168.5 ± 5.3*	83.8 ± 2.5*	0.750 ± 0.039*	0.020 ± 0.007*†
CDCA	47.2 ± 3.3†	49.0 ± 2.2†	0.535 ± 0.055†‡	0.038 ± 0.010*†
DCA	96.7 ± 8.3*†	91.4 ± 1.6*	0.482 ± 0.052‡	0.047 ± 0.010†

Notes: Values bearing different superscripts for each parameter are significantly different ($P < 0.02$). Data are mean ± SEM. Data represents peak total bile acid concentrations (TBA); percent cholesterol distributes in micelles; cholesterol absorption (ABS); free fractional cholesterol synthetic rate (FSR) for 9 subjects completing all interventions.

Abbreviations: BA, bile acid; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid.

previous work.^{10,39,40} Reductions of biliary cholesterol secretion in normal subjects after CDCA treatment may be moderate and thus do not have a significant impact on the amount of intestinal cholesterol absorption. Although currently unknown, a possibility also exists that changes in bile acid composition impose differential impact on the absorption of endogenous and exogenous cholesterol.⁴¹ Isotope methods only permit the estimation of exogenous cholesterol absorption.^{10,42} The determination of biliary cholesterol absorption would be important to understanding the impact of different bile acids on cholesterol absorption. The cholesterol absorption and serum cholesterol-reducing effect of DCA enrichment have been reported elsewhere and were supported, to a certain extent, by this study.^{39,42,43}

The authors note that large variations of cholesterol absorption observed in study 1 and study 2 might have resulted in the finding of no significant changes after CDCA and DCA treatments, particularly with DCA supplement. In the case of DCA supplementation and, to a lesser extent, with CDCA supplementation, the sample size may be too small to appreciate these differences and potentially lead to type II statistical error. In addition, none of the previous studies controlled for known variations in cholesterol absorption associated with apo E or apo A-IV genotypes and all had less stringent dietary control than in this investigation.^{16,44,45} Although a recent study showed no difference between different apo E alleles on cholesterol absorption and metabolism,⁴⁶ this observation awaits further confirmation because previous studies demonstrated apo E genotype-related differences in cholesterol absorption.^{44,45,47,48}

In summary, in a healthy population with apo A-IV 1/1 and apo E 3/3 genotypes, supplementation with CDCA or DCA enriched bile with the corresponding bile acid. CDCA, but not DCA consumption, decreased total intraluminal bile acid concentrations after a meal stimulus. CDCA supplementation inconsistently in-

creased post-meal micellar cholesterol; however, CDCA supplementation did not produce a significant effect on cholesterol absorption and plasma lipid concentrations. Supplementation of DCA tended to increase subphase and micellar cholesterol, but paradoxically it tended to decrease cholesterol absorption and plasma LDL-C and HDL-C concentrations. Cholesterol solubilization in the intraluminal aqueous phase and micelles seemed not to be a rate-limiting step for its absorption.

The authors have summarized the results of this study with the 2 previously published studies in which all 9 subjects completed each of the interventions (Table III, comparisons were performed using a paired *t*-test).^{4,15} Enrichment of the bile acid pool with UDCA, CDCA, and DCA had no effect on total intraluminal bile acid concentration, percent micellar cholesterol, cholesterol absorption, and synthesis. CA increased intraluminal total bile acids and cholesterol absorption, although it did not alter cholesterol incorporated into micelles. Significant differences were detected among the 4 different bile acids; however, they were inconsistent. CA supplementation resulted in higher total bile acid concentrations compared with supplementation with CDCA and UDCA. Cholesterol solubilized in micelles after CA feeding was higher than after CDCA treatment but similar to treatment with UDCA and DCA. CA supplementation increased cholesterol absorption compared with control, CDCA, and DCA but not UDCA treatments. FSR was decreased after UDCA supplementation compared with DCA. Cholesterol absorption was decreased with DCA feeding compared with UDCA feeding. The amount of cholesterol distributed in micelles was reduced after CDCA feeding compared with feeding with UDCA, CA, and DCA. Reciprocal changes of FSR relative to absorption observed in these studies serve as internal validations of the measurements of cholesterol absorption. Results of these 4 studies collectively suggest that CA increases cholest-

terol absorption, whereas CDCA, DCA, and UDCA have no effect. Bile acids affect cholesterol absorption through influencing intraluminal total bile acids and cholesterol solubilization in micelles. However, it seems that cholesterol solubilization in micelles is not a rate-limiting factor on cholesterol absorption. Other unknown mechanisms must have also played roles in regulating cholesterol absorption in response to quantitative and qualitative changes of intraluminal bile acid composition.

The authors wish to thank the research subjects; Christopher Vanstone; the nursing bionutrition staff of the GCRC; and, particularly, Suzanne Gilley, RD, Cindy Deeks, RD, Victoria Henize, RD, and Shanthi Rajan for their help completing this research project. The authors would like to acknowledge the Interventional Radiology staff at CCHMC including Lane Donnelly, MD, John Racadio, MD, and Neil Johnson, MD for their assistance in completing this study. The authors appreciate Christine Gurekian and Wing-Yee Fung for the contributions to the measurements of cholesterol absorption and biosynthesis.

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