

27-Hydroxycholesterol: production rates in normal human subjects

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Abstract We attempted to quantitate production of bile acid via the 27-hydroxylation pathway in six human subjects. After bolus intravenous injection of known amounts of [24-¹⁴C]cholic acid and [24-¹⁴C]chenodeoxycholic acid, each subject underwent a constant intravenous infusion of a mixture of [22,23-³H]-27-hydroxycholesterol and [²H]-27-hydroxycholesterol for 6–10 h. Production rate of 27-hydroxycholesterol was calculated from the infusion rate of [²H]-27-hydroxycholesterol and the serum ratio of deuterated/protium 27-hydroxycholesterol, which reached a plateau level by 4 h of infusion. Conversion of 27-hydroxycholesterol to cholic and chenodeoxycholic acids was determined from the ³H/¹⁴C ratio of these two bile acids in bile samples obtained the day after infusion. In five of the six subjects, independent measurement of bile acid synthesis by fecal acidic sterol output was available from previous studies. Endogenous production of 27-hydroxycholesterol averaged 17.6 mg/day and ranged from 5.0 to 28.2 mg/day, which amounted to 8.7% (range 3.0–17.9%) of total bile acid synthesis. On average 66% of infused 27-hydroxycholesterol was converted to bile acid, of which 72.6% was chenodeoxycholic acid. These data suggest that relatively little bile acid synthesis takes place via the 27-hydroxylation pathway in healthy humans. Nevertheless, even this amount, occurring predominantly in vascular endothelium and macrophages, could represent an important means for removal of cholesterol deposited in endothelium.—Duane, W. C., and N. B. Javitt. 27-Hydroxycholesterol: production rates in normal human subjects. *J. Lipid Res.* 1999. 40: 1194–1199.

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Bile acid synthesis occurs in the liver via one of two pathways. The classical pathway begins with 7 α -hydroxylation of cholesterol in hepatic microsomes. This step, which is rate-limiting, is regulated in part by return of bile acids to the liver (1–4). An alternate pathway in humans begins with 27-hydroxylation of cholesterol. The hydroxylase responsible for this reaction is a polyfunctional mitochondrial enzyme that also catalyzes subsequent oxidation to 3 β -5-cholestenoic acid (5, 6). This pathway, which produces both primary bile acids but favors chenodeoxycholic acid over cholic acid (7–9), is less responsive to bile

acid negative feedback than is cholesterol 7 α -hydroxylase (2–4).

Recent evidence indicates that the initial steps in this pathway, 27-hydroxylation of cholesterol and further oxidation to 3 β -5-cholestenoic acid, can take place outside the liver in vascular endothelium and macrophages (6, 10). Both intermediates presumably can be released into serum for transport to the liver and conversion to bile acids, a scenario supported by relatively high serum levels of the intermediates (2, 11, 12). Several investigators have suggested that this process may help counteract development of atherosclerosis (6, 13), which is believed to involve accumulation of modified LDL in macrophages with subsequent transformation to foam cells (14, 15). Further supporting the hypothesis that generation of 27-hydroxycholesterol is important to the prevention of cholesterol accumulation is the finding that its concentration relative to cholesterol in normal human aorta is about 1.4 mg/g cholesterol (16), at least 10-fold greater than in plasma (12), and that even larger gradients occur in atheromas (6). The continual egress of 27-hydroxycholesterol, together with its metabolites, from vascular tissues into plasma may thus represent an important component of “reverse cholesterol transport” contributing to overall cholesterol homeostasis. This hypothesis could also help to explain the propensity for patients with cerebrotendinous xanthomatosis, a disease caused by an inborn error in 27-hydroxylase, to accumulate excess cholesterol in xanthomata and atheromata despite normal serum levels of cholesterol (17, 18).

