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Effects of Ursodeoxycholic Acid on Synthesis of Cholesterol and Bile Acids in Healthy Subjects

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Key Words

Bile acid synthesis · Cholesterol metabolism · Serum lipids · Ursodeoxycholic acid

Abstract

Background/Aims: Ursodeoxycholic acid (UDCA) decreases biliary secretion of cholesterol and is therefore used for the dissolution of cholesterol gallstones. It remains unclear whether these changes in biliary cholesterol excretion are associated with changes in cholesterol synthesis and bile acid synthesis. We therefore studied the activities of rate-limiting enzymes of cholesterol synthesis and bile acid synthesis, 3-hydroxy-3-methylglutaryl-coenzyme A reductase and cholesterol 7 α -hydroxylase, respectively, in normal subjects during UDCA feeding. **Methods:** UDCA was given to 8 healthy volunteers (5 men, 3 women; age 24–44 years) in a single dose of 10–15 mg/kg body weight for 40 days. Before and during (days 3, 5, 10, 20, 30 and 40) UDCA treatment, urinary excretion of mevalonic acid and serum concentrations of 7 α -hydroxy-4-cholesten-3-one (7 α -HCO) were determined as markers of cholesterol and bile acid synthesis, respectively. The Wilcoxon signed rank test and Spearman's rank correlation coefficient were used for statistical analysis. **Results:** Cholesterol synthesis and serum lipid concentrations remained unchanged during UDCA treatment for 40 days. However, synthesis of bile acids

increased during long-term treatment with UDCA as reflected by an increase in 7 α -HCO serum concentrations from 39.7 \pm 21.3 ng/ml (median 32.8 ng/ml) before treatment to 64.0 \pm 30.4 ng/ml (median 77.5 ng/ml) at days 30–40 of UDCA treatment ($p < 0.05$). **Conclusions:** UDCA treatment does not affect cholesterol synthesis in the liver, but does increase bile acid synthesis after prolonged treatment. This may represent a compensatory change following decreased absorption of endogenous bile acids as observed with UDCA therapy.

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Introduction

In patients with cholesterol gallstones, treatment with ursodeoxycholic acid (UDCA) decreases biliary secretion of cholesterol and cholesterol saturation of gallbladder bile [1, 2]. Moreover, impaired intestinal absorption of cholesterol has been found by some [3–5], but not all authors [6, 7] during UDCA treatment. It remains unclear, however, whether UDCA therapy also affects cholesterol metabolism. Both stimulatory [8] and inhibitory effects of UDCA on 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase [9, 10], the rate-limiting enzyme of cholesterol synthesis, have been reported in patients with gallstones. The enzymatic activity as well as mRNA levels of cholesterol 7 α -hydroxylase, the rate-lim-

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iting enzyme of the catabolic conversion of cholesterol into bile acids, were found to be unchanged in human liver tissue after short-term treatment with UDCA [11, 12]. However, increased bile acid synthesis rates were observed in one study after ingestion of UDCA for 6 weeks [1]. Studies in rats have shown that mRNA levels of HMG-CoA reductase and cholesterol 7 α -hydroxylase do not change significantly during UDCA feeding [13], while enzymatic activities of HMG-CoA reductase in liver microsomes may increase [14]. In primary human hepatocytes incubated with UDCA, mRNA levels of cholesterol 7 α -hydroxylase remained unchanged [15]. Hence, it remains unclear whether UDCA, either directly by its pharmacological action or secondary to effects on biliary excretion or intestinal absorption of cholesterol, affects the hepatic metabolism of cholesterol.

We therefore studied the activities of two key enzymes of cholesterol and bile acid synthesis during ingestion of UDCA in normal subjects. Urinary excretion of mevalonic acid (MVA), the enzymatic product of HMG-CoA reductase, has been shown to reliably reflect HMG-CoA reductase activity in the liver and was used as a semiquantitative marker of cholesterol synthesis [16]. The activity of cholesterol 7 α -hydroxylase was assessed by determination of serum concentrations of 7 α -hydroxy-4-cholesten-3-one (7 α -HCO), an intermediate of the major metabolic pathway of bile acid synthesis [17, 18]. In addition, we evaluated the effects of UDCA on serum lipid and apolipoprotein (Apo) concentrations.

