Bile acids regulate hepatic low density lipoprotein receptor activity in the hamster by altering cholesterol flux across the liver

(cholesterol synthesis/cholesterol esters/chenodeoxycholic acid/ursodeoxycholic acid/cholic acid)

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ABSTRACT The effect of different bile acids on receptor-dependent and receptor-independent low density lipoprotein (LDL) uptake in the liver and intestine was investigated. When fed at the 0.1% level for three weeks, cholic acid and chenodeoxycholic acid suppressed hepatic cholesterol synthesis in the rat by 80% and 50%, respectively, while ursodeoxycholic acid had no effect. In contrast, hepatic cholesteryl ester levels, rates of hepatic LDL transport, and concentrations of plasma LDL–cholesterol were not affected by bile acid feeding in this species. Cholic acid and chenodeoxycholic acid also suppressed hepatic cholesterol synthesis in the hamster. However, since basal rates of hepatic cholesterol synthesis in this species, as in man, are very low, the absolute reduction in hepatic synthesis could not compensate for the change in hepatic sterol balance induced by bile acid feeding. Hence, in the hamster the feeding of cholic acid and chenodeoxycholic acid increased hepatic cholesteryl ester levels 660% and 39%, respectively, reduced hepatic receptor-dependent LDL uptake by 50% and 32%, respectively, and elevated plasma LDL–cholesterol levels by 160% and 50%, respectively. Ursodeoxycholic acid feeding did not alter any of these processes, and none of the bile acids changed the rate of hepatic receptor-independent LDL transport. In the intestine, none of the bile acids altered rates of cholesterol synthesis or LDL uptake. When cholic acid, chenodeoxycholic acid, or ursodeoxycholic acid was infused continuously for 8 hr in supranormal amounts into control hamsters or rats or into animals pretreated with cholestyramine, there were no changes in LDL transport or any other parameter of hepatic cholesterol metabolism. Thus, these studies indicated that cholic acid and chenodeoxycholic acid have no acute, direct effect on rates of receptor-dependent LDL transport or cholesterol synthesis but do alter these processes indirectly by inducing changes in cholesterol balance across the liver. Ursodeoxycholic acid, in contrast, does not affect these processes either directly or indirectly and so causes no change in plasma LDL levels.

The use of chenodeoxycholic acid and ursodeoxycholic acid in the medical treatment of cholesterol gallstone disease has lead to renewed interest in the effects of chronic bile acid administration on cholesterol and lipoprotein metabolism. That bile acids play a regulatory role in lipoprotein metabolism is suggested by the finding that the administration of chenodeoxycholic acid, but not ursodeoxycholic acid, leads to a gradual increase in plasma low density lipoprotein (LDL) levels in man (1, 2). A major determinant of plasma LDL levels is the rate at which LDL is removed from the plasma and degraded. In most species, including man, approximately 70% of total LDL catabolism is receptor dependent (3–5). Furthermore, in all species examined 80–90% of the receptor-dependent LDL transport activity demonstrable in vivo is found in the liver (3, 6, 7). Therefore, changes in circulating LDL levels associated with bile acid feeding almost certainly reflect a change in either hepatic LDL receptor activity or the rate of LDL synthesis.

Bile acids might effect LDL catabolism by acutely altering the rate of receptor-dependent LDL transport in the liver. Indeed, it has been reported that a 4–6 hr infusion of taurocholic acid completely abolishes specific LDL binding to liver membranes in the dog (8). On the other hand, more chronic administration of bile acids could change LDL metabolism indirectly by altering cholesterol balance across the liver. For example, it is well recognized that cholic acid and chenodeoxycholic acid promote cholesterol absorption from the intestine and suppress bile acid synthesis in the liver more effectively than does ursodeoxycholic acid (9–14). Both effects would tend to increase the available pool of cholesterol within the hepatocyte. This, in turn, would lead to a reduction in the rate of de novo synthesis and, if this reduction were quantitatively inadequate to compensate for the increased sterol pool, a reduced rate of LDL–cholesterol uptake from the plasma.

