Significance of Plasma 7α-Hydroxy-4-cholesten-3-one and 27-Hydroxycholesterol Concentrations as Markers for Hepatic Bile Acid Synthesis in Cholesterol-Fed Rabbits

Akira Honda, Tadashi Yoshida, Guorong Xu, Yasushi Matsuzaki, Sugano Fukushima, Naomi Tanaka, Mikio Doy, Sarah Shefer, and Gerald Salen

Plasma 7α-hydroxy-4-cholesten-3-one has been used as an index of hepatic bile acid synthesis. The aim of the current study was to ascertain whether the level of this oxysterol reflects hepatic cholesterol 7α-hydroxylase activity when plasma cholesterol concentrations are markedly changed. In addition, the relationship of hepatic sterol 27-hydroxylase activity with plasma concentrations of 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid was studied. We used New Zealand white rabbits fed 2% cholesterol for 5 or 10 days and/or constructed bile fistula. Feeding cholesterol markedly increased and bile drainage reduced plasma cholesterol concentrations. Initially, in these models there was no correlation between plasma 7α-hydroxy-4-cholesten-3-one concentrations and hepatic cholesterol 7α-hydroxylase activities ($r = -0.24$, n = 10). Cholesterol feeding was associated with downregulated 7α-hydroxylase activities, while plasma 7α-hydroxy-4-cholesten-3-one concentrations were elevated in the presence of increased plasma cholesterol levels. However, this discrepancy was overcome and significant correlation was observed ($r = 0.73$, $P < .05$, n = 10) by expressing 7α-hydroxy-4-cholesten-3-one levels relative to cholesterol. In contrast, hepatic sterol 27-hydroxylase activities were not significantly correlated with plasma absolute ($r = 0.23$, difference not significant [NS], n = 10) nor cholesterol-related levels of 27-hydroxycholesterol ($r = -0.13$, NS, n = 10), or 3β-hydroxy-5-cholestenoic acid concentrations ($r = 0.30$, NS, n = 10). In conclusion, plasma 7α-hydroxy-4-cholesten-3-one concentrations reflected hepatic cholesterol 7α-hydroxylase activities when the sterol levels were adjusted to plasma cholesterol concentrations in rabbits with hypercholesterolemia. The results suggest that plasma 7α-hydroxy-4-cholesten-3-one relative to cholesterol is a better marker for hepatic cholesterol 7α-hydroxylase activity than the absolute concentration when hypercholesterolemia is present. In contrast, 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid levels in plasma did not reflect hepatic sterol 27-hydroxylase activities even if the levels were adjusted to plasma cholesterol concentrations.

THE BILE ACID biosynthetic pathway is initiated by either hepatic 7α-hydroxylation or hepatic and extrahepatic 27-hydroxylation of cholesterol[1] (Fig 1). The former is catalyzed by microsomal cholesterol 7α-hydroxylase (CYP7A1), which is the rate-limiting enzyme in the classic pathway, while the latter is catalyzed by mitochondrial sterol 27-hydroxylase (CYP27A1), a key enzyme in the alternative pathway. Since the conversion of cholesterol to bile acids is a major reaction for the catabolism of cholesterol in the body,[2] measurements of these enzyme activities are clinically useful for exploring the mechanisms of hypercholesterolemia[3] and atherosclerosis.[4,5]

As determining cholesterol 7α-hydroxylase activity in liver specimens is potentially dangerous because liver biopsies are required, plasma concentrations of 7α-hydroxycholesterol[6-8] and 7α-hydroxy-4-cholesten-3-one[9,10] have been proposed as markers of this enzyme's activity. In addition, it was proven that serum 7α-hydroxycholesterol[11] and 7α-hydroxy-4-cholesten-3-one levels[12] reflected bile acid synthesis in humans. We have compared the 2 markers and have chosen 7α-hydroxy-4-cholesten-3-one as the preferred index of hepatic cholesterol 7α-hydroxylase activity because unlike 7α-hydroxycholesterol, this sterol is not formed by cholesterol autoxidation and the coefficient of correlation with cholesterol 7α-hydroxylase activity was higher than 7α-hydroxycholesterol.[10]

A single enzyme, sterol 27-hydroxylase, catalyzes the conversions of cholesterol to 27-hydroxycholesterol and 27-hydroxycholesterol into 3β-hydroxy-5-cholestenoic acid[13] in the alternative pathway. Plasma concentrations of 27-hydroxycholesterol[14-16] or 3β-hydroxy-5-cholestenoic acid[17,18] have been measured in several studies. However, production of bile acids by the alternative pathway may constitute more than 50% of total bile acid synthesis in rats[19] and mice,[20] but only contributes less than 10% in humans.[21] In addition, sterol 27-hydroxylase is expressed not only in the liver, but also in extrahepatic tissues including vascular endothelium and macrophages.[22,23] A recent report showed that up to 30% to 40% of 27-hydroxycholesterol and more than 80% of 3β-hydroxy-5-cholestenoic acid in human plasma originated from the lung.[24] Thus, the interpretation of plasma levels of 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid to bile acid synthesis is limited.

