# Cholecystectomy prevents expansion of the bile acid pool and inhibition of cholesterol 7α-hydroxylase in rabbits fed cholesterol

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**Abstract** **To study the effect of cholecystectomy on the regulation of classic and alternative bile acid syntheses, gallbladder-intact (n = 20) and cholecystectomized (n = 20) New Zealand White rabbits were fed either chow or chow with 2% cholesterol (3 g/day). After 10 days, bile fistulas were constructed in half of each rabbit group to recover and measure the bile acid pool and biliary bile acid flux. After cholesterol feeding, the bile acid pool size increased from 268 ± 55 to 444 ± 77 mg ($P < 0.01$) with a 2-fold rise in the biliary bile acid flux in intact rabbits but did not expand the bile acid pool (270 ± 77 vs. 276 ± 62 mg), nor did the biliary bile acid flux increase in cholecystectomized rabbits. Ileal apical sodium-dependent bile acid transporter protein increased 46% from 93 ± 6 to 136 ± 23 units/mg ($P < 0.01$) in the intact rabbits but did not change in cholecystectomized rabbits (104 ± 14 vs. 99 ± 19 units/mg) after cholesterol feeding. Cholesterol 7α-hydroxylase activity was inhibited 59% ($P < 0.001$) while cholesterol 27-hydroxylase activity rose 83% ($P < 0.05$) after cholesterol feeding in the intact rabbits but neither enzyme activity changed significantly in cholesterol-fed cholecystectomized rabbits. Fecal bile acid outputs reflecting bile acid synthesis increased significantly in the intact but not in the cholecystectomized rabbits fed cholesterol.** **Removal of the gallbladder prevented expansion of the bile acid pool after cholesterol feeding as seen in intact rabbits because ileal bile acid transport did not increase. As a result, cholesterol 7α-hydroxylase was not inhibited.**—Xu, G., G. Salen, B. L. Shneider, M. Ananthanarayanan, S. Shefer, L. Ma, A. Batta, L. B. Nguyen, J. J. Lingutla, G. S. Tint, M. Pcolinsky, and F. J. Suchy. **Cholecystectomy prevents expansion of the bile acid pool and inhibition of cholesterol 7α-hydroxylase in rabbits fed cholesterol.** ***J. Lipid Res.*** **2001.** 42: **1438–1443.**

**Supplementary key words** apical sodium-dependent bile acid transporter • sodium/taurocholate cotransporting polypeptide • bile acid synthesis • cholesterol 27-hydroxylase • bile acid transport

It is well established that cholesterol often causes hypercholesterolemia when fed. However, some individuals are not sensitive to the intake of dietary cholesterol and, thus, might consume more in the diet without risk (1, 2). The different plasma responses to dietary cholesterol have been also observed in various animal species. For example, rats fed a high cholesterol diet are resistant and do not develop hypercholesterolemia because cholesterol 7α-hydroxylase, the rate-controlling enzyme for the classic bile acid synthesis pathway, is upregulated (3–6) to utilize the extra cholesterol to make more bile acids. In contrast, New Zealand White rabbits usually are sensitive to dietary cholesterol and accumulate large amounts in plasma (7). We have described marked inhibition of cholesterol 7α-hydroxylase after 10 days of cholesterol feeding to New Zealand White rabbits (8). We believe that the inhibition of cholesterol 7α-hydroxylase and classic bile acid synthesis contributed to hypercholesterolemia in the cholesterol-fed rabbit model. This finding suggested that cholesterol 7α-hydroxylase was a key factor in regulating cholesterol homeostasis. We hypothesize that a fundamental difference in regulation of cholesterol 7α-hydroxylase and classic bile acid synthesis between rats and rabbits is responsible for the difference in susceptibility to develop plasma hypercholesterolemia. More recently, we found that a significantly expanded bile acid pool in New Zealand White rabbits fed cholesterol was responsible for the inhibition of cholesterol 7α-hydroxylase (9, 10), whereas in rats, cholesterol feeding did not enlarge the bile acid pool. Furthermore, another difference between these species related to ileal apical sodium-dependent bile acid transporter (ASBT) protein, which was increased in rabbits but did not change in rats after feeding cholesterol (11). Cholesterol 7α-hydroxylase (classic bile acid synthesis) was inhibited in cholesterol-fed rabbits because the bile acid pool

Abbreviations: ASBT, apical sodium-dependent bile acid transporter; BBMV, brush border membrane vesicles; NTCP, sodium/taurocholate cotransporting polypeptide.

