Ileal bile acid transport regulates bile acid pool, synthesis, and plasma cholesterol levels differently in cholesterol-fed rats and rabbits

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Abstract **We investigated the effect of ileal bile acid transport on the regulation of classic and alternative bile acid synthesis in cholesterol-fed rats and rabbits. Bile acid pool sizes, fecal bile acid outputs (synthesis rates), and the activities of cholesterol 7α-hydroxylase (classic bile acid synthesis) and cholesterol 27-hydroxylase (alternative bile acid synthesis) were related to ileal bile acid transporter expression (ileal apical sodium-dependent bile acid transporter, ASBT). Plasma cholesterol levels rose 2.1-times in rats (98 ± 19 mg/dl) and 31-times (986 ± 188 mg/dl) in rabbits. The bile acid pool size remained constant (55 ± 17 mg vs. 61 ± 18 mg) in rats but doubled (254 ± 46 to 533 ± 53 mg) in rabbits. ASBT protein expression did not change in rats but rose 31% ($P < 0.05$) in rabbits. Fecal bile acid outputs that reflected bile acid synthesis increased 2- and 2.4-times ($P < 0.05$) in cholesterol-fed rats and rabbits, respectively. Cholesterol 7α-hydroxylase activity rose 33% (24 ± 2.4 vs. 18 ± 1.6 pmol/mg/min, $P < 0.01$) and mRNA levels increased 50% ($P < 0.01$) in rats but decreased 68% and 79%, respectively, in cholesterol-fed rabbits. Cholesterol 27-hydroxylase activity remained unchanged in rats but rose 62% ($P < 0.05$) in rabbits. Classic bile acid synthesis (cholesterol 7α-hydroxylase) was inhibited in rabbits because an enlarged bile acid pool developed from enhanced ileal bile acid transport. In contrast, in rats, cholesterol 7α-hydroxylase was stimulated but the bile acid pool did not enlarge because ASBT did not change. Therefore, although bile acid synthesis was increased via different pathways in rats and rabbits, enhanced ileal bile acid transport was critical for enlarging the bile acid pool size that exerted feedback regulation on cholesterol 7α-hydroxylase in rabbits.**—Xu, G., B. L. Shneider, S. Shefer, L. B. Nguyen, A. K. Batta, G. S. Tint, M. Arrese, S. Thevananther, L. Ma, S. Stengelin, W. Kramer, D. Greenblatt, M. Pcolinsky, and G. Salen. **Ileal bile acid transport regulates bile acid pool, synthesis, and plasma cholesterol levels differently in cholesterol-fed rats and rabbits.** ***J. Lipid Res.*** **2000.** 41: **298–304.**

Supplementary key words bile acids • absorption • biosynthesis • cholesterol 7α-hydroxylase • cholesterol 27-hydroxylase

Humans show wide variations in plasma cholesterol levels in response to dietary cholesterol intake (1, 2). Similarly, great differences in plasma cholesterol levels have also been noted in various species of animals fed cholesterol. For example, rats fed a high cholesterol diet are resistant to developing hypercholesterolemia (3). In contrast, NZW rabbits are very sensitive to dietary cholesterol and accumulate large amounts in plasma (4). It is well known that feeding cholesterol to rats up-regulates both activity and mRNA levels of cholesterol 7α-hydroxylase (5–7), the rate-controlling enzyme for the classic bile acid synthetic pathway. In rabbits, the opposite was observed; cholesterol 7α-hydroxylase activity and mRNA levels were inhibited after cholesterol feeding with substantially elevated plasma cholesterol levels (8). However, in a special group of rabbits, Overturf et al. (9) discovered that feeding 0.1% cholesterol for 7 months did not produce hypercholesterolemia. In these "hypercholesterolemia-resistant" rabbits, both activity and mRNA levels of cholesterol 7α-hydroxylase were higher than in regular NZW rabbits fed either chow or chow plus 0.1% cholesterol (10). The inhibition of cholesterol 7α-hydroxylase after cholesterol feeding was also found in other animal species. Rudel et al. (11) reported that African green monkeys fed cholesterol develop marked plasma hypercholesterolemia associated with inhibited cholesterol 7α-hydroxylase. Horton, Cuthbert, and Spady (12) and Pandak et al. (13) also showed that cholesterol-fed hamsters manifested plasma hypercholesterolemia with suppressed cholesterol 7α-hydroxylase

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; NZW, New Zealand white; ASBT, apical sodium-dependent bile acid transporter.