Since the major part of plasma oxysterols is found in lipoprotein fractions,[25] not only production rates of oxysterols but also plasma cholesterol level seem to affect plasma oxysterol levels. Therefore, it may be possible that plasma 7α-hydroxy-4-cho-

From the Ibaraki Prefectural Institute of Public Health, Mito, Japan; Department of Gastroenterology and Hepatology, University of Tsukuba, Tsukuba; Tsukuba Memorial Hospital, Tsukuba, Japan; Department of Medicine, Gastrointestinal Division, and Liver Center, University of Medicine and Dentistry of New Jersey-New Jersey Medical School, Newark, NJ; and the Veterans Affairs Medical Center, East Orange, NJ.

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Address reprint requests to Akira Honda, MD, PhD, Ibaraki Prefectural Institute of Public Health, 993-2 Kasahara-cho, Mito, Ibaraki 310-0852, Japan.

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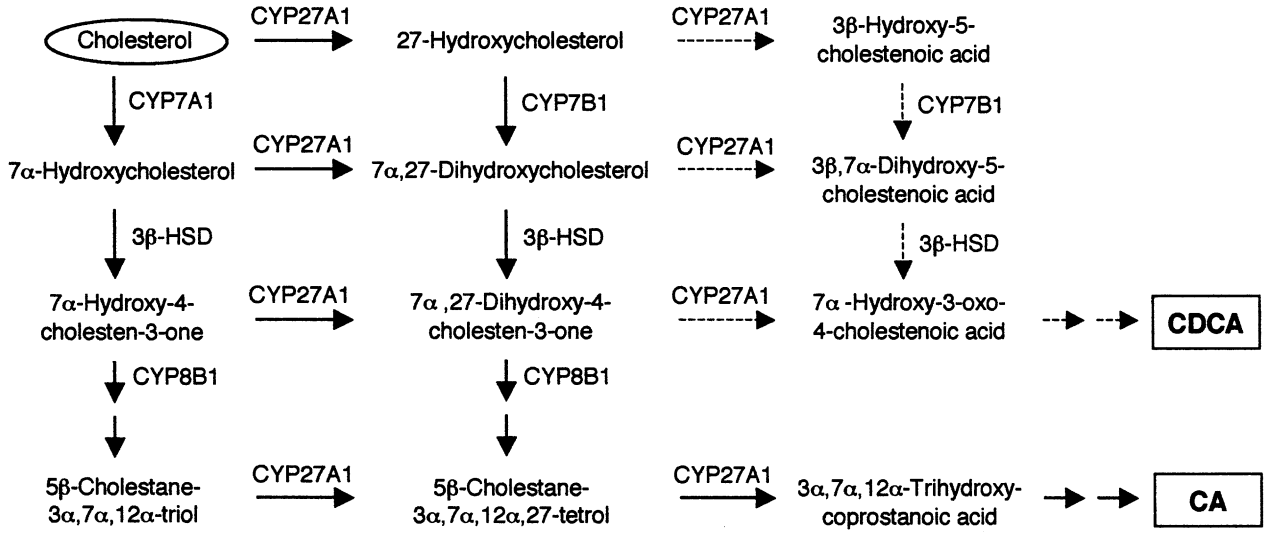

Fig 1. Bile acid biosynthesis from cholesterol initiated either by 7α-hydroxylation (classic pathway) or 27-hydroxylation (alternative pathway). Solid arrows represent major pathways while broken arrows represent minor pathways in rabbits. CYP7A1, cholesterol 7α-hydroxylase; CYP27A1, sterol 27-hydroxylase; CYP7B1, oxysterol 7α-hydroxylase; 3β-HSD, 3β-hydroxy-Δ^5-C_{27}-steroid dehydrogenase/isomerase; CYP8B1, 12α-hydroxylase; CDCA, chenodeoxycholic acid; CA, cholic acid.

lesten-3-one concentration may not reflect hepatic cholesterol 7α-hydroxylase activity in patients with hypercholesterolemia. The present study was undertaken to ascertain in rabbits if plasma 7α-hydroxy-4-cholesten-3-one levels reflect hepatic cholesterol 7α-hydroxylase activities when plasma cholesterol levels are perturbed. In addition, the relationship between hepatic sterol 27-hydroxylase activity and plasma concentrations of 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid were studied.

