Genetic differences in cholesterol absorption in 129/Sv and C57BL/6 mice: effect on cholesterol responsiveness

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Jolley, Christopher D., John M. Dietschy, and Stephen D. Turley. Genetic differences in cholesterol absorption in 129/Sv and C57BL/6 mice: effect on cholesterol responsiveness. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1117–G1124, 1999.—This study compared the cholesterolemie response of two strains of mice with genetically determined differences in cholesterol absorption. When fed a basal low-cholesterol diet, 129/Sv mice absorbed cholesterol twice as efficiently as did C57BL/6 mice (44% vs. 20%). Total lipid absorption, in contrast, averaged 80–82% in both strains. The higher level of cholesterol absorption in the 129/Sv animals was reflected in an adaptive reduction in hepatic and intestinal sterol synthesis. When fed lipid-enriched diets, the 129/Sv mice became significantly more hypercholesterolemic and had twofold higher hepatic cholesterol concentrations than did the C57BL/6 animals even though the conversion of cholesterol to bile acids was stimulated equally in both strains. The difference in cholesterol absorption between these mouse strains was not the result of physicochemical factors relating to the size and composition of the intestinal bile acid pool but more likely reflects an inherited difference in one or more of the biochemical steps that facilitate the translocation of sterol across the epithelial cell.

INCREASED LEVELS OF PLASMA low-density lipoprotein (LDL)-cholesterol (LDL-C) constitute a major risk for the development of atherosclerosis (22, 23). The cholesterol carried in LDL, like all cholesterol throughout the body, is derived ultimately from de novo synthesis and by absorption from the diet (38). In humans plasma LDL-C levels correlate positively with the level of intestinal cholesterol absorption (13, 21). This is also the case in various primate models that have been identified as being either hypo- or hyperresponsive to a dietary cholesterol challenge (2, 18, 33, 41).

The major biochemical steps involved in the translocation of cholesterol from the intestinal lumen to the lymph have been described in detail (36). It is also well documented that the level of cholesterol absorption can be dramatically changed by manipulating some, but not all, of these steps (9, 10). In particular, changes in the physicochemical environment within the intestinal lumen that result from either spontaneous or induced shifts in the size and composition of the bile acid pool, or in the phospholipid content of bile entering the lumen, bring about profound changes in cholesterol absorption (5, 28, 39, 42, 43). Similar effects result from the pharmacological manipulation of the activity of acyl-CoA:cholesterol acyltransferase (ACAT) (9).

Despite all of these findings, it is not clear which step(s) in the absorption process may differ inherently among individuals in any population in a way that explains their differences in dietary cholesterolemic response. This understanding is essential for the development of more efficacious compounds for reducing hypercholesterolemia in the general population through the inhibition of cholesterol absorption (9, 17). One of the challenges in fulfilling this objective is the availability of suitable animal models. Although there are several species of primates that would be ideal for such research, various factors relating mainly to expense and concerns about disease transmission usually exclude their use.

During the course of recent studies on bile acid and sterol metabolism in different strains of mice fed a low-cholesterol basal rodent diet (40), we found that the level of cholesterol absorption, as measured by a fecal isotope ratio method, was twice as high in 129/Sv mice as in C57BL/6 mice. Further investigation of this key metabolic difference was warranted because mice bearing the genetic makeup of these two strains have been used extensively for gene targeting experiments and because evaluation of the phenotype of animals generated by these experiments can be confounded by genetic strain differences (31, 32, 35). Therefore, the objective of the present studies was to fully characterize all aspects of cholesterol metabolism in these two strains, to test their response to challenge with cholesterol- and fat-enriched diets, and to explore possible mechanisms that might account for the propensity of the 129/Sv mice to absorb cholesterol so much more efficiently than their C57BL/6 counterparts.