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enlarged as a result of enhanced ileal bile acid transport. In contrast, feeding cholesterol to rats did not inhibit, but rather stimulated, cholesterol 7α-hydroxylase because ASBT (ileal bile acid transport) was not upregulated and the bile acid pool remained unchanged.

It is also known that, unlike rabbits, rats have no gallbladders. We questioned whether this anatomical difference, that is, having or not having a gallbladder, might also contribute to the different response of the bile acid pool to cholesterol feeding. Therefore, this study was organized to compare the response of cholesterol 7α-hydroxylase and bile acid pool size to cholesterol feeding in control rabbits with that in cholecystomized rabbits. The results suggested that after removal of the gallbladder, cholesterol feeding was no longer associated with inhibited cholesterol 7α-hydroxylase because ileal bile acid transport was not increased such that the bile acid pool size did not enlarge.

## MATERIALS AND METHODS

### Materials

Cholesterol and 5α-cholestane were obtained from Sigma (St. Louis, MO) and used as standards for the measurements of cholesterol by capillary gas-liquid chromato-graphy. Ursocholic acid (a gift from Gipharmex, Milan, Italy) was conjugated with glycine according to the method of Tserng, Hackey, and Klein (12) and used as internal standard for measurements of bile acids. Cholic and deoxycholic acids were purchased from Steraloids (Wilton, NH). Rabbit chow containing 2% cholesterol was prepared by Purina Mills (St. Louis, MO).

### Animal experiments

The experiments were carried out in 40 male New Zealand White rabbits (Convance, Denver, PA): 20 controls (rabbits with intact gallbladders) and 20 cholecystectomized rabbits (without gallbladders). Rabbits were purchased when they were about 2 months old and weighing 1.0–1.2 kg. One week after arrival, 20 rabbits underwent cholecystectomy via a 3-cm transverse incision 0.5 cm below the right costal margin under anesthesia (ketamine, 50 mg/kg; xylazine, 4 mg/kg, and acepromazine, 0.5 mg/kg, administered intramuscularly). Rabbits recovered from the surgery and their growth and weight were similar to those of the controls. Two months after the cholecystectomy, half of the controls (n = 10) and half of the cholecystectomized rabbits (n = 10) were fed 2% cholesterol rabbit chow (about 3 g of cholesterol per day) for 10 days. The remaining 10 rabbits from each group were fed regular rabbit chow. After completion of the feeding, bile fistulas were constructed in half of the cholesterol-fed controls (n = 5) and chow-fed controls (n = 5) and in half of the cholesterol-fed cholecystectomized rabbits (n = 5) and chow-fed cholecystectomized rabbits (n = 5), to collect the bile and measure the bile acid pool and biliary bile acid fluxes. The remaining rabbits (n = 5 in each group) were killed. The feces during the last 2 days of each treatment were collected for measurements of fecal bile acid outputs. Blood samples were taken for determination of plasma cholesterol concentrations. The livers were removed and portions were frozen immediately for measurements of mRNA of sodium/taurocholate cotransporting polypeptide (NTCP), activities of microsomal cholesterol 7α-hydroxylase and HMG-CoA reductase, and mitochondrial cholesterol 27-hydroxylase, and for hepatic cholesterol concentration determinations. At sacrifice, mucosa from the terminal ileum (20 cm) was collected and frozen immediately for Western blot analysis of ASBT protein. In the rabbits with bile fistulas, bile drainage was continued for 5 days to recover the deoxycholic acid pool. The bile acid concentration and composition were measured. The total bile acid pool sizes were calculated from measurements of total recovered deoxycholic acid in the bile collected by bile drainage divided by the percentage of deoxycholic acid in the initial bile collected during the first 30 min after the construction of the bile fistula (9). The biliary bile acid flux (mg/h) was calculated as the total bile acid concentration (mg/ml) in the bile collected within 30 min after the common bile duct was cannulated multiplied by the bile flow rate (ml/h) during that time.

The animal protocol was approved by the Subcommittee on Animal Studies at the Veterans Affairs 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).