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activity and mRNA levels. The information from NZW rabbits and other animal species suggests that feeding cholesterol caused suppression of cholesterol 7α-hydroxylase and was associated with plasma hypercholesterolemia while in species like rats or "hypercholesterolemia-resistant rabbits," increased dietary cholesterol did not inhibit cholesterol 7α-hydroxylase and plasma cholesterol levels increased only slightly. The question to consider is why cholesterol 7α-hydroxylase responded differently to dietary cholesterol in rats and rabbits. We recently reported that the bile acid pool size was significantly enlarged in cholesterol-fed NZW rabbits (14). We hypothesized that the increased bile acid pool size not cholesterol was responsible for the inhibition of cholesterol 7α-hydroxylase in cholesterol-fed rabbits. Further, by gradually increasing the intake of dietary cholesterol in rabbits, we showed an inverse relationship between the size of the bile acid pool and cholesterol 7α-hydroxylase activity (15). The mechanism for the expansion of the bile acid pool in cholesterol-fed rabbits is not yet known although alternative bile acid synthesis was significantly increased. Also, the effect of cholesterol feeding on the bile acid pool size in rats has not been determined.

An important component of the enterohepatic circulation is the active reabsorption of conjugated bile acids in the terminal ileum (16). The first step in this active process is mediated by ileal apical sodium-dependent bile acid transporter (ASBT) (17, 18). Björkhem, Eggertson, and Andersson (19) suggested that, in rats, cholesterol feeding might result in intestinal bile acid malabsorption that would stimulate cholesterol 7α-hydroxylase because of diminished bile acid return to the liver. Consistent with this idea, Torchia, Cheema, and Agellon (20) reported that, in mice, cholesterol feeding significantly decreased ASBT mRNA. However, little is known about the effect of dietary cholesterol on ASBT in rats or rabbits. Ileal lipid binding protein (ILBP) was suggested to be involved in the transport of bile acids from the ileum (21, 22). However, the exact role that ILBP plays remains controversial and the effect of cholesterol feeding on ILBP has not yet been examined.

In this study, we compared the effect of dietary cholesterol on bile acid metabolism and ileal bile acid reabsorption in rats and rabbits. In rats, cholesterol feeding stimulated cholesterol 7α-hydroxylase and classic bile acid synthesis, but ileal bile acid transport did not change and the bile acid pool size was not expanded. In contrast, cholesterol feeding in rabbits was associated with increased ASBT expression that enlarged the bile acid pool so that cholesterol 7α-hydroxylase and classic bile acid synthesis was inhibited.

MATERIALS AND METHODS

Materials

Cholesterol (cholest-5-en-3β-ol) and 5α-cholestane were obtained from Sigma Chemical Co., St. Louis, MO and used as standards for the measurements of cholesterol by capillary gas–liquid chromatography. Ursocholic acid (a gift from Gipharmex, Milan, Italy) was conjugated with glycine according to the method of Tserng, Hackey, and Klein (23). Rat and rabbit chows containing 2% cholesterol were prepared by Purina Mills Inc., St. Louis, MO, and contained similar fatty acid composition.

Animal experiments

The experiments were carried out in 17 male Sprague-Dawley rats (Charles River, Wilmington, MA) weighing 220–250 g and 20 male NZW rabbits (Hazleton Labs, Denver, PA) weighing 2.3–3.2 kg. Nine rats were fed 2% cholesterol rat chow (about 0.5 g cholesterol per day) and 10 rabbits were fed 2% cholesterol rabbit chow (about 3 g cholesterol per day) for 10 days. The remaining 8 rats and 10 rabbits were used as controls and were fed regular rat or rabbit chow, respectively. After completion of the feeding, 4 cholesterol-fed and 4 control rats, and 5 cholesterol-fed and 5 control rabbits were killed. Blood samples were taken from each animal for determination of plasma cholesterol concentrations. The livers were removed and portions were frozen immediately for measurements of activities and mRNA levels of microsomal cholesterol 7α-hydroxylase and HMG-CoA reductase, and mitochondrial sterol 27-hydroxylase and for hepatic cholesterol concentration determinations. In addition, bile fistulas were constructed in 5 cholesterol-fed and 4 control rats, and 5 cholesterol-fed and 5 control rabbits after the feeding. The surgical procedure established a bile fistula for the recovery of bile acids in rats and was similar to that for rabbits as described previously (24). Bile was collected continuously for 2 days in rats and 5 days in rabbits as the bile acid pool is completely depleted in 24 h in rats and in 3 days in rabbits.

