Inhibiting intestinal NPC1L1 activity prevents diet-induced increase in biliary cholesterol in Golden Syrian hamsters

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Address for reprint requests and other correspondence: S. D. Turley, Dept. of Internal Medicine, Univ. of Texas Southwestern Medical School, Dallas, TX 75390-9151 (e-mail: stephen.turley@utsouthwestern.edu).

Valasek MA, Repa JJ, Quan G, Dietschy JM, Turley SD. Inhibiting intestinal NPC1L1 activity prevents diet-induced increase in biliary cholesterol in Golden Syrian hamsters. Am J Physiol Gastrointest Liver Physiol 295: G813–G822, 2008. First published August 21, 2008; doi:10.1152/ajpgi.90372.2008.—Niemann-Pick C1-Like 1 (NPC1L1) facilitates the uptake of sterols into the enterocyte and is the target of the novel cholesterol absorption inhibitor, ezetimibe. These studies used the Golden Syrian hamster as a model to delineate the changes in the relative mRNA expression of NPC1L1 and other proteins that regulate sterol homeostasis in the enterocyte during and following cessation of ezetimibe treatment and also to address the clinically important question of whether the marked inhibition of cholesterol absorption alters biliary lipid composition. In hamsters fed a low-cholesterol, low-fat basal diet, the abundance of mRNA for NPC1L1 in the small intestine far exceeded that in other regions of the gastrointestinal tract, liver, and gallbladder. In the first study, female hamsters were fed the basal diet containing ezetimibe at 1 mg·kg body wt−1·day−1. At this dose, cholesterol absorption fell by 82%, fecal neutral sterol excretion increased by 5.3-fold, and hepatic and intestinal cholesterol synthesis increased more than twofold, but there were no significant changes in either fecal bile acid excretion or biliary lipid composition. The ezetimibe-induced changes in intestinal cholesterol handling were reversed when treatment was withdrawn. In a second study, male hamsters were given a diet enriched in cholesterol and safflower oil without or with ezetimibe. The lipid-rich diet raised the absolute and relative cholesterol levels in bile more than fourfold. This increase was largely prevented by ezetimibe. These data are consistent with the recent finding that ezetimibe treatment significantly reduced biliary cholesterol saturation in patients with gallstones.

The liver plays a central role in the maintenance of sterol balance across the whole body because not only is it the organ that receives most of the cholesterol absorbed by the small intestine, but also it is the site for the degradation and excretion of cholesterol through the bile (40). The liver also actively synthesizes cholesterol but the rate at which it does so varies widely depending on numerous factors and, in particular, the amount of cholesterol being delivered to it from the small intestine (40). It is well documented that dietary or pharmacological manipulation of the enterohepatic flux of either cholesterol or bile acids can potentially cause marked changes in the rate at which the liver synthesizes cholesterol, converts cholesterol to bile acids, incorporates cholesterol into very low-density lipoproteins, esterifies and stores cholesterol, or secretes unesterified cholesterol directly into bile (18, 22, 40). Such changes in the intrahepatic handling of cholesterol can, in turn, lead to clinically significant shifts in the circulating low-density lipoprotein-cholesterol (LDL-C) concentration and in the degree of biliary cholesterol saturation (15, 43, 48).