### Chemical analysis

*Assays for bile acids and sterols.* Bile acids in the bile were quantitatively measured by the capillary gas chromatography method as previously described (13). For measurement of fecal bile acids, internal standard (norcholic acid, 20 μg) in 200 μl of *n*-butanol (10–15 mg) was added to the freeze-dried feces. Concentrated hydrochloric acid (20 μl) was then added and the suspension was heated at 55°C for 4 h, after which solvents were evaporated. The residue was subjected to trimethylsilylation, taken in 200 μl of *n*-hexane, and then an aliquot was subjected to capillary gas chromatography for quantitative analysis.

In the presence of an internal standard, 5α-cholestane, plasma or bile cholesterol was quantitated by capillary gas-liquid chromatography as described previously (14). For hepatic sterols, 0.5 g of liver tissue with 200 μg of 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. Two milliliters of this solution was then 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 by the method of saponification and then was quantitated by gas chromatography as mentioned previously (14).

*Assays for ASBT protein (Western blotting).* Crude brush border membrane vesicles (BBMV) were prepared by a modification of a previously reported divalent cation precipitation (15). Protein concentrations were determined by the Bradford method (16) with bovine serum albumin as the standard. Western blotting for ASBT was performed with BBMV and homogenate, respectively. Similar amounts of protein were loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel and subjected to electrophoresis. After electrotransfer onto nitrocellulose membranes, the blots were blocked overnight at 4°C with Tris-buffered saline containing 0.1% Tween and 5% nonfat dry milk and then incubated for 2 h at room temperature with the respective primary antibodies. The antibody for ASBT was a gift from W. Kramer (Aventis Pharma Deutschland GmbH, Disease Group Metabolic Diseases, Frankfurt, Germany). Immune complexes were detected by using $^{125}$I-labeled protein A as secondary antibodies. Immunoreactive bands were detected with a PhosphorImager screen and quantified with a Phosphor-Imager and ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

*Hepatic microsomal cholesterol 7α-hydroxylase and HMG-CoA reductase and mitochondrial cholesterol 27-hydroxylase activities.* Hepatic microsomes and mitochondria were prepared by differential ultracentrifugation (17), and the protein was determined according to Lowry et al. (18).

Hepatic microsomes were used to determine the activities of

HMG-CoA reductase and cholesterol 7α-hydroxylase. The assay for HMG-CoA reductase activity was the same as described previously (10), which was based on the methods of George et al. (19). 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 cofactors by the isotope incorporation method of Shefer, Salen, and Batta (17). Mitochondrial sterol 27-hydroxylase activity was assayed by an isotope incorporation method as described previously by Shefer et al. (20).

*Assay for hepatic NTCP mRNA levels.* Total RNA from samples of frozen liver was isolated by acid guanidinium thiocyanate- phenol-chloroform extraction (21). The total RNA pellet was dissolved in 100 μl of diethylpyro-carbonate-treated water. Poly($A^+$) was isolated by oligo(dT)-cellulose chromatography (22). The relative mRNA level of NTCP was quantitated by Northern blotting analysis as previously described by Arrese et al. (23).

### Statistical study

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

## RESULTS

After 10 days of 2% cholesterol feeding (3 g/day), plasma cholesterol concentrations increased 41.6 times in rabbits with intact gallbladders (**Table 1**) and 25 times in cholecystectomized rabbits. Although elevated, plasma cholesterol concentrations were 23% ($P$ = NS) lower after removal of the gallbladder than in the intact rabbits (800 ± 266 vs. 1,040 ± 163 mg/dl).

Hepatic cholesterol concentrations increased five times in the intact rabbits and 3.6 times in cholecystectomized rabbits after cholesterol feeding (Table 1).

After cholecystectomy, the biliary bile acid flux did not change significantly as compared with untreated intact rabbits (**Table 2**). However, cholesterol feeding increased the biliary bile acid flux 2-fold (32.3 ± 9.6 to 64.6 ± 16.1 mg/h, $P < 0.01$) in intact rabbits but did not increase the flux (29.2 ± 8.2 vs. 32.1 ± 1.0, $P$ = NS) in cholecystectomized rabbits. Removal of the gallbladders did not significantly reduce the percent deoxycholic acid in the bile acid pool as compared with the intact rabbits (Table 2). After cholesterol feeding, percent deoxycholic acid did not change in the intact rabbits but decreased from 87.2 ± 8.9 to 71.7 ± 11.5% ($P < 0.05$) in cholecystectomized rabbits.