Despite the potential importance of the 27-hydroxylase pathway, the relative activity of this pathway in vivo is uncertain. Studies in animal models suggest that the contribution of this pathway to total bile acid synthesis can be substantial. In rats, virtually complete suppression of 7 α -hydroxylase resulted in only about a 50% reduction of bile acid production, which subsequently increased to nearly

Abbreviations: ANCOVA, analysis of covariance; LDL, low density lipoprotein; NMR, nuclear magnetic resonance; FFM, fat-free mass.

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normal levels over 48 h (3). Moreover, although the cholesterol 7 α -hydroxylase knock-out mouse (CYP 7 $^{-/-}$) has a high neonatal mortality rate, those capable of generating sufficient levels of oxysterol 7 α -hydroxylase can survive to adulthood with biliary bile acid concentrations in the normal range and fecal bile acid concentrations about 35% of normal levels, suggesting substantial use of the alternate pathway (19).

In humans data are more limited. Recent studies indicate that cholesterol 7 α -hydroxylase activity is not detectable in normal human neonates (20) supporting previous evidence that activity of this enzyme is also not detectable in fetal life (21, 22). This suggests that initially bile acid synthesis occurs predominantly via the 27-hydroxylation pathway and that a transition to substantial or predominant use of the cholesterol 7 α -hydroxylation pathway occurs as part of growth and development. Axelson and Sjovall (2), based on plasma concentrations of intermediates of both pathways, have concluded that when the classical pathway is suppressed, the alternate pathway may become dominant. Lund et al. (23) have demonstrated a hepatic arterial-venous gradient for both 27-hydroxycholesterol and 3 β -5-cholestenoic acid, indicating a combined net hepatic uptake of these intermediates in the range of 20 mg/day. This experiment demonstrated substantial peripheral activity of the 27-hydroxylase, but did not provide an estimate of total production of 27-hydroxycholesterol.

In the present study we have performed experiments in which isotopic 27-hydroxycholesterol was infused intravenously at a constant rate to achieve a constant isotopic enrichment in six human subjects. Subsequent measurement of isotope ratios of 27-hydroxycholesterol enabled calculation of endogenous production of 27-hydroxycholesterol by standard formulas (24).

METHODS

Subjects

Study procedures were approved by committees overseeing use of human subjects in research at both the Minneapolis VA Medical Center and the University of Minnesota. All subjects gave written informed consent to participate. We studied six male subjects. Two of these had hypertriglyceridemia. With that exception all subjects were in good health according to previously published criteria (25). Detailed characterization of the subjects is provided in **Table 1**. We attempted to study subjects for whom a previously determined value of bile acid synthesis was available. This was the case for five of the six subjects for whom fecal acidic sterol outputs had been measured as part of two earlier studies (26, 27).

Isotopes

[24- 14 C]cholic acid and [24- 14 C]chenodeoxycholic acid were purchased from DuPont NEN (Boston, MA). Both had specific activity of approximately 50 mCi/mmol and both were determined to be >98% radiochemically pure by thin-layer chromatography.

[22,23- 3 H]-27-hydroxycholesterol was custom synthesized by DuPont NEN (Boston, MA) using a recently described method based on Julia condensation of a sulfone intermediate (28). The final product had a specific activity of 55 Ci/mmol and was >99% radiochemically pure by thin-layer chromatography both

TABLE 1. Characteristics of study subjects

Subject	Age	Wt	Ht	BMI	Serum CH	Serum TG
	yr	kg	m	kg/m ²	mg/dl	mg/dl
1	52	100	1.75	32.7	200	148
2	52	105	1.70	36.5	216	186
3	41	85	1.76	27.2	161	132
4	59	95	1.71	32.5	183	326
5	62	100	1.70	34.6	178	388
6	72	83	1.70	28.6	216	116

Abbreviations: Wt, weight; Ht, height; BMI, body mass index; CH, cholesterol; TG, triglyceride.

in our laboratory and the laboratories of DuPont NEN. NMR analysis performed at DuPont NEN revealed that an appreciable proportion of the 3 H was located on carbons other than C22 and C23. Although strict quantitation was not possible, these NMR spectra suggested that approximately 20–25% of the 3 H was on C24 with less than 2% on C25–27. Because protons on C24 and C25 would be reactive during side-chain cleavage, about 25% of the 3 H could have been released as 3 H $_2$ O during biosynthesis of C24 bile acids from this isotopic material.