The animal protocol was approved by the Subcommittee on Animal Studies at VA Medical Center, East Orange, NJ and Institutional Animal Care and Use Committee at UMD-New Jersey Medical School, Newark, NJ.

Bile acid pool size and hepatic bile acid flux

In both rats and rabbits, bile collected within the first 30 min after the construction of the bile fistula was analyzed to determine baseline bile acid composition and percentage of deoxycholic acid in the bile. Hepatic bile acid flux was calculated by multiplying the total bile acid concentration (mg/ml) in the bile collected within the first 30 min by the relevant bile flow rate (ml/h).

The bile acid pool sizes in rats were measured by the method of Mok, Perry, and Dowling (25) and in rabbits by the method of Xu et al. (14).

Chemical analysis

Assays for bile acids and sterols. Bile acids were quantitatively measured by capillary gas chromatography method as previously described. (14)

In the presence of an internal standard, 5α-cholestane, plasma or bile cholesterol was quantitated by capillary gas–liquid chromatography as described previously (26). For hepatic sterols, 0.5 g of liver tissue with 200 μg 5α-cholestane (internal standard) was homogenized and its neutral sterols were extracted with chloroform. After evaporation, the residue was dissolved in 10 ml of ethyl acetate. Then 2 ml of this solution was used directly for free cholesterol measurement by way of gas chromatography without hydrolysis. For the total cholesterol measurement, another 3 ml of the solution was hydrolyzed using the method of saponification and then was quantitated by gas chromatography as mentioned previously (26).

Assays for ASBT transient transfection/transport. COS-7 cells were transiently transfected with ASBT according to a previously published method (27). Forty-eight hours after transfection, sodium-dependent taurocholate uptake was assayed by examin-

ing the difference in cell-associated radioactivity after incubation with either 116 mm Na or choline chloride. The effect of cholesterol upon this process was measured by the addition of 100 μm cholesterol to the incubation media at the time of the transport assay.

Assays for ASBT-ILBP protein (Western blotting). Crude BBMV were prepared by a modification of a previously reported divalent cation precipitation (28, 29). Aliquots for Western blotting were stored at −70°C for up to 1 month. Protein concentrations were determined using the Bradford method (30) with bovine serum albumin as the standard. Western blotting for ASBT and for ILBP were performed with BBMV and homogenate, respectively, using an amount of protein that was found to give a phosphorimager signal in the linear range of the response curve for the given antibody and antigen. Similar amounts of protein (5 μg of homogenate for ILBP and 15 μg of BBMV for ASBT) were loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel (31) and subjected to electrophoresis. After electrotransfer onto nitrocellulose membranes (Optitran, Schleicher, & Schuell, Keene, NH) the blots were blocked overnight at 4°C with Tris-buffered saline containing 0.1% Tween and 5% non-fat dry milk and then incubated for 2 h, at room temperature with one of the following primary antibodies: i) for rat: carboxy-terminal anti-peptide antibody against ASBT (18) rabbit: 26 amino acid carboxyterminal fusion protein and ii) anti-ILBP polyclonal antibody (22, 28: generous gift from Michael Crossman, Washington University, St. Louis, MO). Immune complexes were detected using ^{125}I-lableled anti-rabbit IgG as secondary antibodies. The protein size was estimated by prestained SDS-PAGE Molecular Weight Standards (Bio-Rad Laboratories, Hercules, CA). Immunoreactive bands were detected using a Phosphorimager screen (Molecular Dynamics, Sunnyvale, CA) and quantified using a Phosphorimager and the Imagequant software (Molecular Dynamics).

Hepatic microsomal cholesterol 7α-hydroxylase and HMG-CoA reductase and mitochondrial cholesterol 27-hydroxylase activities. Hepatic microsomes and mitochondria were prepared by differential ultracentrifugation (32), and the protein was determined according to Lowry et al. (33).