Subjects and Methods

Subjects and Study Protocol

UDCA was given to 8 healthy subjects (5 men, 3 women; age 24–44 years) for 40 days as a single dose of 10–15 mg/kg body weight in the evening. The body weight of the subjects was between 54 and 95 kg (median 77 kg), with a body mass index between 21.4 and 30.7 kg/m² (median 23.2 kg/m²). Blood was drawn in the morning after a fasting period overnight, and urine was collected overnight for 12 h on days 3, 5, 10, 20, 30 and 40 of UDCA treatment. Mean values from two time points (before treatment, days 3 and 5, days 10 and 20, days 30 and 40) were used for further calculations. At all of these time points, 20 ml of serum was preserved for determinations of serum lipoprotein profiles, Apo B and Apo A1.

Analytical Methods

Urinary MVA was assayed as a marker of whole-body cholesterol synthesis by combined gas chromatography-mass spectrometry using the isotope dilution method described by Lindenthal et al. [16] and Lindenthal and von Bergmann [19]. Briefly, 100 ng of ²H₇-MVA (MSD Isotopes, Montreal, Canada) was added as internal standard to 400 μ l of urine. After extraction, purification and derivatization with

methyl-tertiary-butyl-dimethylsilyl-trifluoroacetamide, the concentration of MVA was analyzed by gas chromatography-mass spectrometry on a Finnigan 4000 quadrupole mass spectrometer, SIM mode. The ion m/z 433 (base peak) was scanned in the selected ion monitoring mode for endogenous MVA and the ion m/z 440 for ²H₇-MVA. Creatinine in urine was measured by a standard laboratory procedure.

7 α -HCO in serum as a marker of bile acid synthesis was analyzed by high-performance liquid chromatography (HPLC) according to the method of Axelson et al. [20]. Briefly, 7 α -HCO was extracted in jacketed glass columns connected to a water bath on octadecylsilane-bonded silica at 64°C. After a wash with water and 65% aqueous methanol, 7 α -HCO was eluted with hexane-chloroform (80:20, vol/vol). Analysis was performed by HPLC on a column (250 \times 4.5 mm) of LiChrospher 100 RP-18 (Merck, Darmstadt, Germany) connected to a UV detector (SPD-10A, Shimadzu, Kyoto, Japan) at a wavelength of 254 nm. Acetonitrile-water (98:2, vol/vol) was used as the mobile phase at a flow rate of 1 ml/min. Serum concentrations of 7 α -HCO were calculated from known amounts of 7 β -HCO as internal standard.

Very-low-density lipoproteins (VLDL) were separated by ultracentrifugation (45,000 rpm, 20 h, 4°C, Beckman rotor Ti 50, density 1.006 g/ml). High-density lipoprotein (HDL) cholesterol was determined in the infranatant after heparin-manganese precipitation of Apo B-containing lipoproteins. Low-density lipoprotein (LDL) cholesterol was calculated by subtracting HDL cholesterol from total infranatant cholesterol. Triglyceride and cholesterol levels in serum and lipoprotein fractions were measured enzymatically by an auto-analyzer (EPOS, Eppendorf, Hamburg, Germany) using reagents from Boehringer (Mannheim, Germany). Apo B and Apo A1 concentrations were determined by nephelometry (Behring, Marburg, Germany) using antibodies against human Apo B and Apo A1 (anti-Apo B, anti-Apo A1 from rabbit, Dade Behring, Marburg, Germany).

Data are given as mean \pm SD or median. The Wilcoxon signed ranks test for paired samples and Spearman's rank correlation coefficient were used for statistical analysis. Data analysis was performed with the SPSS for Windows program 10.0 (SPSS Inc., Chicago, Ill., USA). *p* values of less than 0.05 were considered statistically significant.

Results

Concentrations of urinary MVA were not affected by UDCA treatment (fig. 1). In contrast, serum concentrations of 7 α -HCO increased from 39.7 \pm 21.3 ng/ml (median 32.8 ng/ml) before treatment to 64.0 \pm 30.4 ng/ml (median 77.5 ng/ml) at days 30–40 of UDCA treatment (*p* < 0.05) (fig. 2). Serum lipoprotein concentrations (cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, VLDL cholesterol) remained unchanged with the exception of VLDL triglycerides, which transiently decreased from 85 to 64 mg/dl at days 10–20 of UDCA treatment (*p* < 0.05) (table 1). Before treatment, serum concentrations of VLDL triglycerides were positively correlated with serum concentrations of MVA (*n* = 8; *r* = 0.79, *p* =