**Figure 1** shows that the bile acid pool size expanded 65.7% ($P < 0.01$) in the cholesterol-fed rabbits with gallbladders (444 ± 77 vs. 268 ± 55 mg) but the pool size did not change in cholesterol-fed cholecystectomized rabbits (276 ± 62 vs. 270 ± 77 mg). The baseline bile acid pool size in rabbits after cholecystectomy was similar to the baseline value in the intact rabbits.

TABLE 1. Hepatic and plasma cholesterol in intact and cholecystectomized rabbits

| | Intact | Intact + Ch | CS | CS + Ch |
|---|---|---|---|---|
| Plasma (mg/dl) | 25 ± 5 | 1,040 ± 163 | 32 ± 7 | 800 ± 266 |
| Hepatic (mg/g) | 1.9 ± 0.2 | 9.7 ± 2.0 | 2.6 ± 0.5 | 9.5 ± 2.3 |

Data are presented as means ± SD (n = 5). Intact, rabbits with intact gallbladder; Ch, 2% cholesterol fed for 10 days; CS, rabbits after cholecystectomy.

TABLE 2. Biliary bile acid composition and flux in intact and cholecystectomized rabbits

| | Intact | Intact + Ch | CS | CS + Ch |
|---|---|---|---|---|
| Bile acid flux (mg/h) | 32.3 ± 9.6 | 64.6 ± 16.1[a] | 29.2 ± 8.2 | 32.1 ± 1.0 |
| Cholic acid flux (mg/h) | 3.2 ± 0.9 | 10.2 ± 2.6[b] | 4.8 ± 0.9 | 8.2 ± 2.3[c] |
| DCA (%) | 87.2 ± 8.9 | 86.1 ± 5.3 | 83.5 ± 4.5 | 71.7 ± 11.5[d] |

Data are presented as means ± SD (n = 5). Intact, rabbits with intact gallbladder; Ch, 2% cholesterol fed for 10 days; CS, rabbits after cholecystectomy; bile acid flux, total biliary bile acid secretion rate; cholic acid flux, biliary cholic acid secretion rate; DCA (%), deoxycholic acid in total bile acids in the initial bile collected within the first 15 min after the common bile duct is cannulated.

[a] $P < 0.01$ as compared with controls or CS + Ch.
[b] $P < 0.001$ as compared with control.
[c] $P < 0.01$ as compared with CS.
[d] $P < 0.05$ as compared with intact, intact + Ch, and CS.

After cholesterol feeding, fecal bile acid outputs increased 47% (from 32.8 ± 6.7 to 48.2 ± 12.2 mg/day, $P < 0.05$) in the intact rabbits and rose 25% (from 27.5 ± 11.2 to 34.3 ± 17.9 mg/day, $P$ = NS) in cholecystectomized rabbits.

In rabbits with gallbladders, ileal ASBT protein was 46% more abundant (from 93 ± 6 to 136 ± 23 units/μg, $P < 0.01$) after cholesterol feeding than the untreated baseline value (**Fig. 2**). The Western blots for ASBT protein in the intact rabbits with and without cholesterol feeding are shown in **Fig. 3**. After cholecystectomy, ASBT protein tended to rise ($P$ = NS), but was not increased further by cholesterol feeding (from 104 ± 14 to 99 ± 19 units/μg).

The NTCP mRNA level (**Fig. 4**) for sinusoidal bile acid transport declined 30% (from 0.057 ± 0.025 to 0.040 ± 0.011 unit, $P$ = NS) in cholesterol-fed intact rabbits. However, after cholesterol feeding, the mRNA level of NTCP in cholecystectomized rabbits was similar to that of the untreated intact rabbits (0.052 ± 0.010 vs. 0.057 ± 0.025 unit).

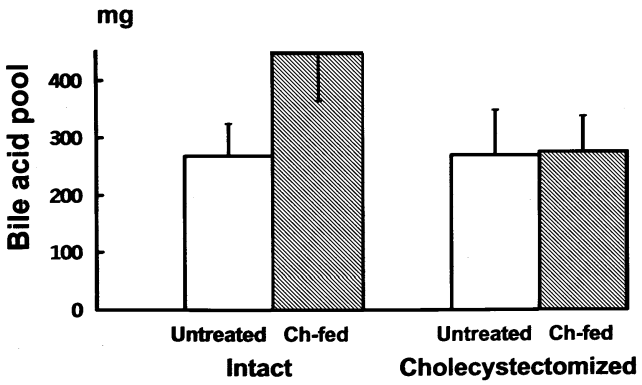


**Fig. 1.** Bile acid pool size in intact and cholecystectomized rabbits. After 10 days of 2% cholesterol feeding (Ch-fed), the bile acid pool expanded by 65.7% ($P < 0.01$) in the intact rabbits but did not change in cholecystectomized rabbits.