Hepatic microsomes were used for determination of the activities of HMG-CoA reductase and cholesterol 7α-hydroxylase. The assay for HMG-CoA reductase activity was the same as described previously (15) which was based on the methods of George et al. (34). Cholesterol 7α-hydroxylase activity was measured in hepatic microsomes after removal of endogenous lipid by acetone and reconstituting the microsomal protein with cholesterol and optimal amounts of co-factors by the isotope incorporation method of Shefer, Salen, and Batta. (32) Mitochondrial sterol 27-hydroxylase activity was assayed by an isotope incorporation method as described previously by Shefer et al. (35).

Assay for hepatic cholesterol 7α-hydroxylase and HMG-CoA reductase mRNA levels. The relative levels of HMG-CoA reductase and cholesterol 7α-hydroxylase mRNAs were quantitated by Northern blotting analysis as previously described by Ness, Keller, and Pendelton (36).

Statistical study

Data are shown as means ± SD and were compared statistically by the Student's *t* test (unpaired). The BMDP Statistical Software (BMDP Statistical Software, Inc., Los Angeles, CA) was used for statistical evaluations.

RESULTS

In rats, feeding cholesterol increased plasma concentrations 2.1 times (from 46 ± 5 mg/dl to 98 ± 19 mg/dl). In comparison, feeding cholesterol to rabbits raised plasma levels 31 times (from 31 ± 11 mg/dl to 986 ± 188 mg/dl). Therefore, dietary cholesterol accumulated more rapidly in the plasma of rabbits than in rats (**Fig. 1**).

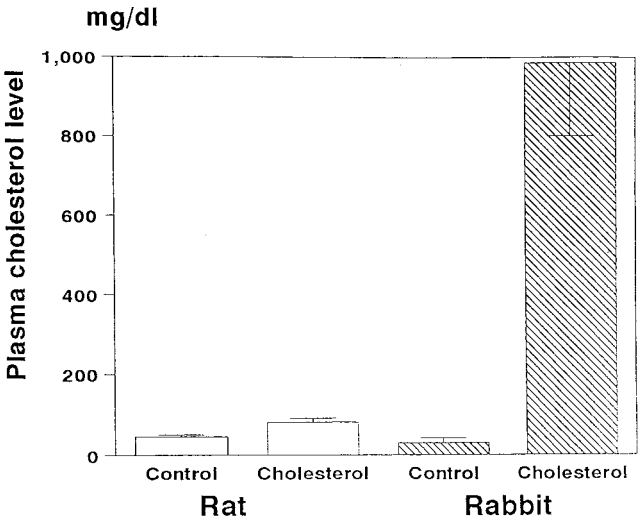

Fig. 1. Plasma cholesterol concentrations after cholesterol feeding in rats and rabbits. The concentrations increased only slightly in rats as compared with rabbits where cholesterol accumulated in the plasma 27 times more rapidly than in rats. The open bars represent results from rats and the crosshatched bars represent those from rabbits. Control: the animals were fed regular chow (without cholesterol). Cholesterol: the animals (rats and rabbits) were fed chow containing 2% cholesterol for 10 days.

Hepatic cholesterol concentrations increased 4.27 times ($P < 0.001$, from 1.70 ± 0.62 to 7.27 ± 1.62 mg/g) in rats and 5 times ($P < 0.0001$, from 1.94 ± 0.15 to 9.74 ± 2.04 mg/g) in rabbits after cholesterol feeding.

The biliary bile acid flux measured immediately after construction of bile fistula was increased 32% (from 11.9 ± 4.4 mg/h to 15.7 ± 4.7 mg/h, P = NS) in cholesterol-fed rats and rose 44% in cholesterol-fed rabbits (from 40.7 ± 15.6 mg/h to 58.8 ± 18.5 mg/h, P = NS). After cholesterol feeding, the proportion of primary bile acids (cholic, chenodeoxycholic, and muricholic acids in rats, and cholic acid in rabbits) were significantly increased.(**Table 1**). Biliary cholesterol secretion was also increased 66% and 3.7 times in rats ($P < 0.001$) and rabbits ($P < 0.01$), respectively.