We synthesized two batches of [2 H]-27-hydroxycholesterol using the Clemmensen reduction method as previously described (29). One of these had a maximum of eight deuteriums per molecule of 27-hydroxycholesterol (d8) and the other had a maximum of four deuteriums per molecule (d4). These were analyzed by mass spectroscopy as previously described (29). The most intense peak in the protium compound (d0) was at m/z 426 accounting for 73.8% of the mass. The exact percentage of the d8 compound (m/z 434) in the first deuterated batch was 20.8% while the percentage of d4 compound (m/z 430) in the second batch was 23.4%.

Study protocol and analyses

After an overnight fast, each subject was given 50 ml of normal saline containing a known amount (2–5 μ Ci each) of [24- 14 C]cholic acid and [24- 14 C]chenodeoxycholic acid by intravenous infusion over about 10 min. We then started a constant intravenous infusion of a mixture of [22,23- 3 H]-27-hydroxycholesterol and [2 H]-27-hydroxycholesterol suspended in 5% human serum albumin. The infusion continued for 6–10 h and delivered the following approximate amounts: 25 ml/h, 1 μ Ci/h of [22,23- 3 H]-27-hydroxycholesterol, and 150 μ g/h of [2 H]-27-hydroxycholesterol. To determine exact infusion rates of each isotope, quadruplicate timed samples were taken from the infusion line at the beginning and end of the constant infusion. These were assayed for radioactivity by liquid scintillation counting and for mass of 27-hydroxycholesterol by capillary gas-liquid chromatography using a modification of methods previously described (30).

Samples of serum (1–3 cc) obtained during infusion were analyzed for deuterium enrichment of 27-hydroxycholesterol using gas-liquid chromatography/mass spectrometry (GC/MS) of the acetate derivatives as previously described (29). Briefly, 27-hydroxycholesterol was extracted from serum using chloroform-methanol and derivatized using acetic acid-acetic anhydride. GC/MS was used to determine the ratio of m/z 434 to m/z 426 of samples containing the d8 compound or the ratio of m/z 430 to m/z 426 of samples containing the d4 compound. By calculation from relative mass intensities a 1:1 mixture of the protium and d8 compounds would yield a ratio of m/z 434 to m/z 426 of (20.8/73.8 = 0.282) while a 1:1 mixture of protium and d4 compounds would yield a ratio of m/z 430 to m/z 426 of (23.4/73.8 =

0.317). The mass ratios together with the mass infusion rate of 27-hydroxycholesterol (I) were used to calculate production rate of 27-hydroxycholesterol (P) according to the equation of Gurpide, Mann, and Lieberman (24). For infusions of the d8 compound the calculation was:

$$P = (0.282 \times I) \div (d8/d0)$$

For infusions of the d4 compound the calculation was:

$$P = (0.317 \times I) \div (d4/d0)$$

About 3 h after cessation of infusion, the subject emptied his bladder and urine was subsequently collected for several hours. Duplicate aliquots of urine were analyzed by liquid scintillation counting both before and after complete evaporation of water. The difference between wet and dry counts was divided by the aliquot volume to calculate concentration of tritium in body water. To calculate the total amount of tritium in body water, we estimated total body water as 72% of fat-free body mass (FFM), calculated as 16.7 kg/m², according to the data of Baarends et al. (31). Total tritium released as ³H₂O during infusion of [22, 23-³H]-27-hydroxycholesterol was then calculated by multiplying total body water times concentration of tritium in body water. We assumed that all tritium not released as ³H₂O would have been recoverable in bile acid biosynthesized from the infused [22, 23-³H]-27-hydroxycholesterol.