The intestinal absorption of cholesterol has long been a key target in the management of dyslipidemia primarily because of the substantial quantity of cholesterol that reaches the liver daily from the small bowel (5, 35). In the average adult consuming a typical Western diet, ~1,200–1,700 mg of cholesterol enters the lumen of the small intestine each day (18). Approximately 300–500 mg of this cholesterol comes from the diet and the remainder is derived largely from bile (19). Given that in humans fractional cholesterol absorption averages ~50%, the amount of intestinally derived cholesterol delivered to the liver throughout the day amounts to hundreds of milligrams (8, 34). This occurs through what is essentially a triphasic process that begins with a series of events within the lumen that culminate in the micellar solubilization of cholesterol and its movement up to the brush border membrane of the absorptive enterocyte (41). The second phase entails the transport of cholesterol, as well as noncholesterol sterols, across this limiting membrane facilitated by Niemann-Pick C1-Like 1 (NPC1L1) (1, 13). Within the enterocyte, cholesterol is esterified and subsequently incorporated, along with other lipids and apolipoprotein B48, into nascent chylomicrons. These latter processes together constitute the third phase of sterol absorption. Although the cellular and molecular events involved in each of these phases are generally well defined, there are nevertheless significant gaps in our knowledge of how cholesterol movement within the enterocyte is facilitated (49). Furthermore, although NPC1L1 clearly plays a fundamental role in sterol absorption, there is currently not unanimous agreement that it is this protein that facilitates the uptake of sterols across the brush border membrane (38).

Although the primary focus of research on the molecular control of cholesterol absorption has been to find more effective ways of modifying the plasma lipoprotein composition to lessen an individual’s risk of developing atherosclerosis, the question of how changes in chylomicron cholesterol uptake by the liver might also alter the rate of biliary cholesterol secretion, and thus a person’s predisposition to cholesterol gallstone formation, has not been as widely investigated. Most of the studies that have been carried out in animal models such as the baboon, squirrel monkey, Golden Syrian hamster, mouse, and prairie dog, as well as in humans, have shown that increases in dietary cholesterol intake result in a rise in biliary cholesterol content (2, 3, 14, 21, 24, 27, 28, 50, 52). However, the extent...
to which this happens varies markedly depending on the type of model and the dietary formulation that were utilized. Comparatively, much less is known about the converse situation involving the use of inhibitors that diminish the amount of cholesterol reaching the liver from the small bowel. Studies with agents that interfere with the intraluminal phase of absorption such as β-sitosterol, sucrose polyester (a nonabsorbable fat substitute), and surfomer (AOMA, a copolymer of maleic acid and an 18-carbon α-olefin) all showed either no change or a modest reduction in biliary cholesterol saturation (6, 10, 11). The impact of agents that act at the intracellular phase of sterol absorption has not been widely studied. However, the focus of attention has now shifted to ezetimibe, an entirely new class of inhibitors that, in milligram doses, markedly reduces cholesterol absorption by inhibiting NPC1L1 activity in the brush border membrane (17). The dose of ezetimibe needed to block cholesterol absorption varies between species because of wide differences in the affinity with which ezetimibe binds to NPC1L1 (20). In the LDL receptor knockout mouse fed a lipid-rich diet, the degree of increase in biliary cholesterol content was blunted substantially by the addition of ezetimibe to the diet (29). Similar findings have been recently reported for mice harboring constitutive hepatic liver X receptor activity and for gallstone-susceptible C57L mice fed a lithogenic diet (45). Ezetimibe has also been found to have beneficial effects in a murine model of cholecystosteatosis (26). There are also now newly published data showing that ezetimibe treatment lowered the degree of biliary cholesterol saturation in small groups of gallstone patients and overweight subjects without gallstones (51).

In the present studies the Golden Syrian hamster has been utilized as an alternate model to determine the impact of ezetimibe treatment on biliary lipid composition during periods of low and high dietary cholesterol intake and to delineate the concomitant biochemical and molecular changes in sterol metabolism within the small intestine and liver. Previous studies have established that this small animal has features of cholesterol metabolism that more closely resemble those seen in the human and nonhuman primate than do species like the mouse and rat (44). Utilizing this hamster model, the data demonstrate that ezetimibe, by blocking the transport of excess chylomicron cholesterol to the liver, was highly effective in preventing an otherwise pathological increase in hepatic cholesterol storage and biliary cholesterol content. The data also show for the first time that the ezetimibe-induced changes in the expression of multiple genes involved in cholesterol handling by the enterocyte are quickly reversed when treatment is withdrawn.