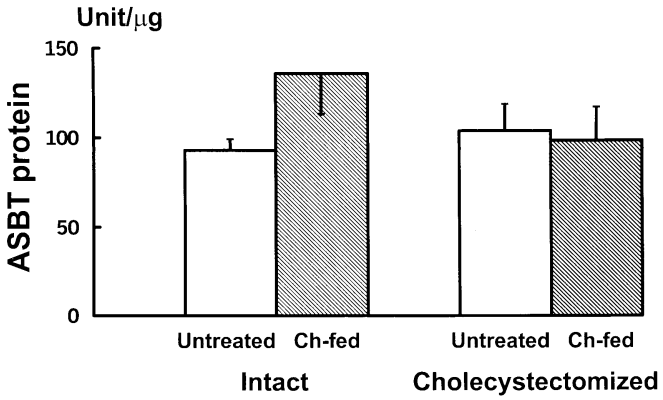


**Fig. 2.** Ileal ASBT protein levels. After 10 days of 2% cholesterol feeding (Ch-fed), ASBT protein rose 46% ($P < 0.01$) in the intact rabbits but did not change in cholecystectomized rabbits.

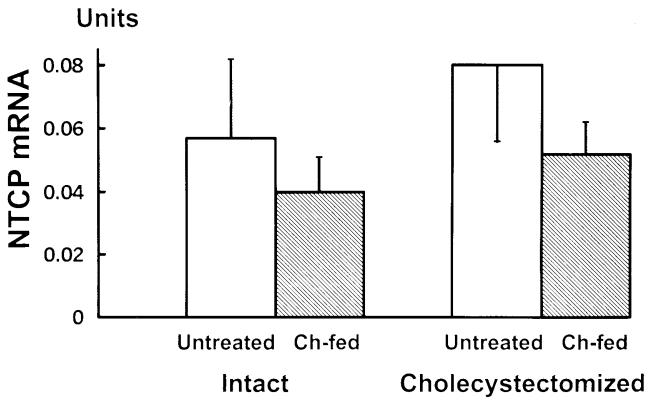


**Fig. 4.** Hepatic sodium/taurocholate cotransporting peptide (NTCP) mRNA levels decreased 30% in intact rabbits after cholesterol feeding (Ch-fed). NTCP mRNA levels in cholesterol-fed cholecystectomized rabbits remained similar to the baseline in untreated intact rabbits.

After 10 days of cholesterol feeding, cholesterol 7α-hydroxylase activity (**Fig. 5**) was inhibited 59% ($P < 0.001$) in rabbits with gallbladders (from 28.1 ± 2.7 to 11.4 ± 4.2 pmol/mg/min) but was not reduced in cholecystectomized rabbits (27.6 ± 5.6 vs. 23.4 ± 6.7 pmol/mg/min). Baseline cholesterol 7α-hydroxylase activities were similar in the untreated intact and cholecystectomized rabbits.

Cholesterol 27-hydroxylase activity (**Fig. 6**) increased 83% ($P < 0.05$) after 10 days of cholesterol feeding in the intact rabbits (from 25.1 ± 8.5 to 46.0 ± 11.2 pmol/mg/min) but did not rise above baseline in cholesterol-fed cholecystectomized rabbits (28.3 ± 5.9 vs. 24.9 ± 8.4 pmol/mg/min).

HMG-CoA reductase activities were significantly inhibited in both intact (−82%, $P < 0.001$, from 44.6 ± 14.7 to 8.1 ± 2.2 pmol/mg/min) and cholecystectomized (−73.5%, $P < 0.01$, from 36.3 ± 25.5 to 9.6 ± 4.9 pmol/mg/min) rabbits after 10 days of cholesterol feeding.