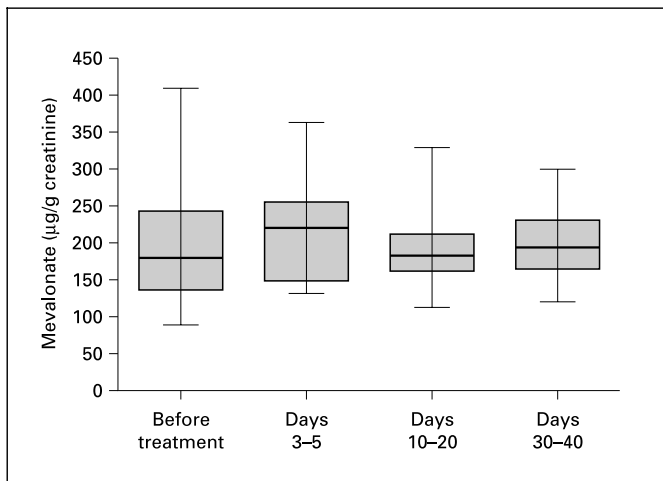


Fig. 1. Urinary MVA excretion as a marker of whole-body biosynthesis of cholesterol before and during treatment with UDCA (box and whiskers plots). Cholesterol synthesis remained unchanged during UDCA feeding.

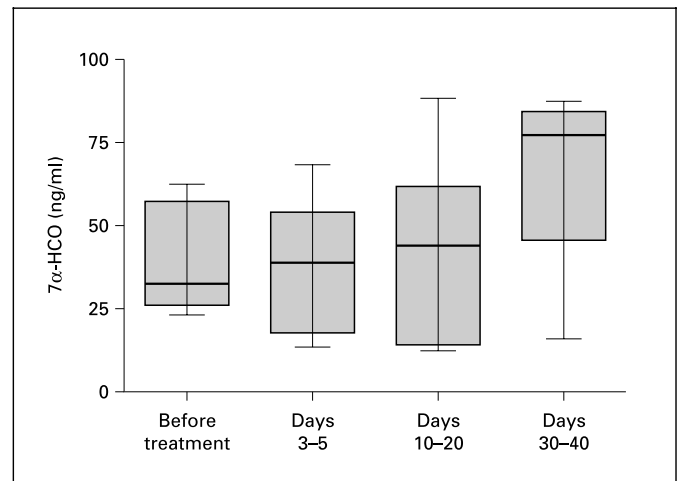


Fig. 2. Serum levels of 7α-HCO as a marker of bile acid synthesis before and during UDCA feeding (box and whiskers plots). Serum levels of 7α-HCO remained constant within the first 3 weeks of UDCA treatment but increased at days 30–40.

Table 1. Serum concentrations of cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, VLDL cholesterol, VLDL triglycerides, Apo A1 and Apo B of 8 volunteers who ingested UDCA for 40 days

	Before treatment	Days 3–5	Days 10–20	Days 30–40
Cholesterol, mg/dl	172 ± 31	170 ± 26	173 ± 21	171 ± 22
Triglycerides, mg/dl	92 ± 50	86 ± 33	91 ± 28	98 ± 22
HDL cholesterol, mg/dl	54 ± 13	53 ± 13	58 ± 13	55 ± 11
LDL cholesterol, mg/dl	104 ± 26	101 ± 24	99 ± 21	100 ± 21
VLDL cholesterol, mg/dl	13 ± 5	15 ± 5	15 ± 6	17 ± 5
VLDL triglycerides, mg/dl	85 ± 43	66 ± 34	64 ± 28*	75 ± 19
Apo A1, mg/dl	157 ± 25	155 ± 23	164 ± 32	161 ± 27
Apo B, mg/dl	91 ± 18	85 ± 19	86 ± 19	87 ± 17

Data are given as mean ± SD. * p < 0.05 compared to pretreatment values.

0.021). Correlations between MVA and 7α-HCO in serum as well as correlations between each of these two parameters and body weight, body mass index and age did not reach statistical significance.

Discussion

In the present study, bile acid synthesis was found to increase in normal subjects after ingestion of UDCA for more than 4 weeks. The activity of HMG-CoA reductase, the rate-limiting enzyme of cholesterol synthesis, remained unchanged during UDCA treatment.

Our interpretations are based on the finding that treatment with UDCA for more than 4 weeks increased serum concentrations of 7α-HCO in healthy subjects. Serum concentrations of 7α-HCO have been shown to correlate closely with hepatic cholesterol 7α-hydroxylase activity in human liver [17] as well as with total synthesis of primary bile acids as measured by the isotope dilution technique [18]. Hence, the present data provide evidence that UDCA enhances the conversion of cholesterol into bile acids after prolonged treatment. Using the synthetic bile acid analogue ⁷⁵selenium homocholeic acid taurine, Eusufzai et al. [21] found a dose-dependent reduction of intestinal bile acid reabsorption during administration of UDCA for 3 weeks. Moreover, Stiehl et al. [22] reported