MATERIALS AND METHODS

Animals and diets. Female and male Golden Syrian hamsters (Mesocricetus auratus) were obtained from either Charles River Laboratories, Wilmington, MA, or, for one experiment, from Harlan Sprague Dawley, Indianapolis, IN, in the weight range of 100–110 g. They were housed individually in plastic cages containing wood shavings and maintained in a room with alternating 12-h periods of light and darkness. The hamsters were adapted to these conditions for 1 week prior to the beginning of the study, the hamsters were fed their respective experimental diet ad libitum for either 10, 21, or 34 days. All animals were studied in the fed state toward the end of the dark phase of their light cycle. All studies were approved by the Institutional Animal Care and Use Committee of The University of Texas Southwestern Medical Center.

Intestinal cholesterol absorption, fecal bile acid and neutral sterol excretion, and biliary lipid composition. The details of the methods used for measuring these parameters are described previously (32). Intestinal cholesterol absorption was determined as a fractional value (percentage) by a fecal dial-isotope ratio method (32). For tissue, plasma, and bile harvesting, the hamsters were anesthetized and exsanguinated from the abdominal aorta. The liver was exposed and the gallbladder accessed. Bile was taken from the gallbladder via a 1-ml plastic syringe fitted with a 25-gauge, 5/8-in, needle. The bile was then transferred to a microfuge tube, after which aliquots were diluted in methanol for lipid composition analysis. The molar ratio of cholesterol in gallbladder bile was expressed as a percentage value. The rate of fecal bile acid excretion was taken as a measure of the rate of bile acid synthesis (44).

mRNA isolation and qRT-PCR gene expression analysis. Hamsters were anesthetized with isoflurane (Baxter Healthcare, Deerfield, IL) and exsanguinated into heparinized syringes via the abdominal aorta. Small intestines were removed, flushed with ice-cold PBS, then cut longitudinally. The intestinal mucosae were removed by gentle scraping, immediately frozen in liquid nitrogen, and stored at −85°C. Total RNA was isolated from tissue samples by use of RNA STAT-60 (Tel-Test, Friendswood, TX). RNA concentrations were determined by absorbance at 260 nm. Quantitative RT-PCR (qRT-PCR) was performed by using an Applied Biosystems Prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA) as described previously (25). Briefly, total RNA was treated with DNase I (RNase-free) (Roche Diagnostics, Indianapolis, IN) and reverse transcribed with random hexamers by use of SuperScript II (Invitrogen, Carlsbad, CA) to generate cDNA.

Primers for each gene were obtained from the literature (16) or were designed with PrimerExpress software (PerkinElmer Life and Analytical Sciences, Boston, MA) and validated by analysis of template titration and dissociation curves (46) (see Table 1 for primer sequences). The gene sequence for hamster ACAT2 was not yet available, so a partial cDNA was cloned by reverse transcription-PCR using hamster RNA as template and the following primers: forward, 5′-ACTTCAATTGATGAGGCGAGG; reverse, 5′-CCCCAGGACGCAA- GCAGAGA. These cloning primers were designed according to regions of highest sequence similarity between the human (NM_003578), mouse (NM_146064), and rat (NM_153728) ACAT2 genes. The resulting 871-bp product was sequenced and found to share 89% nucleotide identity with the mouse ACAT2 mRNA (NM_146064), and this novel sequence was subjected to the Primer Express algorithm to identify qRT-PCR primers for measuring hamster ACAT2 mRNA levels.

Each qRT-PCR reaction contained (final volume of 10 μl): 25 ng of reverse-transcribed RNA, each primer at 150 nM, and 5 μl of 2X SYBRgreen PCR master mix (Applied Biosystems), and each sample was analyzed in triplicate. Data from the PCR reactions were analyzed by the comparative cycle number determined at threshold (Ct) method (User Bulletin no. 2, PerkinElmer Life and Analytical Sciences) with cyclophilin as the invariant control gene. In most cases, mRNA levels in each experiment are expressed relative to those
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proteins, which either fell (ABCG5 and G8) or increased (HMG CoA synthase and reductase) during treatment, had returned to normal values within 6 days of ezetimibe withdrawal (Fig. 3B).