The evening after the infusion, each subject swallowed a polyvinyl tube weighted with a metal aspiration tip and mercury bag. The next morning, a sample of gallbladder bile was obtained via this tube. Specific activities of biliary cholic acid and chenodeoxycholic acid were determined on an aliquot of this bile after enzymatic deconjugation using standard procedures for double label counting of ³H and ¹⁴C. This sampling procedure and the determination of specific activity have been described previously (32). The ratio of ³H/¹⁴C in cholic acid and chenodeoxycholic acid was multiplied by the amount of ¹⁴C of each respective bile acid administered to calculate the actual amount of tritium converted to each of the two bile acids during infusion of [22,23-³H]-27-hydroxycholesterol.

Statistical testing was by unpaired *t*-test and analysis of covariance (ANCOVA) was performed using SAS.

RESULTS

In two subjects the constant infusion of isotopic 27-hydroxycholesterol was continued for 10 h to determine time required to reach constant serum levels. In both cases constant ratios of isotope enrichment were achieved by 4 h of infusion. In addition, ratios of isotope enrichment fell to near baseline within an hour of stopping the infusion of isotopes. Results of one of these prolonged infusions are shown in **Fig. 1**. Based on these results we continued the infusion for only 6 h in the other four subjects. Serum samples at hours 4, 5, and 6 were used for final analysis.

Isotope enrichment ratios for these serum samples are provided in **Table 2** together with infusion rates of [²H]-27-hydroxycholesterol and calculated endogenous production rate of 27-hydroxycholesterol. Endogenous production of 27-hydroxycholesterol averaged 17.6 mg/day and ranged from 5.0 to 28.2 mg/day. In five of the six subjects we had previously estimated total bile acid synthesis

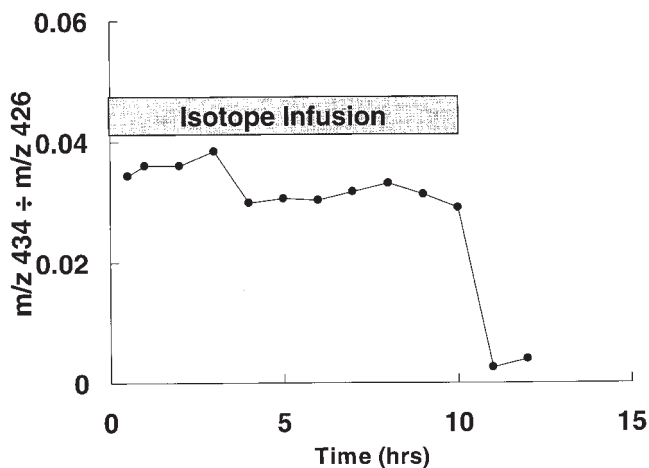


Fig. 1. Time course of the ratio of deuterated/protium 27-hydroxycholesterol during constant intravenous infusion of [²H]-27-hydroxycholesterol (d8) in one subject. The ratio was essentially constant during hours 4–10 of infusion and fell rapidly once the infusion was stopped. The prompt rise of the ratio during the initial hour of infusion is also consistent with rapid turnover of this compound in serum. The explanation for a slightly higher ratio during hours 1–3 is uncertain.

by measuring steady-state output of fecal acidic sterols (26, 27). Using these values we calculated that production of 27-hydroxycholesterol averaged 8.7% (range 3.0–17.9%) of total bile acid synthesis (Table 2).