MATERIALS AND METHODS

Animals and diets. Unless otherwise stated, all the 129/Sv mice used in these studies were generated within our own colony, whereas the C57BL/6 mice were obtained from the Jackson Laboratory (Bar Harbor, ME). This was also the source of additional 129/Sv and C57BL/6 mice that were used in one experiment for direct comparison with our 129/Sv animals. Our colony of 129/Sv mice was derived from 129/SvEvBrd-Hprt<sup>−/−</sup> breeding stock provided by Bradley as described (31) and has remained closed since its establishment in 1994. All mice were housed either as groups or individually in plastic colony cages containing wood shavings. The animals were maintained in a room at 72–74°F with alternating 12-h periods of light (11 AM to 11 PM) and dark (11 PM to 11 AM). They had access to drinking water at all times and were fed ad libitum a pelleted cereal-based rodent diet (no. 8604; Harlan Teklad, Madison, WI). This formula-
tion (basal diet) had an inherent cholesterol content of 0.02% (wt/wt) and a fatty acid composition as described elsewhere (39). The meal form of this diet was used to prepare various experimental diets that contained either cholic acid (0.01% wt/wt), chenodeoxycholic acid (0.01% wt/wt), cholesterol (1.0% wt/wt; Byron Chemical, Long Island City, NY), cholesterol (1.0% wt/wt) combined with hydrogenated coconut oil (10% wt/wt; ICN Pharmaceuticals, Costa Mesa, CA), or cholesterol (1.0% wt/wt) combined with olive oil (10% wt/wt; Bertolli USA, Secaucus, NJ). The diets with added bile acids were fed for 14 days, whereas those enriched with cholesterol and oil were fed for 21 days. In all experiments the mice were ~3 mo of age and were studied in the fed state, except in one case in which gallbladder bile was harvested from mice that had been fasted for 4 h. All experiments were approved by the Institutional Animal Care and Research Advisory Committee.

Measurement of intestinal cholesterol and lipid absorption. Cholesterol absorption was measured by a fecal dual-isotope ratio method using [4-14C]cholesterol (NEN, Boston, MA) and [5,6-3H]sitostanol (stigmastanol) (American Radiolabeled Chemicals, St. Louis, MO) as described (28). The stools were collected from each animal over a 72-h period immediately after dosing with the labeled sterols contained in medium-chain triacylglycerol (MCT) oil. The mice were dosed toward the end of the dark phase of the lighting cycle. Aliquots of ground stool and the dosing mixture were extracted, and the ratio of 14C to 3H in each was determined. The percent cholesterol absorption was calculated from these data as described (28). In one study the cholesterol absorption measurements were made on stools that were collected during only the first 24 h after dosing, and in another experiment the dosing mixture was prepared using corn oil in place of MCT oil. The mice were dosed toward the end of the dark phase of the lighting cycle. Aliquots of ground stool and the dosing mixture were extracted, and the ratio of 14C to 3H in each was determined. The percent cholesterol absorption was calculated from these data as described (28).

Measurement of intestinal sterol synthesis. The rate of hepatic and intestinal sterol synthesis was measured in vivo as described previously (28). The mice were given an intraperitoneal injection of ~40 mCi of 3H-labeled water (NEN) and after 1 h were anesthetized and exsanguinated. Aliquots of liver and the whole small intestine were digested, and their content of digitonin-precipitable sterols was measured. The rate of sterol synthesis in each organ was expressed as nanomoles of 3H-labeled water incorporated into sterol per hour per gram of tissue.

Measurement of plasma, hepatic, biliary, and dietary cholesterol. Plasma total cholesterol concentrations were determined enzymatically, whereas dietary and hepatic and biliary cholesterol levels were measured by gas-liquid chromatography as previously described (28, 39).

Analysis of data. The data are presented as means ± SE of measurements in the specified number of individual animals. Differences between these mean values were tested for statistical significance by the two-tailed Student’s t-test.

RESULTS

The data in Table 1 show that, when fed a basal rodent diet without added cholesterol, the two strains of mice exhibited very similar characteristics, except for plasma cholesterol levels, which were marginally higher in the 129/Sv animals. In gallbladder bile obtained from separate groups of mice that had been fasted for 4 h, there was no strain difference in the absolute concentrations of either cholesterol [3.8 ± 0.3 µmol/ml in 129/Sv (n = 8) vs. 3.3 ± 0.3 µmol/ml in C57BL/6 (n = 7)] or bile acid [180 ± 17 µmol/ml in 129/Sv (n = 8) vs. 175 ± 12 µmol/ml in C57BL/6 (n = 7)].