The present studies were, therefore, undertaken to examine the effect of bile acid feeding on LDL transport and cholesterol synthesis in the liver and small intestine and to determine if such effects were due to the direct regulation of these two metabolic pathways by bile acids or to a compensatory response to alterations in cholesterol balance across the liver. These studies were carried out in vivo in the rat, which can alter its rate of hepatic cholesterol synthesis over a very wide range (3), and in the hamster, which, like man, is much more limited in this regard and responds to changes in cholesterol balance and other dietary manipulations with a change in circulating plasma LDL–cholesterol levels (6, 7, 15).

MATERIALS AND METHODS

Animals and Diets. Female Sprague–Dawley rats and male Golden Syrian hamsters (Charles River Breeding Laboratories) were light cycled and fed a control rodent diet (Allied Mills, Chicago) for 3 weeks prior to use. At that time the rats and hamsters weighed 180–220 g and 110–140 g, respectively. The experimental diets were prepared by mixing 0.1% (wt/wt) cholic acid, chenodeoxycholic acid, and ursodeoxycholic acid with ground rodent diet, following which the diet was repelleted. Each diet was fed ad libitum to different groups of animals for 3 weeks. Weight gain over the feeding period was not significantly different in any of the groups. At the end of this 3-week period, all experimental measurements were carried out during the mid-dark phase of the light cycle.

Determination of Sterol Synthesis Rates in Vivo. As described (16–18), animals were administered 3H2O (approximately 50 mCi; 1 Ci = 37 GBq) intravenously and killed 1 hr

Abbreviations: LDL, low density lipoprotein.
later. Aliquots of plasma were taken for the determination of plasma water specific activity, and aliquots of liver were obtained for the isolation of digitonin precipitable sterols. The entire small intestine was saponified, and aliquots also were taken for the isolation of sterols. Rates of synthesis were expressed as the nmol of \(^3\)H\(_2\)O incorporated into digitonin precipitable sterols per hr per g of tissue (nmol/hr per g).

**Lipoprotein Preparation.** Rat, hamster, and human LDL was isolated from plasma by preparative ultracentrifugation in the density range of 1.020-1.055 g/ml and labeled with \[^{14}C\]sucrose (Amersham) (19). The hamster and human LDL in this density range contained almost exclusively apolipoprotein B-100 on polyacrylamide gels. Rat LDL, however, contained significant amounts of apolipoprotein E and required further purification on Geon-Pevikon slabs (20). The human LDL was methylated as described (21). All lipoprotein fractions were used within 24 hr of preparation and were filtered through a 0.45-μm pore sized Millipore filter immediately prior to use.

**Determination of Tissue LDL Uptake Rates in Vivo.** Rates of tissue LDL clearance were determined by using a primedcontinuous infusion of \[^{14}C\]sucrose-labeled LDL (3, 6). Groups of animals were killed at 10 min, 2 hr, 4 hr, and 6 hr by exsanguination through the abdominal aorta, and aliquots of plasma and tissue were obtained and solubilized (6, 22). The radioactivity in the liver and small intestine at each time point was expressed as the number of μl of plasma that would contain an equivalent amount of radioactivity. The increase in this tissue space with time equaled the number of μl of plasma cleared entirely of its LDL content per hr per g wet weight of tissue (μl/hr per g). When these clearance values were multiplied by the plasma LDL–cholesterol concentration, the absolute rate of LDL–cholesterol uptake was obtained (μg/hr per g).

**Analytic Procedures.** Plasma LDL-cholesterol concentrations (density of 1.020-1.063 g/ml) were measured colorimetrically (23). Hepatic free and esterified cholesterol levels were measured by using silicic acid/celite columns as described (24). The total content of bile acids extractable from the intestine and liver was assayed by using the 3a-hydroxyesteroid dehydrogenase assay described (25), and these total pools were expressed as the μmol of bile acid per kg of body weight. To identify the individual bile acids present in this pool, aliquots were also diluted with 100 mM NaOH, filtered through a 0.45-μm pore size Millipore filter and prepped using a Sep-Pak cartridge (Waters Associates). HPLC analysis was then performed by using a Waters Associates system with a differential refractometer and a \(\mu\)Bondapak C\(_{18}\) reversed-phase column. The mobile phase used was methanol/water/glacial acetic acid (73:25:2, vol/vol) adjusted to a pH of 4.5 with NaOH and run at a pump speed of 1.3 ml/min.