Because calculation of production rate for 27-hydroxycholesterol in these experiments depends on the assumption that the isotope enrichment ratio in the serum reasonably reflects the ratio in the liver, we sought to measure isotope enrichment in fresh bile (unmixed with bile produced prior to plateau of the serum isotope ratio during infusion). This was accomplished by performing a constant intravenous infusion of [²H]-27-hydroxycholesterol (d4) at a rate of 460 µg/hr in a 66-year-old male subject with a t-tube, placed 3 weeks earlier during common bile duct exploration for choledocholithiasis. After 6 h of infusion, simultaneous samples of blood and bile were ob-

TABLE 2. Deuterium analyses and production of 27-hydroxycholesterol

Subject	Infusion Rate ^a µg/h	Peak	Isotope Ratio ^b	Production of 27-Hydroxycholesterol		Acidic Sterols ^c mg/day
				mg/day	% ^d	
1	125	d8	0.03185	28.2	6.6	425
2	117	d4	0.17733	5.0	—	—
3	150	d4	0.05809	19.6	17.9	109
4	156	d4	0.07735	15.3	3.0	520
5	155	d4	0.11182	10.5	6.3	167
6	137	d8	0.03514	26.9	9.8	273
Mean				17.6	8.7	299
SEM				3.7	2.5	77

^a Intravenous infusion rate of [²H]-27-hydroxycholesterol.

^b For d8, ratio of *m/z* 434 to *m/z* 426; for d4, ratio of *m/z* 430 to *m/z* 426.

^c Available from one of two previous studies (26, 27).

^d Percent of acidic sterol output.

TABLE 3. Tritium dispersal after infusion of [22,23-³H]-27-hydroxycholesterol

Subject	³ H Infused	³ H in Body Water		³ H in Bile Acid ^a	
	μCi	μCi	%	μCi	%
1	18.0	4.19	23.3	8.21	45.6
2	4.16	0.57	13.7	2.23	53.6
3	5.34	0.69	12.9	2.47	46.2
4	5.56	0.95	17.1	2.63	47.3
5	5.51	0.81	14.6	2.36	42.8
6	19.8	4.13	20.9	11.53	58.4
Mean			17.1		49.0
SEM			1.7		2.4

^a Sum of ³H in cholic acid and chenodeoxycholic acid.

tained. The ratios of *m/z* 430 to *m/z* 426 in the serum and bile were 1.12 and 1.39, respectively, indicating nearly complete equilibration of infused isotope between serum and liver pools.

Dispersal of tritium from infused [22,23-³H]-27-hydroxycholesterol is shown in **Table 3**. On average, 17.1% (range 13.7–23.3%) of infused tritium was converted to ³H₂O. This observation is consistent with NMR data from DuPont NEN estimating that 20–25% of the tritium on the [22,23-³H]-27-hydroxycholesterol was actually on carbons 24 and 25, carbons with reactive protons during conversion to bile acid.

On average, 49% (range 42.8–58.4%) of the infused [22,23-³H]-27-hydroxycholesterol was converted to bile acid. Of the 27-hydroxycholesterol converted to bile acid there was a clear-cut preferential conversion to chenodeoxycholic acid. Thus, mean \pm SEM of the tritiated bile acid generated from [22,23-³H]-27-hydroxycholesterol was $27.4 \pm 1.4\%$ for cholic acid and $72.6 \pm 1.4\%$ for chenodeoxycholic acid ($P < 10^{-9}$).

DISCUSSION

The cholesterol 27-hydroxylase pathway for bile acid synthesis is potentially important in at least two respects. First, it may constitute a substantial proportion of total bile synthesis, 50% or more in some animal models (3, 19). Second, the presence of this enzyme in vascular endothelium and macrophages may represent a defense against atherosclerosis by providing a route for conversion of excess local cholesterol to intermediates that can be further transformed to bile acid in the liver (6, 10, 13). Fundamental to assessing the importance of the cholesterol 27-hydroxylase pathway in human health and disease is quantitating the actual *in vivo* activity of this pathway in human subjects, a measurement which has been difficult to accomplish.