Lipid composition of gallbladder bile was unchanged in hamsters given ezetimibe with a low-cholesterol, low-fat diet. In many of the hamsters used in the preceding studies, gallbladder bile was harvested. The absolute concentrations of cholesterol, phospholipid, and bile acid in the bile from hamsters fed the basal diets containing ezetimibe at doses up to and including 2 mg·day⁻¹·kg body wt⁻¹ are given in Fig. 4, A, B, and C, respectively. Although there was a trend toward marginally higher cholesterol concentrations in the bile of the hamsters given ezetimibe, this trend was not dose related. Moreover, when the data for biliary cholesterol content were expressed as a molar percent of total biliary lipid content, none of the values for the ezetimibe-treated hamsters was significantly different from that for the group given the basal diet alone (Fig. 4D).

Diet-induced cholesterol accumulation in liver and plasma was prevented by ezetimibe. Western diets typically contain substantial amounts of triacylglycerol and cholesterol, particularly relative to endogenous synthesis rates. Thus, to examine the effect of this drug in a more relevant situation, female hamsters were fed for 28 days a diet containing cholesterol (1% wt/wt) and safflower oil (10% wt/wt) without or with ezetimibe at a dose of just 1 mg·day⁻¹·kg body wt⁻¹. The livers depicted
The molar ratio of cholesterol was 0.9 in the group fed the lipid-rich diet with ezetimibe. In the male of this species, the molar ratio of cholesterol to the liver. The efficacy of ezetimibe in treating liver cholesterol to the liver. The efficacy of ezetimibe in treating liver cholesterol accumulation that resulted from the ezetimibe-induced inhibition of intestinal cholesterol absorption and the diminished delivery of chylomicron cholesterol to the liver.

DISCUSSION

The discovery and development of the novel sterol absorption inhibitor, ezetimibe, has provided not only an effective new strategy for the management of primary hypercholesterolemia in the general population (5), but also a treatment for sitosterolemia, a rare disorder resulting from mutations in ABCG5 or G8 that lead to the hyperabsorption of dietary sterols and premature atherosclerosis (30, 31). More recently, ezetimibe was found to be effective also in ameliorating liver dysfunction in a murine model of Niemann-Pick type C (NPC) disease, a multisystem disorder resulting from mutations in the NPC1 gene that encodes a protein involved in intracellular cholesterol transport (7). Those studies showed that the sooner in life mice deficient in npc1 received daily treatment with ezetimibe, the better was the improvement in their plasma liver enzyme levels. This benefit correlated directly with the degree of reduction in hepatic cholesterol accumulation that resulted from the ezetimibe-induced inhibition of intestinal cholesterol absorption and the diminished delivery of chylomicron cholesterol to the liver.

Fig. 3. Fractional cholesterol absorption and the relative expression level of mRNA for various proteins in the small intestine of female Golden Syrian hamsters after withdrawal of ezetimibe treatment. As described in MATERIALS AND METHODS, female hamsters were given 1 of 3 treatments over a 10-day period. One group received only the basal diet, while a second group was fed the same diet containing ezetimibe (2 mg·day⁻¹·kg body wt⁻¹). A third group received the diet with ezetimibe for 10 days but was started on this diet 3 days ahead of the other group. These hamsters were switched back to the basal diet alone 3 days before they, and the other 2 groups, were dosed intragastrically with labeled sterols for the measurement of fractional cholesterol absorption (A). For all 3 groups, stools were collected for 3 days after dosing. The hamsters were then terminated and the small intestine was removed for the measurement of the relative expression level of mRNA for various proteins (B). Values are means ± SE of data from 4 animals (no treatment) and 7 animals in each of the ezetimibe and ezetimibe (withdrawn) groups. *Significantly different from corresponding value for group not given ezetimibe, P < 0.05.