**RESULTS**

Preliminary studies were done to determine the effects of bile acid feeding at the 0.1% level on total plasma cholesterol concentrations and on bile acid pool sizes and composition in the rat and hamster. As summarized in Table 1, in control rats the plasma cholesterol level equaled 56 mg/dl and was not affected by bile acid feeding. In control hamsters, the plasma cholesterol level equaled 115 mg/dl and increased significantly with cholic acid (51%) and chenodeoxycholic acid (23%) feeding. In contrast, ursodeoxycholic acid had no effect. The total bile acid pool size increased only marginally in the bile acid fed rats but increased approximately 50% in all three groups of bile acid fed hamsters. In the rats, the major bile acids were cholic acid, muricholic acid, and chenodeoxycholic acid. With bile acid feeding, the fed bile acid accounted for 80%, 31%, and 37% of the total bile acid pool in the rats given cholic acid, chenodeoxycholic acid, and ursodeoxycholic acid, respectively. In control hamsters the major bile acids were cholic acid and chenodeoxycholic acid, and in hamsters fed the various bile acids, the fed bile acid accounted for >70% of the total bile acid pool.

Studies were next undertaken to examine the effects of bile acid feeding on rates of cholesterol synthesis and LDL transport in the liver. As has been noted (18), the liver of control rats synthesized cholesterol at the very high rate of approximately 2000 nmol/hr per g, as shown in Fig. 1A. Cholic acid and chenodeoxycholic acid feeding suppressed the rate of hepatic synthesis by nearly 80% and 50%, respectively, while Ursodeoxycholic acid had no effect. However, despite these effects on synthesis, none of the bile acids significantly altered hepatic cholesterol ester levels (Fig. 1B), hepatic LDL clearance rates (Fig. 1C), or plasma LDL–cholesterol concentrations (Fig. 1D) in this species.

In the hamster, the relative changes in rates of hepatic cholesterol synthesis induced by bile acid feeding were similar to those found in the rat in that cholic acid and chenodeoxycholic acid inhibited synthesis by approximately

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**Table 1. Plasma cholesterol concentration and the composition of the bile acid pool in rats and hamsters fed different bile acids**

<table>
<thead>
<tr>
<th>Bile acid fed</th>
<th>Plasma cholesterol concentration, mg/dl</th>
<th>Bile acid pool</th>
<th>Total, μmol/kg</th>
<th>MCA, %</th>
<th>UDCA, %</th>
<th>CA, %</th>
<th>CDCA, %</th>
<th>DCA, %</th>
<th>LCA, %</th>
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</thead>
<tbody>
<tr>
<td>Rat</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>56 ± 3</td>
<td>565 ± 26</td>
<td>31</td>
<td>4</td>
<td>48</td>
<td>14</td>
<td>3</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CA</td>
<td>54 ± 2</td>
<td>620 ± 42</td>
<td>11</td>
<td>1</td>
<td>80</td>
<td>3</td>
<td>5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CDCA</td>
<td>53 ± 4</td>
<td>594 ± 21</td>
<td>37</td>
<td>8</td>
<td>22</td>
<td>31</td>
<td>1</td>
<td>—</td>
<td>—</td>
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<tr>
<td>UDCA</td>
<td>57 ± 4</td>
<td>628 ± 22</td>
<td>21</td>
<td>37</td>
<td>38</td>
<td>28</td>
<td>12</td>
<td>1</td>
<td>—</td>
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<tr>
<td>Hamster</td>
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</tr>
<tr>
<td>Control</td>
<td>115 ± 6</td>
<td>128 ± 17</td>
<td>—</td>
<td>72</td>
<td>22</td>
<td>5</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>CA</td>
<td>174 ± 10*</td>
<td>212 ± 17*</td>
<td>—</td>
<td>74</td>
<td>24*</td>
<td>24</td>
<td>—</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>CDCA</td>
<td>141 ± 9*</td>
<td>189 ± 15*</td>
<td>—</td>
<td>7*</td>
<td>87*</td>
<td>2</td>
<td>4</td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>UDCA</td>
<td>112 ± 5</td>
<td>198 ± 8*</td>
<td>70</td>
<td>15</td>
<td>3</td>
<td>2</td>
<td>—</td>
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</table>