MATERIALS AND METHODS

Materials

Cholesterol was purchased from Sigma Chemical Co (St Louis, MO) and was purified 3 times by recrystallization. 7α-Hydroxycholesterol was obtained from Steraloids (Wilton, NH). 7α-Hydroxy-4-cholesten-3-one was a gift from Drs T. Hoshita and K. Kihira (Pharmaceutical Institute, Hiroshima University, Hiroshima, Japan). 27-Hydroxycholesterol was synthesized from diosgenin[26] and the pure compound was obtained by preparative thin-layer chromatography (TLC). [$^2H_7$]cholesterol was obtained from MSD Isotopes (Montreal, Canada). [$^2H_7$]7α-hydroxy-4-cholesten-3-one,[10,27] [$^2H_7$]27-hydroxycholesterol,[28] and [$^2H_7$]3β-hydroxy-5-cholestenoic acid[28] were synthesized by previously described methods.

Regular rabbit chow that contained less than 0.001% (wt/wt) cholesterol and chow containing 2% cholesterol were obtained from Purina Mills (St Louis, MO). Both chow diets contained 0.57% (wt/wt) saturated fatty acids, 0.66% (wt/wt) monounsaturated fatty acids, and 0.97% (wt/wt) polyunsaturated fatty acids.

Animal Experiments

Sixteen male New Zealand white rabbits (2.5 to 3.2 kg body weight) were purchased from Hazleton Labs (Denver, PA) and fed a regular rabbit chow diet. Eight of the rabbits were fed 2% cholesterol, which was incorporated into the regular chow so that about 3 g/d of cholesterol was fed for 5 or 10 days. After completion of cholesterol feeding, bile fistulas were constructed in 4 rabbits as described previously[29] under anesthesia (ketamine, 40 mg/kg body weight, combined with xylazine, 4 mg/kg body weight, administered intramuscularly). During 7 days of bile drainage, the animals were fed regular rabbit chow (without cholesterol) and were given lactated Ringer's solution with 5% dextrose intravenously at 12 mL/h in the first 24 hours, but this was then replaced by 0.9% NaCl. This infusion was continued until the experiment was finished to replace the loss of body fluid and electrolyte and to prevent acidosis. All rabbits were then killed under anesthesia and liver and blood were collected. Liver was immediately frozen and stored at −70°C until used. Plasma was stored at −20°C until analyzed. The animal protocol was approved by Subcommittee on Animal Studies at VA Medical Center (East Orange, NJ) and Institutional Animal Care and Use Committee at University of Medicine and Dentistry of New Jersey-New Jersey Medical School (Newark, NJ).

Determination of Plasma Cholesterol Concentration

Plasma cholesterol was measured by high-resolution gas chromatography–mass spectrometry (GC-MS) with selected-ion monitoring (SIM). Fifty micrograms of [$^2H_7$]cholesterol was added to 10 to 100 μL of plasma as an internal recovery standard, and alkaline hydrolysis was carried out in 1 mL of 1N ethanolic KOH at 60°C for 1 hour. After addition of 0.5 mL of distilled water, sterols were extracted twice with 2 mL of *n*-hexane, and the extract was evaporated to dryness under nitrogen. The extracted sterols were then converted into trimethylsilyl (TMS) ethers with 100 μL of TMSI-H (GL Sciences, Tokyo, Japan) for 15 minutes at 55°C. GC-MS with SIM was performed with a JMS-SX102 instrument equipped with a JMA DA-6000 data-processing system (JEOL, Tokyo, Japan). The accelerating voltage was 10 kV, the ionization energy was 70 eV, the trap current was 300 μA, and the mass spectral resolution was about 10,000. An Ultra Performance capillary column (25 m × 0.32 mm inner diameter) coated with methylsilicone (Agilent Technologies, Palo Alto, CA) was used at a flow rate of helium carrier gas of 1.0 mL/min. The column oven was programmed to change from 100°C to 260°C at 30°C/min, after a 1-minute delay from the start time. The multiple ion detector was focused on *m/z* 329.3208 for cholesterol and *m/z* 336.3647 for [$^2H_7$]cholesterol.