Total fecal bile acid outputs that reflect new bile acid synthesis were significantly increased 2.1 times from 49 ± 21 (baseline) to 102 ± 43 mg/d ($P < 0.05$) in cholesterol-fed rats and 2.4 times from 21 ± 9 mg/d to 50 ± 18 mg/d ($P < 0.05$) in the cholesterol-fed rabbits.

However, after cholesterol feeding, the bile acid pool size remained constant in rats (55 ± 17 mg vs. 61 ± 18 mg) but enlarged more than 2-fold over baseline, from 254 ± 46 mg to 533 ± 53 mg ($P < 0.01$), in rabbits (**Fig. 2**).

In rats, 2% cholesterol feeding for 10 days resulted in no significant change in the steady state level of either the ileal apical sodium-dependent bile acid transporter (ASBT) protein expression (control 105 ± 42 vs. 101 ± 26 phosphorimager unit/μg protein, n = 4) or ileal lipid binding protein (ILBP) expression (control 90 ± 56 vs.

TABLE 1. Biliary bile acids and cholesterol in rats and rabbits

	Bile Flow	Bile Acid Composition				Bile Acid Secretion	Cholesterol Secretion
		DCA	CDCA	CA	MCA		
	ml/h	*%*				*mg/h*	*mg/h*
Rat							
Control	1.80 ± 0.18	2.0 ± 1.0	5.0 ± 0.5	70.0 ± 2.8	23.0 ± 7.5	11.9 ± 4.4	0.15 ± 0.03
Ch-fed	2.55 ± 0.37[a]	2.8 ± 1.2	13.1 ± 7.5[b]	28.0 ± 11.0[c]	55.0 ± 12.0[a]	15.7 ± 4.7	0.25 ± 0.01[c]
Rabbit							
Control	27.8 ± 11.1	88.9 ± 3.1	1.8 ± 0.5	9.3 ± 2.7		40.7 ± 15.6	0.52 ± 0.21
Ch-fed	32.3 ± 6.0	82.9 ± 3.1[b]	1.9 ± 0.3	15.4 ± 2.0[a]		58.8 ± 18.5	2.48 ± 0.95[a]

Ch-fed: 2% cholesterol for 10 days; DCA: deoxycholic acid; CDCA: chenodeoxycholic acid; CA: cholic acid; MCA: total muricholic acids.

[a] $P < 0.01$ as compared with the controls; [b] $P < 0.05$ as compared with the controls; [c] $P < 0.001$ as compared with the controls.

90 ± 19 phosphorimager unit/μg protein, n = 4). Moreover, in a separate experiment, 100 μm cholesterol produced no inhibitory effect on sodium-dependent taurocholate uptake into COS-7 cells that were transiently transfected with ASBT (control 118 ± 53 vs. 110 ± 50 pmol/mg protein, n = 3). In contrast, in rabbits fed cholesterol, ASBT protein expression (**Fig. 3**) significantly increased 31%, from 100 ± 5 to 131 ± 24 unit/μg protein ($P < 0.05$), signifying enhanced ileal bile acid transport. However, there was no difference in the ILBP expression in the control and cholesterol-fed rabbits (control 77 ± 13 vs. 90 ± 26 unit/μg).

Cholesterol 7α-hydroxylase activity, the rate-limiting enzyme for classic bile acid synthesis (**Fig. 4**) rose 33% (24 ± 2.4 vs. 18 ± 1.6 pmol/mg/min, $P < 0.01$) and mRNA levels increased 50% (from 6.3 ± 1.3 to 9.4 ± 0.3 units/μg mRNA, $P < 0.01$) in cholesterol-fed rats. However, cholesterol 7α-hydroxylase activity decreased 68% (50.2 ± 12.3 to 29.0 ± 5.5 pmol/mg/min, $P < 0.01$) and mRNA levels dropped 79% (29.1 ± 15.6 to 6.2 ± 3.7 units/μg mRNA, $P < 0.05$) respectively in cholesterol-fed rabbits.

Cholesterol 27-hydroxylase activity, the rate-controlling enzyme for alternative bile acid synthesis (**Fig. 5**) did not change in cholesterol-fed rats (56 ± 8.8 to 49.6 ± 3.8 pmol/mg/min) while in rabbits, enzyme activity increased 62%, from 29 ± 6 to 47 ± 10 pmol/mg/min ($P < 0.01$) after cholesterol feeding.