In both strains, the level of intestinal total lipid absorption averaged 80–82% (Fig. 1A), but the efficiency of cholesterol absorption in the 129/Sv mice (44 ± 3%) was substantially greater than in matching C57BL/6 animals (20 ± 3%) (Fig. 1B). The comparatively high level of cholesterol absorption found in 129/Sv mice from our own colony was equally apparent in 129/Sv and 129 strains obtained directly from Jackson Laboratory [41 ± 4% in 129/Sv mice (n = 9) and 52 ± 4% in 129 mice (n = 10)]. When stools were collected for only 24 h instead of 72 h after dosing, the strain difference in cholesterol absorption was still evident [63 ± 9% (n = 5) in 129/Sv mice vs. 39 ± 4% (n = 4) in C57BL/6 mice], but the values were higher in each case than those found from analysis of stools collected over 72 h. A similar result was obtained when corn oil replaced MCT oil in the dosing mixture. The more efficient absorption of cholesterol in 129/Sv mice was reflected in a marked compensatory reduction in the rate of cholesterol synthesis in both the liver and small intestine (Fig. 2).

Although bile acid pool size was not different in these two types of mouse (Fig. 3A), the proportion of cholic to muricholic acid in the pool of the 129/Sv mice was ~30% higher (P < 0.05) than in the C57BL/6 mice. In other animal models, enrichment of the bile acid pool with cholic acid raises the level of intestinal cholesterol absorption.

Table 1. Food consumption, stool output, and total cholesterol concentration in plasma and tissues in C57BL/6 and 129/Sv mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>C57BL/6</th>
<th>129/Sv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>28 ± 1</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td>1.4 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>Small intestine weight, g</td>
<td>1.0 ± 0.04</td>
<td>0.9 ± 0.04</td>
</tr>
<tr>
<td>Food consumption, g·day−1·100 g body wt−1</td>
<td>11.8 ± 0.9</td>
<td>13.6 ± 0.5</td>
</tr>
<tr>
<td>Stool output, g·day−1·100 g body wt−1</td>
<td>2.7 ± 0.1</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Plasma total cholesterol concn, mg/dl</td>
<td>541 ± 17</td>
<td>77.3 ± 35*</td>
</tr>
<tr>
<td>Hepatic total cholesterol concn, mg/g</td>
<td>2.5 ± 0.1</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Small intestine total cholesterol concn, mg/g</td>
<td>2.9 ± 0.1</td>
<td>2.9 ± 0.1</td>
</tr>
</tbody>
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Values represent means ± SE of 10 animals. Mice were 3-mo-old adult males fed a basal rodent diet with no added cholesterol. *P < 0.05 vs. corresponding value for C57BL/6 mice.
absorption (28, 39). To determine whether the higher proportion of cholic acid in the pool of the 129/Sv animals accounted for their inherently higher levels of cholesterol absorption compared with that manifest in the C57BL/6 animals, the pool composition in each strain was manipulated so as to bring the ratio of cholic to muricholic acid in the pool of each type of mouse toward more similar values. This was achieved by adding chenodeoxycholic acid (a precursor of muricholic acid) to the diet of the 129/Sv mice and cholic acid to the diet of the C57BL/6 mice. Each bile acid was incorporated into the diet at a level of only 0.01% (wt/wt) so as to facilitate a subtle shift in pool composition without expanding pool size. Intestinal cholesterol absorption was measured in the bile acid-fed animals, as well as in matching groups of mice of both strains that were fed only the plain rodent diet. Levels of intestinal total lipid and cholesterol absorption were measured as described in MATERIALS AND METHODS. Values represent means ± SE of data from 9 mice of each strain. *P < 0.05 vs. corresponding value for C57BL/6 mice.

The next study was designed to determine whether the inherent difference in cholesterol absorption between these two strains of mice was reflected in the propensity with which they accumulated cholesterol in their plasma and liver when challenged with a diet that was enriched with cholesterol alone or cholesterol combined with either coconut or olive oil. As shown in Fig. 5A, plasma cholesterol levels were raised by all three diets in both strains, but in every case a significantly greater level of hypercholesterolemia was manifest in the 129/Sv mice compared with matching C57BL/6 mice fed the same diet. Although the addition of cholesterol alone to the diet resulted in a twofold increase in hepatic cholesterol levels in both types of mice, the inclusion of either coconut or olive oil in the cholesterol-rich diet resulted in substantially more cholesterol accumulation in the liver of the 129/Sv mice than was the case in their C57BL/6 counterparts (Fig. 5B). This was particularly the case with the cholesterol and olive oil diet, which raised hepatic cholesterol concentrations in the 129/Sv animals to levels that were double those in the C57BL/6 animals (28.9 ± 1.0 vs. 14.2 ± 0.7 mg/g, respectively). Although the data are not shown, essentially all of the excess hepatic cholesterol that accumulated in both strains of mice was present as cholesteryl ester. In a final study, it was further demonstrated that this strain difference in hepatic cholesterol accumulation was not the result of a markedly reduced in the bile acid-fed animals, the strain difference in cholesterol absorption prevailed (Fig. 4C).
difference in the ability of each type of mouse to convert excess cholesterol to bile acid. Thus, as shown in Fig. 6, the rate of fecal bile acid excretion was stimulated by about the same extent in both strains of mice when fed the diet with added cholesterol and olive oil.