Determination of Plasma 7α-Hydroxy-4-cholesten-3-one Concentration

Plasma 7α-hydroxy-4-cholesten-3-one levels were measured as described previously by Yoshida et al.[10] In brief, [$^2H_7$]7α-hydroxy-4-cholesten-3-one (7 ng) was added to 200 μL of plasma as an internal standard. Sterols were extracted from plasma by salting-out procedure and 7α-hydroxy-4-cholesten-3-one was purified by serial solid-phase extractions using Bond Elut C18 and SI cartridges (Varian, Harbor City, CA). After the addition of *O*-methylhydroxylamine hydrochloride and dimethylethylsilyl (DMES) imidazole (Tokyo Kasei Kogyo, Tokyo, Japan), high-resolution GC-SIM analysis was performed.

Determination of Plasma 27-Hydroxycholesterol and 3β-hydroxy-5-cholestenoic Acid Concentrations

Determination of plasma 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid levels was based on the methods of Honda et al[30] with minor modifications. Briefly, 64 ng of [$^2H_7$]27-hydroxycholesterol and 13 ng of [$^2H_7$]3β-hydroxy-5-cholestenoic acid were added to 200 μL of plasma as internal recovery standards, and alkaline hydrolysis was performed in 1 mL of 1N ethanolic KOH at 60°C for 1 hour. After saponification, 27-hydroxycholesterol was extracted with *n*-hexane, purified by Bond Elut SI cartridge, derivatized to DMES ether, and measured by high-resolution GC-SIM. The mass spectral resolution was about 10,000. The resulting aqueous phase of n-hexane extraction was diluted with 5 mL of 0.5 mol/L sodium phosphate buffer (pH 7.0) and applied to Bond Elut C18 cartridge (500 mg).[31] The cartridge was washed with 6 mL of water, and 3β-hydroxy-5-cholestenoic acid was then eluted with 5 mL of ethanol. After ethylation and dimethylethylsilylation performed as described previously,[31] the sample was analyzed by high-resolution GC-SIM.[30]

Assay for Cholesterol 7α-Hydroxylase and Sterol 27-Hydroxylase Activities

Hepatic microsomes and mitochondria were prepared by differential ultracentrifugation[32] and the protein determined according to the method of Lowry et al.[33] The activities of microsomal 7α-hydroxylation of cholesterol and mitochondrial 27-hydroxylation of cholesterol were measured by isotope incorporation methods by using endogenous cholesterol as substrate.[34,35]

Statistics

Data are reported here as the mean ± SEM. Correlation between hepatic enzyme activity and plasma level of corresponding sterol was checked by simple linear regression. For all comparisons, significance was accepted at the level of $P < .05$.

RESULTS

The data presented in Table 1 show hepatic cholesterol 7α-hydroxylase activity and plasma concentrations of cholesterol and 7α-hydroxy-4-cholesten-3-one in each rabbit. Feeding 2% cholesterol (3 g/d) for 5 or 10 days markedly increased plasma cholesterol levels and inhibited hepatic cholesterol 7α-hydroxylase activity. Bile drainage after cholesterol feeding reduced the elevated plasma cholesterol levels and upregulated hepatic cholesterol 7α-hydroxylase activities. Plasma 7α-hydroxy-4-cholesten-3-one concentrations were increased not only after bile drainage but also before drainage after cholesterol feeding. Therefore, plasma 7α-hydroxy-4-cholesten-3-one concentrations were not significantly correlated with hepatic cholesterol 7α-hydroxylase activities ($r = -0.24$, difference not significant [NS], n = 10; Fig 2A). However, when 7α-hydroxy-4-cholesten-3-one levels were expressed relative to cholesterol instead of the absolute concentrations, significant positive correlation between plasma relative 7α-hydroxy-4-cholesten-3-one levels and hepatic cholesterol 7α-hydroxylase activities were obtained ($r = 0.73$, $P < .05$, n = 10; Fig 2B). Correlation coefficient between plasma concentrations of cholesterol and 7α-hydroxy-4-cholesten-3-one was calculated ($r = 0.36$, n = 10), but it was not statistically significant.