Groups of six animals were fed either control diet or this same diet containing 0.1% (wt/wt) cholic acid (CA), chenodeoxycholic acid (CDCA), or ursodeoxycholic acid (UDCA) for three weeks. Mean values ± 1 SEM are shown for the plasma cholesterol concentrations and total bile acid pool sizes. Only mean values are given for the percentage distribution of the individual bile acids in each pool. LCA, lithocholic acid; MCA, muricholic acid; DCA, deoxycholic acid.

*Significantly different (P < 0.05) from the appropriate control value.
The effect of bile acid feeding on rates of cholesterol synthesis and LDL clearance in the small intestine of these animals also was measured, and the results are summarized in Fig. 3. The rate of cholesterol synthesis in the small intestine was unaffected by bile acid feeding in either the rat (Fig. 3A) or the hamster (Fig. 3C). In the rat, the rate of LDL clearance in the small intestine was also unaffected (Fig. 3B); however, in the hamster the rate of LDL clearance in the small intestine was modestly decreased by cholic acid and chenodeoxycholic acid feeding but remained unchanged with ursodeoxycholic acid (Fig. 3D).

Since plasma LDL cholesterol concentrations were elevated in the hamsters fed chenodeoxycholic acid and cholic acid, it was impossible to interpret whether the observed decreases in LDL clearance reflected an increase in the production rate of LDL and increased saturation of LDL receptors, or actual suppression of receptor-dependent LDL transport. To distinguish these two possibilities, the kinetic characteristics of the receptor-dependent and receptor-independent transport processes in the liver and small intestine were defined over the range of plasma LDL–cholesterol concentrations seen in the bile acid fed animals. Thus, rates of LDL clearance were measured in control hamsters whose plasma LDL–cholesterol levels were acutely raised and maintained at values ranging from 25 mg/dl to 100 mg/dl by adding various amounts of unlabeled LDL to the primed-continuous infusions of [14C]sucrose-labeled LDL. Such studies were performed by using both homologous LDL (to measure total LDL transport) and methylated human LDL (to measure receptor-independent transport) (6, 7, 15). The data were analyzed by using least-square, nonlinear regression methods as described (15), and the best-fit curves ± 2 SD are presented in Fig. 4 in two different ways. Fig. 4A and C show the relationship between total LDL clearance (stippled areas) and receptor-independent clearance (hatched areas) and the plasma LDL-cholesterol concentration in both the liver (A) and small intestine (C). These same data are presented in Fig. 4B and D as total and receptor-independent LDL–cholesterol uptake. In both cases the areas between the two curves represent the receptor-dependent component of LDL transport. The observed rates of LDL transport in the bile acid fed hamsters are superimposed upon these standard kinetic curves for LDL transport in control animals.
cholesterol synthesis and LDL transport could be detected. Rats and hamsters were placed in restraining cages and administered an intravenous infusion of the taurine conjugates of cholic acid and chenodeoxycholic acid (100 μmol/hr per kg of body weight) for 8 hr at which time rates of hepatic cholesterol synthesis and LDL transport were determined in vivo. In these studies, no suppression was observed in either hepatic cholesterol synthesis or hepatic LDL transport in the animals infused with cholic acid or chenodeoxycholic acid. Even when the animals were pretreated with cholestyramine (3%, wt/wt) for 10 days to derepress rates of hepatic cholesterol synthesis (rats and hamsters) and LDL transport (hamsters), there was no suppression of hepatic cholesterol synthesis or LDL transport by the 8 hr infusion of cholic acid or chenodeoxycholic acid.