DISCUSSION

Over the past decade, both C57BL/6 and various sublines of 129 mice, as well as crosses of these two strains, have been used extensively for generating genetically manipulated models in which numerous proteins involved in regulating the transport and metabolism of cholesterol have been either deleted or overexpressed (6, 11, 16, 24, 26, 28, 30, 44). The finding that the level of cholesterol absorption is so different in these two strains may be of particular importance when such models are used for cholesterol balance studies. Clearly, data obtained from such studies may vary significantly depending on whether a genetically altered model is derived from a pure 129 or C57BL/6 background or from a cross of these two strains. Mixed-background mice are likely to show greater animal-to-animal variability, particularly for data relating to intestinal cholesterol absorption.

Several other points pertaining to the difference in absorption between these two widely used strains warrant emphasis. First, the lower level of cholesterol absorption in the C57BL/6 mice was manifest in animals that had essentially the same level of total lipid absorption as was found in the 129/Sv strain. Furthermore, when the animals were challenged with the cholesterol- and fat-enriched diets, there was no evidence of lipid malabsorption in either strain. Second, the propensity of male 129/Sv mice from our colony to absorb cholesterol more efficiently than C57BL/6 mice is shared with other sublines of the 129 strain from commercial sources (unpublished observations) and is just as apparent in female as it is in male mice of this strain (3). Third, although there is already extensive literature describing differences between numerous strains of mice in susceptibility to atherosclerosis and choledolithiasis (14, 20, 25), many of these studies used...
lipid-enriched, semisynthetic diets that were supplemented with high levels of cholic acid. In the present studies the metabolic differences between 129/Sv and C57BL/6 mice were seen in animals fed a natural, cereal-based rodent diet. These differences might well have been distorted or masked altogether if the animals had been fed a traditional cholic acid-enriched atherogenic diet. Fourth, the absolute value obtained for percent cholesterol absorption for any group of animals will vary with many factors, including the method by which the measurement is made, the type of oil used as a vehicle for the intragastric administration of labeled sterols, and, in the case of the fecal-isotope ratio technique, the period over which the stools are collected after dosing (37, 39). The levels of absorption reported by other investigators for these two strains, especially the C57BL/6 mice, are generally higher than those found in our studies (3, 15, 29). Although these differences mainly reflect differences in the technique used, other factors, like the gender of the animals, need to be considered when such comparisons are made (40). The difference in absorption in the two strains studied here prevailed irrespective of the type of oil used in the dosing mixture or the period of stool collection after dosing. The lower percent absorption values for both strains found when a 3-day collection period was applied likely resulted because a portion of the labeled cholesterol absorbed during the first 24 h was subsequently excreted on the second or third day after dosing. Be that as it may, it is clear that the difference in cholesterol absorption between these two strains was not an artifact of the conditions of the fecal-isotope ratio method that is routinely utilized in this laboratory.

The data derived from the sterol synthesis and cholesterol feeding experiments provided further evidence attesting to a difference in cholesterol absorption between these two strains of mice. It is well documented that the rate of hepatic cholesterol synthesis varies inversely with the amount of chylomicron cholesterol reaching the liver from the small intestine (38). The rate of sterol synthesis in the intestinal mucosa is also often reflective of the level of cholesterol absorp-
tion (28). In the present studies, the higher-absorbing 129/Sv mice manifested markedly lower rates of hepatic and intestinal cholesterol synthesis than their C57BL/6 counterparts. When the strains were challenged with diets enriched with cholesterol and oil, the difference between the two strains in the magnitude of the increase in their plasma and hepatic cholesterol concentrations was fully consistent with the difference that was manifest in their efficiency of cholesterol absorption. Thus, irrespective of whether predominantly saturated or unsaturated fat was added to the cholesterol-enriched diet, the concentration of cholesterol in the liver was increased twice as much in the high-absorbing 129/Sv mice as it was in the C57BL/6 mice. This difference was particularly striking in the groups fed the diet with cholesterol and olive oil, which raised hepatic cholesterol levels to 14 mg/g in the C57BL/6 mice but to 29 mg/g in the 129/Sv strain.