in Fig. 5A are representative of what was found in hamsters fed the lipid-rich diet without ezetimibe (left) or with ezetimibe (right). The lipid-rich diet alone resulted in massive accumulation of cholesterol in both the liver (Fig. 5B) and plasma (Fig. 5C). The livers from the untreated hamsters had a total cholesterol concentration of 99 ± 4 mg/g and, relative to body weight, were 50% larger than the livers of hamsters given the lipid-rich diet with ezetimibe. The total cholesterol concentration in the livers from the treated animals averaged 2.5 ± 0.2 mg/g. The pale appearance of the livers from the untreated animals presumably reflected the accumulation of cholesteryl esters because hepatic total triacylglycerol concentrations averaged ~5–6 mg/g irrespective of whether the lipid-rich diet contained ezetimibe.

Changes in hepatic relative mRNA levels for multiple proteins induced by lipid-rich diet were attenuated by ezetimibe. The relative mRNA level for multiple proteins was determined in the livers of the hamsters used in the study described in Fig. 5 and these are shown in Fig. 6. The mRNA for ACAT2 was essentially the same in all four experimental groups. In contrast, the level of mRNA expression for ABCA1, ABCG5, and ABCG8 was significantly elevated whereas that for HMG CoA synthase and HMG CoA reductase was markedly reduced in the livers of hamsters given the lipid-rich diet with ezetimibe. These changes in message levels either did not occur or were far less pronounced in the hamsters given ezetimibe with the high-fat, high-cholesterol diet.

Diet-induced increase in biliary cholesterol content was prevented by ezetimibe. In the study with the female hamsters fed the lipid-rich diet (Figs. 5 and 6) the lipid composition of gallbladder bile was also determined (data not shown). The cholesterol, safflower oil-enriched diet caused only a modest rise in the molar ratio of cholesterol in the bile (1.4 ± 0.2 vs. 0.8 ± 0.2% in matching hamsters given the basal diet alone). In the group fed the lipid-rich diet with ezetimibe, the molar ratio of cholesterol was 0.9 ± 0.1%. However, it is known that the male of this species responds much more dramatically to dietary lipids (3). Consequently, these studies were repeated in male hamsters given the diets enriched with cholesterol and safflower oil. As shown by the data in Fig. 7A, this dietary formulation did raise the cholesterol concentration in the gallbladder bile 5.2-fold. There was also a 2.4-fold increase in the phospholipid concentration (Fig. 7B) but no change in the bile acid concentration (Fig. 7C). Thus, although ezetimibe treatment alone did not change the molar ratio of cholesterol in the bile, the lipid-rich diet alone did increase this ratio 4.6-fold (Fig. 7D). When this diet was given with ezetimibe, the increase in biliary cholesterol saturation was greatly diminished. Thus increasing the flow of cholesterol into the liver of the male animal markedly increased the rate of secretion of sterol by the liver into bile and this effect was nearly completely obviated by the administration of ezetimibe.
The second conclusion concerns the lack of change in plasma total cholesterol concentrations (Fig. 1F) under circumstances where biliary cholesterol secretion was markedly reduced in hamsters given ezetimibe. This finding supports the premise that ezetimibe is an effective therapeutic agent for lowering cholesterol concentrations in plasma and bile in hamsters and that this effect is consistent across a wide range of doses.

The third conclusion is based on the observation that ezetimibe treatment resulted in a significant reduction in the molar ratio of cholesterol to bile acid in bile extracts. This finding is consistent with previous studies in dogs and humans, indicating that ezetimibe reduces cholesterol synthesis in the liver and increases cholesterol absorption in the intestine, leading to a net decrease in cholesterol excretion.

The fourth conclusion is based on the observation that ezetimibe treatment resulted in a significant reduction in plasma total cholesterol concentrations (Fig. 1F) under circumstances where biliary cholesterol secretion was markedly reduced in hamsters given ezetimibe. This finding supports the premise that ezetimibe is an effective therapeutic agent for lowering cholesterol concentrations in plasma and bile in hamsters and that this effect is consistent across a wide range of doses.