impaired absorption of endogenous bile acids in patients with ileostomies who had received a single dose of UDCA. These findings are in agreement with kinetic studies, which have shown increased fractional turnover rates of cholic acid and chenodeoxycholic acid during UDCA treatment [1, 2, 4, 23, 24]. These kinetic studies have also shown that pool sizes of endogenous bile acids decrease with UDCA treatment. However, there are conflicting data regarding changes in bile acid synthesis, which would be expected to increase in order to compensate for the increased loss of bile acids. While bile acid synthesis was reported to increase by 40–80% during UDCA treatment for 5–6 weeks in one study [1], unchanged cholesterol 7 α -hydroxylase activity was found under short-term conditions [8, 12, 17], and even decreased synthesis rates have been reported [23]. Hardison and Grundy [4] used a balance technique in which they measured both the daily fecal output of bile acids and the incorporation of injected [¹⁴C]cholesterol into bile acids. They found that mean total bile acid synthesis increased from 650 to 800 mg/day when UDCA (15 mg/kg day) was administered for 1 month.

In the present study, bile acid synthesis remained unchanged within the first weeks of the study, but increased after more than 4 weeks. This finding supports the hypothesis that the effects of UDCA on cholesterol and bile acid metabolism may change over time [8]. The lack of short-term effects indicates that UDCA either has no direct effect on bile acid synthesis or moderately inhibits bile acid synthesis, thereby neutralizing the stimulatory effect of bile acid malabsorption in the first weeks of UDCA treatment. This hypothesis is in agreement with the fact that UDCA binds only weakly to the farnesoid X receptor, which has been shown to inhibit cholesterol 7 α -hydroxylase gene expression after binding of endogenous bile acids [25, 26]. Since UDCA treatment results in a continuous loss of endogenous bile acids, the observed increase in cholesterol 7 α -hydroxylase activity is probably due to an increasing shortage of endogenous bile acids over time.

Whole-body cholesterol synthesis as measured by urinary mevalonate excretion remained unchanged during the 40-day regimen of UDCA treatment in our study. This finding is in agreement with data of Angelin et al. [27], who observed unchanged hepatic HMG-CoA reductase activity in patients with gallstones after 6 weeks of treatment with UDCA. However, Maton et al. [9] found that hepatic HMG-CoA reductase activity was reduced to 35% after 3–5 months of UDCA treatment, and Salen et al. [10] reported a threefold decrease in 2 patients after 1 year of UDCA treatment. Even though data on changes in

hepatic HMG-CoA reductase activity after longer treatment periods are conflicting, decreased biliary excretion of cholesterol obviously does not affect cholesterol synthesis under short-term conditions. These findings correspond to recent observations of Hillebrant et al. [28], who studied the effects of combined treatment with pravastatin and UDCA on hepatic cholesterol metabolism. Pravastatin did not further reduce the cholesterol saturation of bile in patients with gallstones treated with UDCA, although hepatic cholesterol synthesis was inhibited. Our results support the concept that *de novo* synthesis of cholesterol is not particularly important for biliary cholesterol secretion in humans.

The effects of UDCA on serum lipoprotein profiles have mainly been studied in patients with gallstone disease. Erlinger et al. [29] treated 197 patients at daily doses of 2.1–16.2 mg/kg for up to 1 year and observed no significant influence of UDCA treatment on serum cholesterol and triglycerides. Fromm et al. [30] found no change in serum cholesterol and triglyceride in serum in patients with gallstones who were fed UDCA for 1 year at doses of 400–800 mg/day. In the present study on normal subjects, we were unable to detect changes in cholesterol and triglyceride in serum, including LDL cholesterol, HDL cholesterol and VLDL cholesterol. However, there was a transient decrease in VLDL triglycerides at days 10–20. This finding is in agreement with studies demonstrating an inhibitory effect of bile acids, including UDCA, on VLDL lipoprotein secretion in rat hepatocytes in primary culture [31]. Moreover, serum concentrations of VLDL triglycerides were positively correlated with serum concentrations of MVA before, but not during UDCA treatment. A similar correlation between cholesterol synthesis and secretion of VLDL Apo B was described by Watts et al. [32] in normolipidemic subjects, indicating that *in vivo* cholesterol synthesis is a determinant of hepatic secretion of Apo B.

In conclusion, the present study shows that ingestion of UDCA moderately increases bile acid synthesis after treatment periods of 4–6 weeks. Moreover, from the transient decrease in VLDL triglycerides during UDCA feeding, we cannot exclude the possibility that UDCA affects VLDL secretion.

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