**DISCUSSION**

These studies demonstrate that chronic cholic acid and chenodeoxycholic acid feeding in the hamster suppresses hepatic receptor-dependent LDL transport by 50% and 32%, respectively, while the rate of receptor-independent transport remains unchanged. In the hamster, as well as in the other species that have been studied, the liver is the major site for LDL catabolism (3, 6). Furthermore, the uptake of LDL by the liver is mediated largely (>90%) by LDL receptors (6, 7, 15). Thus, a change in hepatic receptor-dependent transport would be expected to have a major effect on LDL levels and, indeed, plasma LDL–cholesterol concentrations increased 160% and 50% in hamsters fed cholic acid and chenodeoxycholic acid, respectively. However, the intravenous infusion of these two bile acids at rates 50% to 100% higher than the normal flux across the liver failed to alter hepatic LDL transport even after 8 hr in the hamster and rat, and this was true even under circumstances where hepatic LDL transport and cholesterol biosynthesis had been increased by pretreatment with cholestyramine. These latter findings, therefore, differ significantly from a previous report that specific LDL binding to hepatic membranes in the dog was abolished by the intravenous administration of taurocholate for 4–6 hr (8) and suggest that there is no acute, direct effect of these two bile acids on the receptor-dependent LDL transport process.

In contrast to these results observed with cholic acid and chenodeoxycholic acid, the chronic feeding of ursodeoxycholic acid had no effect on hepatic LDL uptake or plasma LDL levels. Since the regulation of cholesterol metabolism in the hamster very closely reflects regulation in man, these findings presumably explain the gradual rise in plasma LDL–cholesterol levels that have been reported in patients undergoing gallstone dissolution therapy with chenodeoxycholic acid but not in those receiving ursodeoxycholic acid (1).

In theory, these effects of chronic bile acid administration could have resulted from a direct action of the bile acid on the genetic regulation of the LDL receptor or, alternatively, could have arisen indirectly through an effect on net cholesterol balance across the cell. Two lines of evidence suggest that the latter possibility is correct and that regulation of receptor-dependent LDL transport is mediated by a change in the size of some critical, regulatory pool of sterol in the liver. First, certain bile acids may alter cholesterol flux across the liver by both promoting sterol absorption from the intestine and suppressing the conversion of cholesterol to bile acids. Cholic acid and, to a lesser extent, chenodeoxycholic acid can exert both of these effects while ursodeoxycholic acid may actually lower dietary sterol absorption and has little effect on regulation of bile acid synthesis (9–14). Thus the differential effects of these three bile acids on LDL metabolism in the hamster closely reflect their known effects
on hepatic sterol balance. Second, the liver of the rat has such an exceptionally high rate of sterol synthesis that the 80% suppression seen with cholic acid feeding (Fig. 1) resulted in a decrease in cholesterol production by the liver equal to about 50 μg/hr per g. Presumably this fully compensates for any change that may have occurred in hepatic cholesterol acquisition due to enhanced cholesterol absorption or reduced conversion to bile acids. However, the similar percentage decrease in cholesterol synthesis induced by cholic acid feeding in the hamster (Fig. 2) resulted in an absolute decrease in cholesterol synthesis of only 1 μg/hr per g. Hence, cholesterol accumulated in the liver as esters and receptor-dependent LDL transport was suppressed. Thus, whether or not bile acid feeding causes changes in the rate of receptor-dependent LDL transport appears to depend on whether or not the liver of a particular species can compensate fully for a change in cholesterol flux across that organ by an appropriate change in the rate of cholesterol synthesis.

Finally, the effect of cholic acid and chenodeoxycholic acid feeding was exerted only in the liver and manifested by reduced receptor-dependent LDL uptake in that organ. The secondary increase in circulating LDL concentrations, however, did not result in suppression of receptor-dependent LDL transport in the extrahepatic tissues, as was evident in the intestine (Fig. 4). A similar finding has been reported with cholestyramine feeding in that LDL transport was increased in the liver but not in the extrahepatic organs (6). Thus, manipulations such as bile acid and cholestyramine feeding affect receptor-dependent LDL transport only in the liver.

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