Hepatic HMG-CoA reductase activity, the rate-limiting enzyme for cholesterol synthesis, decreased 81.8% (from 91.7 ± 7.8 pmol/mg/min to 16.7 ± 3.9 pmol/mg/min) in rats and 74% (from 34.0 ± 12.9 pmol/mg/min to 8.8 ± 3.3 pmol/mg/min) in rabbits after cholesterol feeding.

DISCUSSION

The aim of this study was to understand better the mechanism for the different regulation of bile acid synthesis and changes in plasma cholesterol concentrations in response to cholesterol feeding in rats and rabbits. The results demonstrated that cholesterol stimulated cholesterol 7α-hydroxylase activity and mRNA levels without

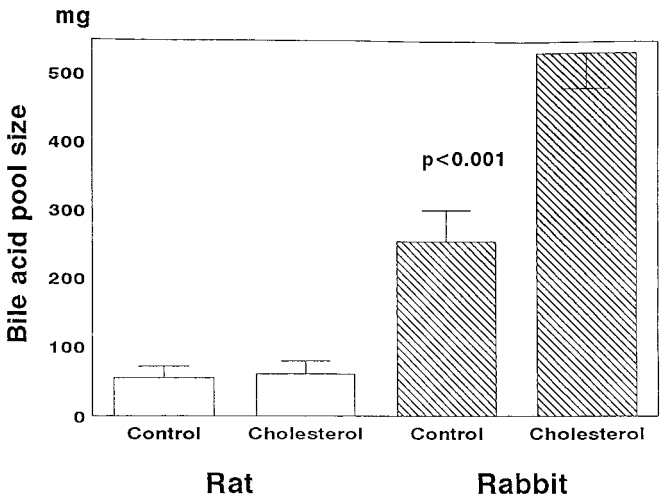

Fig. 2. The bile acid pool size after cholesterol feeding. The pool size did not change in rats but enlarged about 2 times ($P < 0.01$) in rabbits.

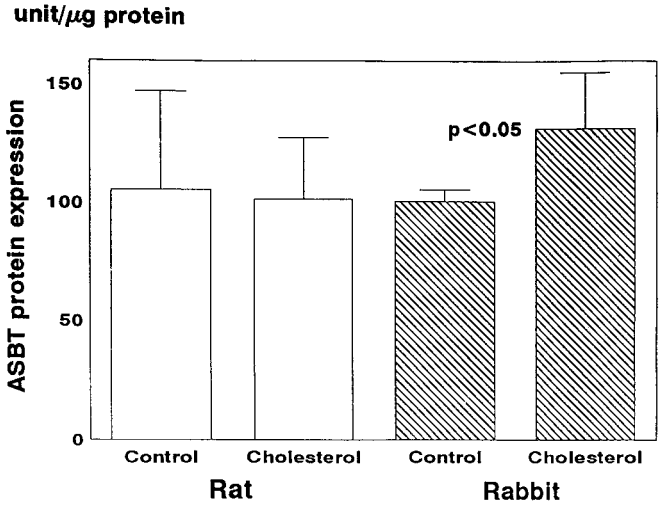

Fig. 3. Ileal apical sodium-dependent bile acid transporter protein after cholesterol feeding. The protein expression remained unchanged in rats but was significantly increased 31% in rabbits.

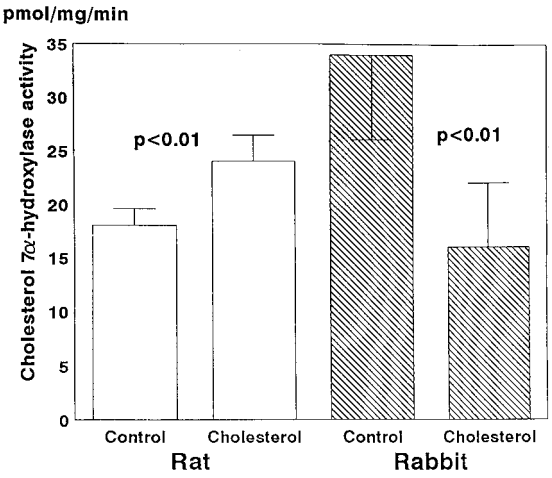

Fig. 4. Hepatic cholesterol 7α-hydroxylase activity after cholesterol feeding. The enzyme activity was increased significantly in rats but was inhibited significantly in rabbits.

changing the bile acid pool size in rats. In comparison, cholesterol-fed rabbits showed inhibited cholesterol 7α-hydroxylase activity and mRNA levels probably due to an expanded bile acid pool. These differences may be related to ileal bile acid reabsorption as ileal ASBT protein increased 31% in cholesterol-fed rabbits but remained unchanged in rats after cholesterol feeding.