Table 1. Hepatic Cholesterol 7α-Hydroxylase Activity and Plasma Concentrations of Cholesterol and 7α-Hydroxy-4-cholesten-3-one in Rabbits Treated With CHOL Feeding and/or Bile Drainage

	Treatments		CHOL 7αOHase Activity	Plasma Sterols	
Rabbit No.	CHOL*	BD†	(pmol/min/mg)	CHOL (mg/dL)	7α-3-one (ng/mL)
1-7	—	−	31 ± 5	41 ± 5	0.46 ± 0.10
8	5 d	−	22	914	2.3
9	5 d	−	19	333	3.8
10	5 d	−	16	215	0.60
11	10 d	−	4.8	859	1.3
12	10 d	−	9.5	500	5.3
13	—	+	78	24	0.51
14	5 d	+	71	196	2.7
15	10 d	+	50	369	3.0
16	10 d	+	36	128	0.65

Abbreviations: CHOL 7αOHase, cholesterol 7α-hydroxylase; CHOL, cholesterol; 7α-3-one, 7α-hydroxy-4-cholesten-3-one; BD, bile drainage.

*Fed 2% cholesterol in chow for 5 or 10 days.

†Continued bile drainage for 7 days after cholesterol treatments.

Hepatic sterol 27-hydroxylase activity and plasma concentrations of 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid are listed in Table 2. Cholesterol feeding upregulated hepatic sterol 27-hydroxylase activity while bile drainage after cholesterol feeding decreased the up-regulation of sterol 27-hydroxylase activity. No correlation was observed between plasma 27-hydroxycholesterol levels and hepatic sterol 27-hydroxylase activities ($r = 0.23$, NS, n = 10; Fig 3A). This observation persisted even when the levels of 27-hydroxycholesterol relative to cholesterol were related to hepatic sterol 27-hydroxylase activities ($r = -0.13$, NS, n = 10; Fig 3B). Also no significant correlation was found between plasma 3β-hydroxy-5-cholestenoic acid concentrations and hepatic sterol 27-hydroxylase activities ($r = 0.30$, NS, n = 10; Fig 3C). 3β-Hydroxy-5-cholestenoic acid is a metabolite of 27-hydroxycholesterol produced by sterol 27-hydroxylase and is not transported in lipoprotein fractions but in lipoprotein-free plasma.[25] We calculated correlation coefficients between plasma cholesterol level and concentrations of 27-hydroxycholesterol ($r = 0.48$, n = 10) or 3β-hydroxy-5-cholestenoic acid ($r = 0.45$, n = 10). However, these values were not statistically significant.

DISCUSSION

A recent study showed that not only abnormal lipoprotein metabolism but also an inherited defect of cholesterol 7α-hydroxylase causes hypercholesterolemia in humans.[3] Therefore, evaluation of cholesterol 7α-hydroxylase activities will become more important for diagnosis of the cause of hyper-