We initially attempted this quantitation by mathematical modeling of serum specific activity decay curves after bolus injection of isotopic 27-hydroxycholesterol. However, this approach quickly proved impractical because of very rapid egress of the compound from the serum compartment, a process reflected in the rapid attainment of a

constant level of isotopic enrichment during constant infusion of isotope (Fig. 1). In addition, such mathematical modeling, while potentially providing information beyond just production rate, would have been encumbered by additional assumptions and inherent complexity. In contrast, the constant infusion technique provided a straightforward, manageable approach to measuring *in vivo* production of 27-hydroxycholesterol. Because of the rapid turnover of 27-hydroxycholesterol, a constant level of isotopic enrichment was achieved quickly and unequivocally (Fig. 1). Calculation of production rate from the infusion rate and plateau level of isotope enrichment is straightforward (24).

Using this approach in our six subjects we found an average production rate for 27-hydroxycholesterol of 18 mg/day (Table 2). Based on direct measurement of total bile acid synthesis as fecal acidic sterol output in five of six subjects, this accounts for an average of 9% of total bile acid synthesis (Table 2). Thus, in the unperturbed state, the alternate 27-hydroxylation pathway accounts for a fairly small proportion of bile acid synthesis. Because the 27-hydroxylation pathway may be less responsive than the 7 α -hydroxylation pathway to negative feedback inhibition (2–4), it is possible that the relative contribution of the alternate pathway would increase during such perturbations as administration of chenodeoxycholic acid.

The relatively low level of 27-hydroxycholesterol production measured in these experiments naturally raises the possibility that production was underestimated. There are several potential ways in which such underestimation might happen. First, the measurement of either deuterated or protium compound in serum might be inaccurate, leading to an erroneously high ratio of deuterated to protium compound. This seems quite unlikely in view of our laboratories extensive experience with these measurements and our practice of routinely checking against added standards. Second, the infusion interval may have been too short to achieve a constant level of isotope enrichment. However, this would lead to a falsely high, not falsely low, estimate of 27-hydroxycholesterol production. Moreover, we documented that the ratio of deuterated to protium compound reached a plateau during the selected infusion interval (Fig. 1). Finally, it is possible that the ratio of deuterated to protium compound measured in the serum is not representative of the ratio in the liver. There seems likely to be some exchange of 27-hydroxycholesterol from liver back into the serum pool because two intermediates in bile acid synthesis, 7 α -hydroxycholesterol and 7 α -hydroxy-4-cholesten-3-one, both of which are produced only in the liver, are found in substantial amounts in serum, and their serum levels vary in proportion to bile acid synthesis (33, 34). To assess whether this exchange is rapid enough to achieve equalization of isotope ratios, we performed a constant intravenous infusion of [²H]-27-hydroxycholesterol in a subject who had a t-tube placed after common bile duct exploration 3 weeks earlier. After 6 h of infusion, the ratio of d4/d0 in this subject's serum was the same as that in his bile (Results). This observation provides strong support for the assumption

that the serum ratio of deuterated to protium compound is similar to that in the liver. Even so, we cannot completely exclude some disequilibrium between subcellular hepatic pools such that 27-hydroxycholesterol might be rapidly converted to distal intermediates before completely mixing with the deuterated compound. However, the similar isotope enrichments in serum and bile greatly reduce the probability that our methods underestimated whole-body production of 27-hydroxycholesterol.

Although 18 mg/day is small relative to total bile acid synthesis, it may nevertheless be large enough to make a substantial impact on reverse cholesterol transport from vascular endothelium and macrophages. Interestingly Lund et al. quantitated the hepatic uptake of 27-oxygenated products by arterial-venous difference and reported a mean uptake of 20 mg/day, which is nearly identical to our measurement for production of 27-hydroxycholesterol. This suggests that at least in unperturbed, healthy human subjects, all or nearly all 27-hydroxycholesterol is produced outside the liver. Quantitative hepatic production of 27-hydroxycholesterol has not been directly measured in vivo even in animal models, although in rats bile acid synthesis via 27-hydroxycholesterol can reach levels high enough to seemingly require some contribution from the liver (3). Moreover, in cultured rat and human hepatocytes selective inhibition of 27-hydroxylase with cyclosporin A substantially reduces total bile acid synthesis (35). On the other hand, hepatic production of 27-hydroxycholesterol may be selectively impaired because the liver does not express steroidogenic acute regulatory (StAR) protein, which catalyzes transfer of cholesterol from outer to inner mitochondrial membrane (36). If this factor played a more prominent role in vivo than in cultured hepatocytes, it could explain the very minimal in vivo hepatic production of 27-hydroxycholesterol suggested by our data.