## DISCUSSION

The results of this investigation show the effect of gallbladder removal on cholesterol and bile acid metabolism in rabbits fed a regular chow (cholesterol-free) diet and after 10 days of high cholesterol intake (3 g/day). When the cholecystectomized rabbits consumed the regular chow diet (cholesterol free), plasma and hepatic cholesterol concentrations as well as cholesterol and bile acid biosyntheses were unchanged as evidenced by nearly equal activities for their respective rate-controlling enzymes, HMG-CoA reductase, cholesterol 7α-hydroxylase, and cholesterol 27-hydroxylase compared with the baseline in the intact rabbits. As expected, feeding cholesterol for 10 days greatly increased plasma and hepatic cholesterol concentrations although less (−23%) cholesterol accumulated in plasma after removal of the gallbladder. The enlarged hepatic cholesterol pools markedly inhibited endogenous hepatic cholesterol synthesis (HMG-CoA reductase activity) in both models. However, unlike rabbits with gallbladders, in which classic bile acid synthesis (cholesterol 7α-hydroxylase) was markedly inhibited and alternative bile acid synthesis (cholesterol 27-hydroxylase) was stimulated, neither rate-controlling enzyme nor the formation of bile acids by the classic and alternative bile acid synthetic pathways were affected by cholesterol feed-

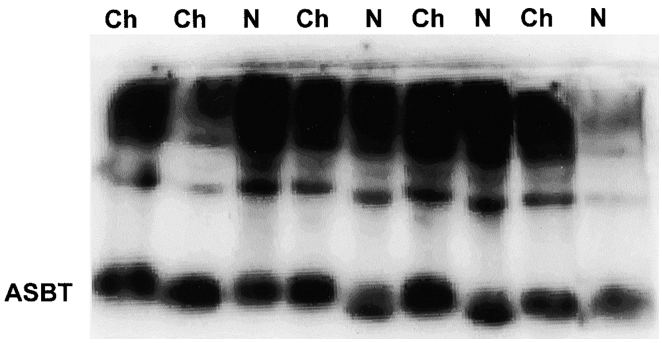


**Fig. 3.** Western blots for measurement of ASBT protein in rabbits with intact gallbladders. Ch, 2% cholesterol feeding for 10 days; N, untreated intact rabbits. Ileal ASBT expression is higher in rabbits fed cholesterol (Ch) than in rabbits without cholesterol feeding (N).

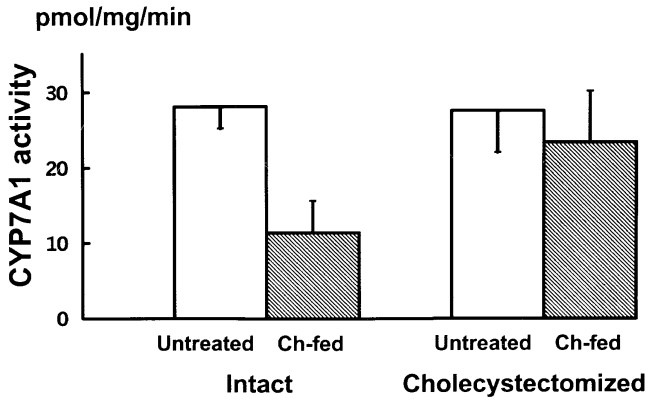


**Fig. 5.** Cholesterol 7α-hydroxylase (CYP7A1) activity in intact and cholecystectomized rabbits. After 10 days of 2% cholesterol feeding (Ch-fed), enzyme activity was inhibited 59% ($P < 0.001$) in the intact rabbits but did not change in cholecystectomized rabbits.

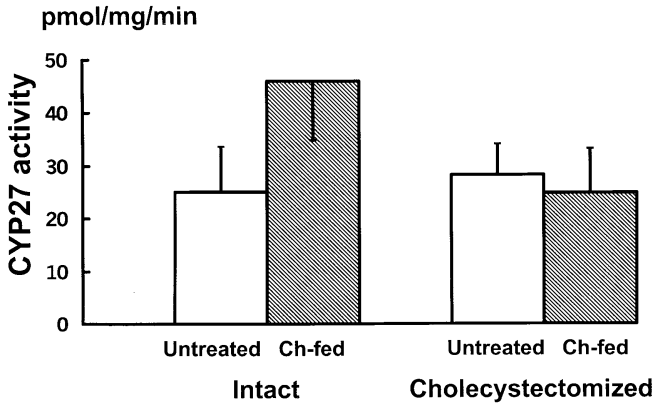


**Fig. 6.** Cholesterol 27-hydroxylase (CYP27) activity in intact and cholecystectomized rabbits. After 10 days of 2% cholesterol feeding (Ch-fed), enzyme activity rose 83% ($P < 0.05$) in intact rabbits but did not change in cholecystectomized rabbits.