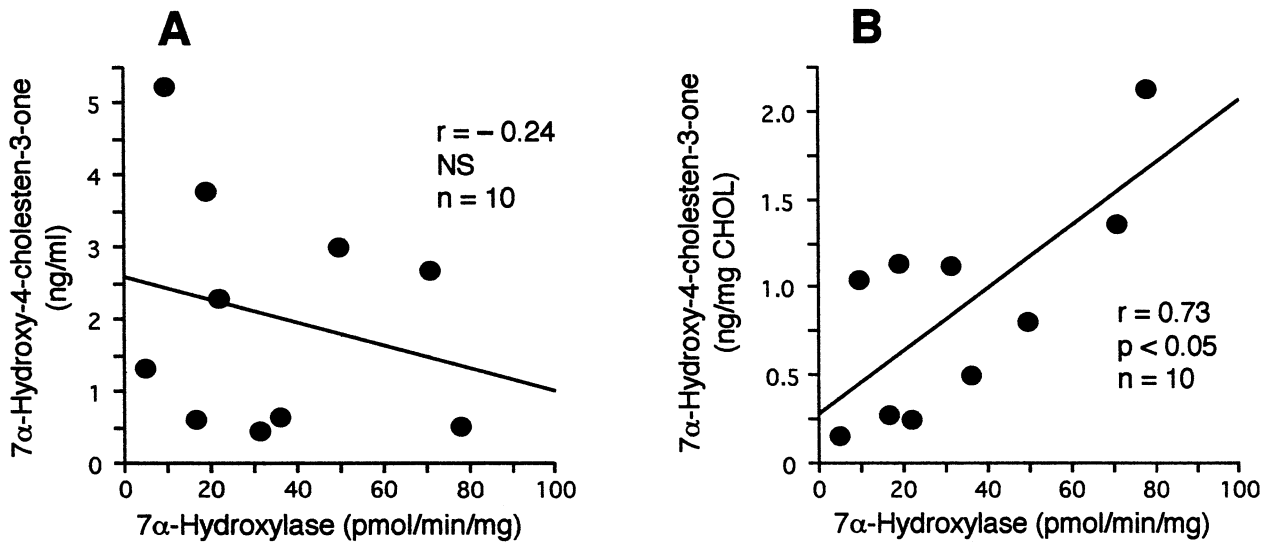

Fig 2. Relationship of hepatic cholesterol 7α-hydroxylase activity with plasma absolute concentration of 7α-hydroxy-4-cholesten-3-one (A) and plasma 7α-hydroxy-4-cholesten-3-one level expressed relative to cholesterol (B) in rabbits fed high-cholesterol diet and/or constructed bile fistula.

cholesterolemia. It is well established that determination of plasma 7α-hydroxy-4-cholesten-3-one concentration is useful as a marker for hepatic cholesterol 7α-hydroxylase activity[9,10] and bile acid synthesis.[12] However, our results demonstrated that plasma 7α-hydroxy-4-cholesten-3-one concentrations were not significantly correlated with hepatic cholesterol 7α-hydroxylase activities in rabbits treated with high-cholesterol diet and/or constructed bile fistula (Fig 2A). Since plasma oxysterols including 7α-hydroxy-4-cholesten-3-one are transported in lipoproteins, it may be possible that plasma 7α-hydroxy-4-cholesten-3-one concentrations are affected by the half-life of plasma lipoproteins. Cholesterol 7α-hydroxylase activities might be overestimated in patients with hyperlipoproteinemia and be underestimated in those with hypolipoproteinemia. The major aim of this study was to test this hypothesis.

Plasma cholesterol concentrations are easily altered by cholesterol feeding or bile drainage in rabbits.[36] Cholesterol feeding increases plasma cholesterol levels markedly and down-regulates hepatic cholesterol 7α-hydroxylase activities in this animal.[37] Note cholesterol feeding in mice and rats does not increase plasma levels because cholesterol 7α-hydroxylase activity is increased to convert cholesterol to bile acids. This feature in rabbits was advantageous for our experiment because we could easily detect an overestimation of cholesterol 7α-hydroxylase activity associated with increased plasma 7α-hydroxy-4-cholesten-3-one levels in cholesterol-fed rabbits.

Another aim of this study was to find the method for correcting the overestimation of cholesterol 7α-hydroxylase activities in cholesterol-fed rabbits. Since most plasma oxysterols are found in the low-density lipoprotein (LDL) and high-density lipoprotein (HDL) fractions,[25] the oxysterols seem to be transported in plasma with cholesterol. Therefore, plasma 7α-

Table 2. Hepatic Sterol 27-Hydroxylase Activity and Plasma Concentrations of Cholesterol, 27-Hydroxycholesterol, and 3β-Hydroxy-5-cholestenoic Acid in Rabbits Treated With Cholesterol Feeding and/or Bile Drainage

	Treatments		Sterol 27OHase Activity	Plasma Sterols		
Rabbit No.	CHOL*	BD†	(pmol/min/mg)	CHOL (mg/dL)	27OH-CHOL (ng/mL)	3βOH-CA (ng/mL)
1-7	—	−	25 ± 3	41 ± 5	17 ± 3	3.5 ± 0.8
8	5 d	−	31	914	339	32
9	5 d	−	54	333	190	17
10	5 d	−	50	215	38	5.0
11	10 d	−	76	859	275	28
12	10 d	−	44	500	765	106
13	—	+	22	24	14	1.5
14	5 d	+	24	196	468	21
15	10 d	+	16	369	147	4.3
16	10 d	+	17	128	26	5.2

Abbreviations: Sterol 27OHase, sterol 27-hydroxylase; 27OH-CHOL, 27-hydroxycholesterol; 3βOH-CA, 3β-hydroxy-5-cholestenoic acid.

*Fed 2% cholesterol in chow for 5 or 10 days.

†Continued bile drainage for 7 days after cholesterol treatments.