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stances in which there was a profound reduction in intestinal cholesterol absorption (Fig. 1A) and a markedly accelerated rate of fecal neutral sterol excretion (Fig. 1B). Presumably, in the ezetimibe-treated hamsters, the compensatory increase in intestinal and hepatic sterol synthesis (Fig. 1, D and E) fully balanced the additional loss of sterol from the body, so that there was no detectable impact on circulating plasma cholesterol levels in the face of a major reduction in cholesterol absorption. The lowest dose of ezetimibe given to the hamsters (0.5 mg·day⁻¹·kg body wt⁻¹) corresponds to about four times the dose typically prescribed to humans (10 mg once daily). In subjects with a very low dietary cholesterol intake, this dose of ezetimibe reduced fractional cholesterol absorption by 58% and increased fecal neutral sterol excretion by 81% (9). There was also a 72% increase in whole body cholesterol synthesis. Despite this, plasma LDL-C levels in these subjects fell, on average, by 17%. Thus, in these individuals, the compensatory increase in cholesterol synthesis, which presumably reflected increases mainly by the liver and small bowel, did not fully balance the increased loss of sterol from the body, thus leading to a fall in circulating LDL-C levels. Although no effect of ezetimibe on plasma cholesterol levels was seen in hamsters fed the basal diet, there was clearly a marked reduction when dietary cholesterol intake was raised (Fig. 3C).

The third conclusion relates to the changes in the relative level of expression of mRNA for a number of key proteins involved in regulating sterol homeostasis in the enterocyte during, and following cessation of treatment with ezetimibe. It is particularly noteworthy that the relative mRNA level for NPC1L1 remained almost unchanged, irrespective of whether the hamsters were on or off ezetimibe treatment (Fig. 3B). This was also true for the transcript level for ABCA1. In contrast, the mRNA level for ABCG5 and G8 fell significantly when ezetimibe was given but returned to baseline values after withdrawal of treatment. Clearly, the reduced level of mRNA for ABCG5 and G8 reflected an ezetimibe-induced deficit of cholesterol within the enterocyte, a condition that also triggered a marked induction of local cholesterol synthesis as evidenced not only by the increased mRNA levels for HMG CoA synthase and HMG CoA reductase but also by direct measurements of the rate of small bowel sterol synthesis in vivo (Fig. 1, D and G). Although these data clearly attest the impact of blocking cholesterol uptake into the enterocyte at the brush border membrane, they also demonstrate that once ezetimibe treatment ceases there is a rapid return toward normal levels of cholesterol absorption (Fig. 3A) and the pathways that govern cholesterol homeostasis in the enterocyte (Fig. 3B).
The fourth conclusion concerns the relative expression of NPC1L1 mRNA in the liver and extrahepatic organs of the Golden Syrian hamster compared with what has been reported for other animal models (1, 36) and humans (1, 12). Although such comparative data remain limited and are based on mRNA rather than protein or activity measurements, it appears that in humans there is a much greater relative expression of mRNA for NPC1L1 in liver compared with other tissues than is the case in the few animal species in which this has been investigated. On the basis of the present data, the Golden Syrian hamster appears to be more like the rat, mouse, and miniature pig in that the dominant site of expression of NPC1L1 message is the small bowel, with comparatively little being present in the liver (Fig. 2; Refs. 1, 36). In the hamster, NPC1L1 mRNA was detected in the gallbladder at levels higher than those seen in the liver but still trivial compared with the level of expression in the small intestine. The pathophysiological significance of NPC1L1 in the liver of humans is not well understood, but studies with hepatocytes from African Green monkeys reveal that the protein localizes in the canalicular membrane (54). Furthermore, other data from a transgenic mouse model show that hepatic overexpression of human NPC1L1 in the liver resulted in a dramatic decrease in biliary cholesterol concentration and, moreover, that ezetimibe treatment of these mice essentially normalized biliary cholesterol concentration (37). These findings have been interpreted to mean that in humans NPC1L1 may function in regulating the amount of cholesterol entering the bile (53).