This study re-emphasized that rats fed a high cholesterol diet showed only mildly increased plasma cholesterol concentrations while NZW rabbits were very sensitive to the large cholesterol intake and accumulated cholesterol in plasma much more rapidly than rats. We believe that this difference was related to the different response of cholesterol 7α-hydroxylase to dietary cholesterol in these two species. Classic bile acid synthesis is the major path for catabolism of cholesterol in the body. Stimulating cholesterol 7α-hydroxylase, the rate-limiting enzyme for

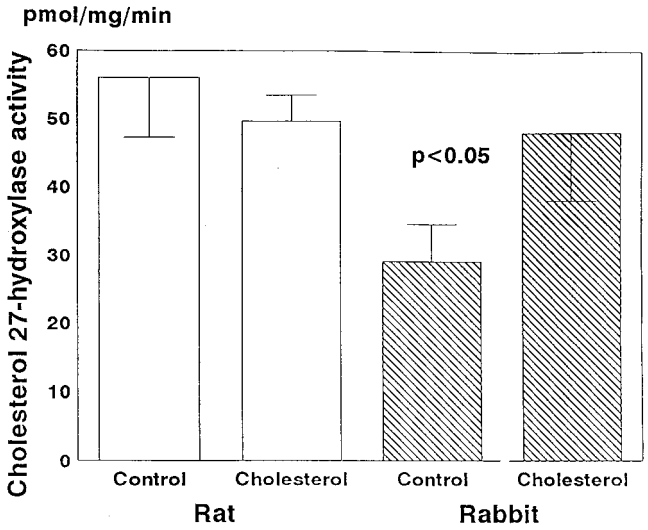

Fig. 5. Hepatic cholesterol 27-hydroxylase activity after cholesterol feeding. The enzyme activity remained constant in rats but was significantly increased 62% in rabbits.

classic bile acid synthesis profoundly enhances the conversion of cholesterol destined for plasma circulating lipoproteins (LDL/VLDL) to bile acids and in turn reduces production of LDL cholesterol (37, 38). Therefore, up-regulated cholesterol 7α-hydroxylase in cholesterol-fed rats resulted in the relatively small increase in plasma cholesterol levels while inhibited cholesterol 7α-hydroxylase in cholesterol-fed rabbits was associated with high plasma cholesterol concentrations. However, in a separate study in rats (39), when cholic acid was added to the cholesterol-containing diet, cholesterol 7α-hydroxylase became inhibited and plasma cholesterol levels increased profoundly, 3 times more than those with dietary cholesterol alone. Thus, in rats when cholesterol 7α-hydroxylase was inhibited by feeding cholic acid, plasma cholesterol levels became more sensitive to dietary cholesterol intake and the cholesterol that could not be converted to bile acids through the classic pathway was diverted to increase plasma cholesterol levels. This fact also supported the idea that cholesterol 7α-hydroxylase plays an important role in the modulation of plasma cholesterol levels. More recently, Wang et al. (40) reported that polymorphisms in cholesterol 7α-hydroxylase gene might be responsible for the heritable variations in plasma LDL cholesterol concentrations in humans. Their work suggested that a genetic linkage between cholesterol 7α-hydroxylase and high plasma LDL cholesterol concentrations existed. However, at this time, there is no evidence that up-regulation of alternative bile acid synthesis (cholesterol 27-hydroxylase) produces an effect similar to that of enhanced cholesterol 7α-hydroxylase to divert cholesterol from the formation of LDL cholesterol and reduce plasma concentrations. These mechanisms need to be clarified.