ing after removal of the gallbladder. The explanation for the failure to downregulate cholesterol 7α-hydroxylase and classic bile acid synthesis after cholecystectomy seems related to the fact that the bile acid pool, which had expanded significantly after cholesterol feeding in rabbits with gallbladders, did not enlarge during cholesterol feeding in rabbits without gallbladders. This study showed that after cholesterol feeding, ileal ASBT protein that reflected ileal bile acid transport increased 46% ($P < 0.01$) in rabbits with gallbladders but not in cholecystectomized rabbits. Furthermore, the transhepatic biliary bile acid flux did not increase in cholesterol-fed cholecystectomized rabbits as in cholesterol-fed rabbits with gallbladders (2-fold increase; see Table 2), in which the bile acid pool enlarged significantly. We hypothesize that in rabbits after cholecystectomy, cholesterol feeding was not associated with an enlarged bile acid pool because ileal bile acid transport did not increase. In addition, percent deoxycholic acid in the bile of cholecystectomized rabbits decreased 17.8% ($P < 0.05$) after cholesterol feeding. Because biliary cholic acid outputs were similar in both cholesterol-fed intact and cholecystectomized rabbits, the decreased proportion of deoxycholic acid in the bile acid pool might also indicate that ileal bile acid transport in cholecystectomized rabbits was less than in intact rabbits after cholesterol feeding.

It was noteworthy that fecal bile acid outputs, which in the steady state are equivalent to hepatic bile acid synthesis, were increased significantly in the intact rabbits with gallbladders after cholesterol feeding. These results suggested that the increased bile acid synthesis occurred via the alternative pathway through cholesterol 27-hydroxylase, as the classic pathway via cholesterol 7α-hydroxylase was inhibited at this time. In contrast, fecal bile acid outputs were not significantly increased in cholesterol-fed cholecystectomized rabbits as compared with their untreated baseline and agreed with our finding that neither cholesterol 7α-hydroxylase (classic synthesis) nor cholesterol 27-hydroxylase (alternative synthesis) activity was upregulated in these rabbits.

This study demonstrated that although hepatic cholesterol concentrations increased 3.6 times after cholesterol feeding in cholecystectomized rabbits, cholesterol 7α-hydroxylase activity was not inhibited. This fact again supports our previous contention (10) that cholesterol 7α-hydroxylase and classic bile acid synthesis are regulated by the hepatic bile acid flux but not the hepatic cholesterol pool. Inhibition of cholesterol 7α-hydroxylase after cholesterol feeding resulted from the significant expansion of the bile acid pool but not because the hepatic cholesterol pool had increased.

These results bring up the important question of how bile acids regulate their synthesis and transport. Discoveries show that the activated orphan nuclear receptor, farnesoid X receptor (FXR), inversely regulates transcription of cholesterol 7α-hydroxylase and is in turn activated by its ligand, bile acids (24, 25). In vitro studies demonstrated (26) that hydrophobic bile acids lithocholic, chenodeoxycholic, and deoxycholic acids were the most potent ligands for FXR. We hypothesize that the different response of cholesterol 7α-hydroxylase to cholesterol feeding in rabbits with or without gallbladders depends on whether the ligand pool (bile acid pool) for FXR is significantly expanded and consequently enhances transhepatic biliary bile acid flux, the ligand flux that activates FXR.

It has been suggested that *NTCP* is also a target gene and that its transcription is negatively regulated by FXR (27, 28). After cholesterol feeding, hepatic NTCP mRNA levels decreased 30% in the intact rabbits whereas in cholecystecomized rabbits, NTCP mRNA levels were similar to the baseline in untreated intact rabbits. This might be because, unlike with intact rabbits, cholesterol feeding of cholecystectomized rabbits did not enlarge the pool of bile acid (ligands for FXR) such that hepatic FXR was not further activated.

In summary, removal of the gallbladder prevented induction of ileal bile acid transport and expansion of the bile acid pool in cholesterol-fed rabbits. As a result, hepatic cholesterol 7α-hydroxylase did not decline and cholesterol 27-hydroxylase did not rise.

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