On average we found that 17% of the tritium on infused [22,23-³H]-27-hydroxycholesterol was converted to ³H₂O and 49% was converted to bile acid (Table 3). Because by NMR analysis 20–25% of the tritium on [22,23-³H]-27-hydroxycholesterol was on carbon-24 (Results), presumably the tritium converted to ³H₂O represents conversion of the sterol to bile acid with release of tritium on carbon-24 as ³H₂O. That would mean that 66% of infused 27-hydroxycholesterol was converted to bile acid. Thus, despite the fact that free [22,23-³H]-27-hydroxycholesterol disappeared from serum in a matter of minutes, presumably as a result of conversion to other compounds, we were nevertheless unable to identify 34% of the products of this metabolic conversion. Although we can only speculate regarding the fate of the remaining 34% of infused isotope, the most likely explanation is that it represents 27-hydroxycholesterol converted to esters or sulfates which would turn over much more slowly than the free sterol. It is well known that 27-hydroxycholesterol exists in vivo in both ester and sulfate forms (12). Moreover, that explanation would also be consistent with the fact that 20–25% of the tritium on the infused compound was located on carbon 24 (tritium which would be released and converted to

³H₂O during conversion to bile acids) yet only 17% of the infused tritium actually emerged as ³H₂O.

Of the [22,23-³H]-27-hydroxycholesterol converted to bile acid, there was on average a 2.6-fold preference for conversion to chenodeoxycholic acid versus cholic acid (Results). That 27-hydroxycholesterol is preferentially converted to chenodeoxycholic acid is well known (7–9). Perhaps the most remarkable feature of the present data is their consistency. Thus, in these six subjects, the 95% confidence interval for percentage of 27-hydroxycholesterol converted to chenodeoxycholic acid versus cholic acid was 69–76%. Although we did not formally measure synthesis of individual bile acids in these six subjects, the mean ratio of cholic acid and metabolites (mainly deoxycholic acid) to chenodeoxycholic acid and metabolites (mainly lithocholic acid) in their stools was 1.6 with a range from 1.2 to 2.2 (data not shown) and typically synthesis of chenodeoxycholic acid is about half that of cholic acid in healthy human subjects (37). This predominance of cholic acid synthesis, together with the consistent 2.6-fold preference of the 27-hydroxylation pathway for chenodeoxycholic acid production, further supports our conclusion that the 27-hydroxylation pathway contributed relatively little to overall bile acid synthesis in these adult male subjects.

Sufficient data do not currently exist to place these findings in perspective with regard to quantitative importance of the cholesterol 27-hydroxylase pathway vis-a-vis its relationship to atherosclerosis. The only current model of individuals completely lacking the cholesterol 27-hydroxylase is cerebrotendinous xanthomatosis, which is associated with accelerated atherosclerosis with accumulation of excess cholesterol in xanthomata and atheromata despite normal serum levels of cholesterol (17, 18, 38). Whether there is a relationship between persistence of the 27-hydroxylation pathway from neonatal life and protection against later development of atherosclerosis is a reasonable question that deserves further investigation. Even the relatively small amount of 27-hydroxycholesterol production measured in this study could represent an important means for removal of cholesterol from the periphery if the bulk of activity occurred in vascular endothelium and macrophages. ■

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