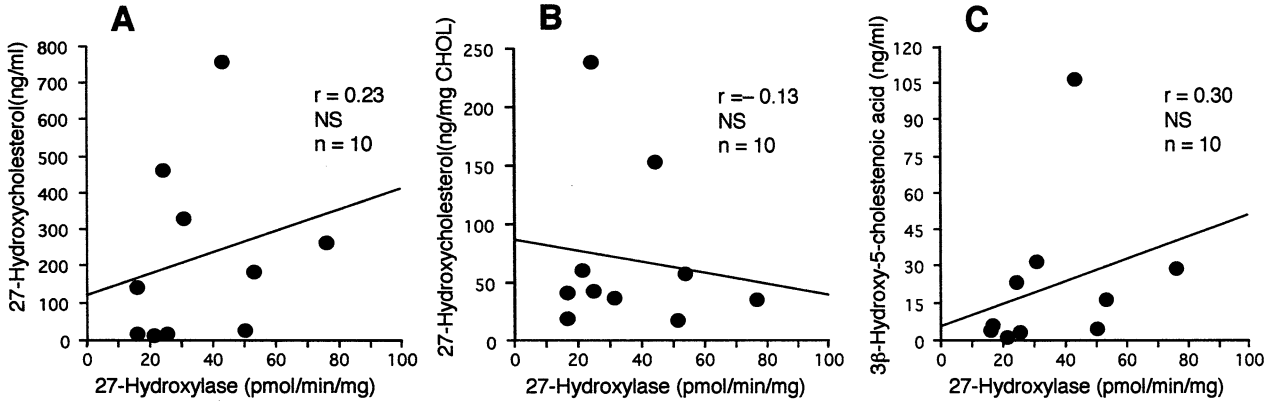

Fig 3. Relationship of hepatic sterol 27-hydroxylase activity with plasma absolute concentration of 27-hydroxycholesterol (A), plasma 27-hydroxycholesterol level expressed relative to cholesterol (B) and plasma absolute concentration of 3β-hydroxy-5-cholestenoic acid (C) in rabbits fed high-cholesterol diet and/or constructed bile fistula.

hydroxy-4-cholesten-3-one levels expressed relative to cholesterol were thought to be a more accurate marker than the absolute 7α-hydroxy-4-cholesten-3-one concentrations. In fact, this ratio did correlate with cholesterol 7α-hydroxylase activities in hypercholesterolemic rabbits (Fig 2B).

We previously measured hepatic cholesterol 7α-hydroxylase activities and plasma 7α-hydroxy-4-cholesten-3-one concentrations in 16 patients with cholelithiasis or early gastrointestinal cancer and reported significant positive correlation ($r = 0.84$, $P < 0.0001$, n = 16) between the 2 parameters.[10] To apply the present rabbits' results to human data, we calculated coefficient of correlation again by using results from 15 patients whose plasma cholesterol data were available. As shown in Fig 4, higher coefficient of correlation ($r = 0.92$ *v* $r = 0.84$) was observed when 7α-hydroxy-4-cholesten-3-one relative to cholesterol ratios were used for the calculation. Thus, the relative plasma 7α-hydroxy-4-cholesten-3-one level was a better index for hepatic cholesterol 7α-hydroxylase activity not only in rabbits but also in humans.

In contrast to 7α-hydroxy-4-cholesten-3-one, the relationship of plasma 27-hydroxycholesterol concentrations to bile acid synthesis is equivocal. In humans, there is a report that production of bile acids by the alternative pathway contributed only 9% to total bile acid synthesis.[21] Thus, determination of 7α-hydroxycholesterol or 7α-hydroxy-4-cholesten-3-one, as markers for classic pathway, is sufficient to assess total bile acid synthesis in humans.[11,12] Therefore, the contribution of cholesterol 27-hydroxylation to whole body cholesterol homeostasis appears to be much less than that of cholesterol 7α-hydroxylation in humans. However, sterol 27-hydroxylase