Taken together, these various observations raise the clinically important question of what the long-term impact of using ezetimibe, either in combination with statins or in some cases as monotherapy, for managing hypercholesterolemia might be on biliary cholesterol content and an individual’s predisposition to cholesterol gallstone formation. The results of the present studies with ezetimibe treatment of hamsters fed a cholesterol- and fat-enriched diet (Fig. 5–7) make two points that bear directly on this question. One is that by placing a major block on absorption at the level of cholesterol uptake by the enterocyte, the dramatic and adverse changes in intrahepatic cholesterol metabolism and biliary cholesterol content associated with consumption of the high-cholesterol, high-fat diet are greatly attenuated (Fig. 5–7). The other is that although there was a detectable increase in the cholesterol concentration in the bile when hamsters fed the low-cholesterol basal diet were given ezetimibe, the corresponding increase associated

Fig. 6. Relative level of expression of mRNA for various proteins in the livers of female hamsters fed a basal or lipid-rich diet without or with ezetimibe. The relative mRNA level of expression for various proteins was determined in the livers of the female hamsters used for the study described in the legend to Fig. 5. For each protein, the mRNA level was expressed relative to the level found in the group fed the basal diet alone, which was arbitrarily set at 1.0. Values are means ± SE of data from 6 or 7 animals in each group. *Significantly different from corresponding value for group fed basal diet without ezetimibe, P < 0.05. ‡Significantly different from corresponding value for group fed lipid-rich diet without ezetimibe, P < 0.05.

Fig. 7. Biliary lipid composition in male Golden Syrian hamsters fed a basal or lipid-rich diet without or with ezetimibe. Gallbladder bile was harvested from male Golden Syrian hamsters that were fed the same diets as were used in the study described in the legend for Fig. 6, and biliary lipid concentrations (A–C) and molar ratio of cholesterol (D) were measured as described in MATERIALS AND METHODS. Values are means ± SE of data from 7 or 8 animals in each group. *Significantly different from corresponding value for group fed basal diet without ezetimibe, P < 0.05. ‡Significantly different from corresponding value for group fed lipid-rich diet without ezetimibe, P < 0.05.
with feeding the lipid-rich diet alone was far greater (Fig. 7A). More important, however, was the finding that ezetimibe treatment, given concurrently with this diet, resulted in a reduction in the absolute (Fig. 7A) and relative (Fig. 7D) cholesterol content of the bile to values at or near those seen in matching hamsters given the basal diet alone. Herein lies the potential relevance of these data in the hamster to the question of long-term ezetimibe treatment and biliary cholesterol levels in humans. Based on our current understanding of all the factors that can impact the level of biliary cholesterol saturation, it seems that any increase in biliary cholesterol levels that might arise from an effect of ezetimibe directly on the activity of NPC1L1 on the canalicular surface of hepatocytes would be more than offset by the marked reduction in the inflow of chylomicron cholesterol to the liver and the potential that this has to drive up biliary cholesterol content. Added to this is the point that ezetimibe is most widely used as an add-on to statins, which are known to reduce biliary cholesterol content (38). That aside, the present data, together with the results of a recent study demonstrating a favorable effect of short-term ezetimibe treatment on biliary cholesterol levels in humans (51), suggest that a clinical trial designed to investigate the potential of ezetimibe for reducing biliary cholesterol saturation in populations with a predisposition to cholelithiasis is now warranted.

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DISCLOSURES

From time to time, Dr. Dietschy has served as a consultant for Merck/Schering-Plough Pharmaceuticals. Dr. Turley has served as a member of the Speaker’s Bureau for Merck & Co., Schering-Plough, and Merck/Schering-Plough Pharmaceuticals.

REFERENCES