The findings obtained in this study provide some answers as to why cholesterol 7α-hydroxylase responded differently to dietary cholesterol in these two species. *a*) In rabbits, the enlarged bile acid pool was a more powerful down-regulator of cholesterol 7α-hydroxylase and classic bile acid synthesis that overrode the initial stimulating effect of increasing substrate (cholesterol) supplied by feeding (15). We now demonstrate that, unlike rabbits, cholesterol feeding in rats does not expand the bile acid pool size so that the stimulating effect of increased substrate (cholesterol) continues to prevail. Therefore, not only were cholesterol 7α-hydroxylase activity and mRNA levels not inhibited but both actually increased. *b*) The different response of ileal bile acid transport (ASBT) to cholesterol feeding in these two species played a key role that permitted the bile acid pool to enlarge in rabbits but remained unchanged in rats. After cholesterol feeding, fecal bile acid outputs that reflected bile acid synthesis increased significantly in both rats and rabbits due to stimulated classic and alternative bile acid synthesis, respectively, as well as increased availability of substrate (cholesterol). However, the bile acid pool size enlarged only in rabbits but remained constant in rats. We believe that the greater ASBT protein signified enhanced bile acid transport with retention that accounted

for the enlargement of the bile acid pool in cholesterol-fed rabbits. The increased ASBT protein played a more important role than increased bile acid synthesis in expanding the bile acid pool size. However, we would also like to emphasize that in cholesterol-fed rabbits, when ileal bile acid transport was enhanced, increased amounts of newly synthesized bile acids, as evidenced by increased fecal bile acid outputs, may also contribute to the enlargement of the bile acid pool. In contrast, ileal bile acid transport (ASBT expression) was not increased in rats after cholesterol feeding which limited ileal bile acid reabsorption despite greater bile acid production. Therefore, excess bile acids were lost in feces. As a result, the bile acid pool remained small in rats, cholesterol 7α-hydroxylase and classic bile acid synthesis were up-regulated, and more cholesterol was diverted to be catabolized as bile acids, which reduced the secretion of LDL cholesterol into the plasma.

We do not know whether dietary cholesterol regulates the ileal ASBT directly or indirectly but one thing seems certain, the ileal ASBT is not inhibited by the increased intestinal bile acid flux. Although bile acids secreted into the intestine were significantly increased in both rats and rabbits after cholesterol feeding, the ileal ASBT was up-regulated only in rabbits. This result suggested that ASBT expression might respond differently to the increased intestinal bile acid fluxes in rats and rabbits. This also emphasizes the different effects produced by cholesterol feeding in various animal species (41–43).

It is tempting to speculate that the gallbladder may also play some role in the different responses of ASBT. The rabbit has a gallbladder which can accommodate extra bile acids for expansion of the bile acid pool while the rat has no gallbladder and continuously secretes bile acids into the intestine. Thus, the expanded bile acid pool in rats might accumulate in the liver with ensuing damage, while storing the enlarged bile acid pool in the gallbladder in rabbits protects the liver.

These investigations that show differences in the pathway of bile acid synthesis in response to dietary cholesterol in rats and rabbits may be also relevant to humans. Some people, like rats, can tolerate large intakes of cholesterol presumably by stimulating cholesterol 7α-hydroxylase and classic bile acid synthesis because their ileal bile acid transporter does not increase and, as a result, the bile acid pool does not enlarge. Others, like rabbits, can raise plasma cholesterol levels significantly in response to dietary cholesterol because newly synthesized bile acids are conserved by increased ileal bile acid reabsorption so that the bile acid pool is expanded and cholesterol 7α-hydroxylase is inhibited. The net result is that plasma cholesterol concentrations rise when cholesterol 7α-hydroxylase is inhibited and decline when cholesterol 7α-hydroxylase is stimulated. Thus, ileal bile acid transport (ASBT), which controls the reabsorption of bile acid, plays an important role in modulating the bile acid pool size and exerting negative feedback regulation of cholesterol 7α-hydroxylase that, in turn, raises plasma cholesterol levels.

This study was supported by VA Research Service and US Public Health Service Grants HL-17818, HL-18094, DK-26756, DK-54165, American Heart Association 9850180T, Fogarty International Center 5 F05TW05082-02, and Fondecyt 1990519. We also thank Bibiana Pcolinsky and Eva Paroulek for their excellent technical assistance and Barbara Rouse for preparing the manuscript.

Manuscript recieved 11 August 1999 and in revised form 22 November 1999.

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