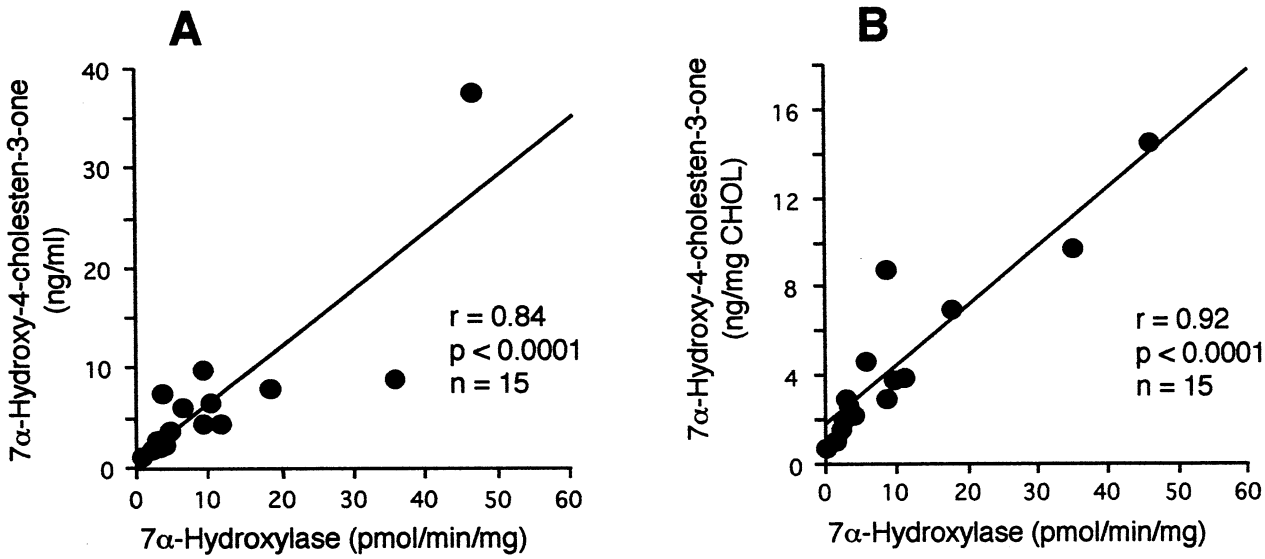

Fig 4. Relationship of hepatic cholesterol 7α-hydroxylase activity with plasma absolute concentration of 7α-hydroxy-4-cholesten-3-one (A) and plasma 7α-hydroxy-4-cholesten-3-one level expressed relative to cholesterol (B) in patients with cholelithiasis or early gastrointestinal cancer.

activity may measure the prevention of atherosclerosis by elimination of intracellular cholesterol in macrophages and endothelial cells.[22,23] These hypotheses are supported by the fact that patients with cerebrotendinous xanthomatosis, which is an inborn error caused by mutations in sterol 27-hydroxylase gene,[5] develop premature atherosclerosis and xanthomas without elevated plasma cholesterol levels.[4]

It has been suggested that the activities of hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme in cholesterol biosynthetic pathway, in nonhepatic tissues behave in a similar manner as hepatic HMG-CoA reductase in different conditions.[38] Although the reports for the simultaneous measurement of hepatic and extrahepatic sterol 27-hydroxylase activites are limited, coordinate regulation of hepatic and adrenal sterol 27-hydroxylase activities has been reported in baboons treated with high-cholesterol diet.[39] In the same report, significant correlation between hepatic sterol 27-hydroxylase activities and plasma absolute 27-hydroxycholesterol concentrations was also demonstrated. Therefore, we also compared absolute or relative plasma 27-hydroxycholesterol levels with hepatic sterol 27-hydroxylase activities in our rabbit models. However, no significant correlation was observed (Fig 3A and B), which suggests that hepatic and extrahepatic sterol 27-hydroxylase activities are not always coordinately regulated in rabbits.

Since 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid are synthesized by a single enzyme, sterol 27-hydroxylase,[13] and the latter is found predominantly in the plasma lipoprotein-free fraction,[25] 3β-hydroxy-5-cholestenoic acid may be a better plasma marker for sterol 27-hydroxylase activity. However, at least in rabbits, no significant correlation was observed between hepatic sterol 27-hydroxylase activity and plasma 3β-hydroxy-5-cholestenoic acid concentration (Fig 3C). In rabbits with bile fistulas, 98% of the newly synthesized bile acids is cholic acid.[29] Since about 85% of intravenously administrated radiolabeled 27-hydroxycholesterol was converted to cholic acid while no more than 8% of 3β-hydroxy-5-cholestenoic acid was metabolized to cholic acid,[40] most of 27-hydroxycholesterol formed by sterol 27-hydroxylase appears to be metabolized to 7α,27-dihydroxycholesterol by microsomal oxysterol 7α-hydroxylase (CYP7B1) in rabbits (Fig 1). Thus, 3β-hydroxy-5-cholestenoic acid concentration may not serve as a marker for sterol 27-hydroxylase activity in rabbits.

In summary, this study demonstrated that plasma 7α-hydroxy-4-cholesten-3-one relative to cholesterol is a better marker for hepatic cholesterol 7α-hydroxylase activity than the absolute concentration especially in hypercholesterolemia. Plasma 27-hydroxycholesterol and 3β-hydroxy-5-cholestenoic acid concentrations may be indicators for extrahepatic sterol 27-hydroxylase activity, but did not reflect hepatic sterol 27-hydroxylase activity in our rabbit models.

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