Three points regarding this exceptional response of the C57BL/6 mice but to 29 mg/g in the 129/Sv strain. Three points regarding this exceptional response of the C57BL/6 mice but to 29 mg/g in the 129/Sv strain. Three points regarding this exceptional response of the C57BL/6 mice but to 29 mg/g in the 129/Sv strain. Three points regarding this exceptional response of the C57BL/6 mice but to 29 mg/g in the 129/Sv strain. Three points regarding this exceptional response of the C57BL/6 mice but to 29 mg/g in the 129/Sv strain.

First, the mice are generally resistant to dietary cholesterol. In most strains, the feeding of high-cholesterol diets usually results in only very modest increases in hepatic cholesterol content (40), unless the mice either lack the LXr receptor or are given a cholesterol-enriched diet that also contains high levels of fat and cholic acid (25, 26). Second, although the data are not shown, almost all of the excess cholesterol that accumulated in the livers of both types of mice was esterified. Third, in both strains bile acid synthesis was induced to the same extent in the groups fed the diet with cholesterol and olive oil. Hence, the strain difference in hepatic cholesterol accumulation in the groups given this diet could not be attributed to a difference in the ability of each type of mouse to upregulate the conversion of excess cholesterol to bile acids.

The question thus arises as to which step(s) in the absorption of cholesterol might be inherently different, not just in the two strains of mice studied here, but in other strains as well. Within the intestinal lumen there are a number of processes involved in the handling of cholesterol from endogenous and exogenous sources that are essential for its subsequent movement into the epithelial cell (36). One of these involves the action of pancreatic cholesterol esterase. Controversy surrounding the importance of this lipolytic enzyme as a regulator of cholesterol absorption was recently settled with the finding that deletion of the gene for this protein in mice did not significantly change the efficiency with which cholesterol was absorbed (10). Thus the difference in absorption between various strains of mice is probably not due to differences in the expression of this particular enzyme.

The other major intraluminal process that is involved in preparing cholesterol for its absorption is its micellization by bile acids and phospholipids (36). The importance of phospholipids in this process has recently been clearly demonstrated in mice lacking the gene for the Mdr2 P-glycoprotein. In these animals, the biliary secretion rates of phospholipid and of cholesterol, but not of bile acid, fall dramatically, and there is a marked reduction in the efficiency of cholesterol absorption (42, 43). Although biliary phospholipid secretion was not measured in the mice studied here, the ratio of the concentrations of cholesterol to bile acid in gallbladder bile was the same in both strains. This can be taken as an indication that the difference in cholesterol absorption between these two strains was, at least, not the result of a difference in the extent to which the labeled cholesterol contained in the dosing mixture was diluted by biliary cholesterol. The strain difference in absorption also could not be attributed to differences in the size or composition of the intestinal bile acid pool. Although pool size was identical in both strains, the partially greater enrichment of the pool with cholic acid in the 129/Sv mice was initially considered a possible cause of the higher levels of absorption in this strain. This followed from numerous earlier studies, including those recently described in the cholesterol 7a-hydroxylase-knockout mouse (28). In that model, bile acid pool size is dramatically reduced and the animals do not absorb cholesterol. This condition is readily reversed by feeding a diet containing 0.2% (wt/wt) cholic acid. In the present study a dietary cholic acid level of only 0.01% (wt/wt) was needed to raise the proportion of this bile acid in the pool of the C57BL/6 mice closer to that which occurred spontaneously in their 129/Sv counterparts. Conversely, feeding the same dietary level of chenodeoxycholic acid to the 129/Sv animals lowered the proportion of cholic acid in their bile acid pool more toward the level characteristic of the C57BL/6 animals. Importantly, these subtle shifts in pool composition were achieved without expanding pool size in either strain. The finding that the level of cholesterol absorption was still as different in the bile acid supplemented groups as it was in matching unsupplemented animals showed unequivocally that the modest difference in hydrophobicity of the bile acid pool between these two strains of mice was not the cause of their inherently different levels of cholesterol absorption. These findings, however, do not preclude the possibility that the level of absorption in the C57BL/6 mice could be raised to at least that which is characteristic of 129/Sv mice by feeding much higher doses of cholic acid.

Thus physicochemical effects within the intestinal lumen clearly play a decisive role in determining the quantity of cholesterol that moves from the lumen into the epithelial cells. However, although genetically determined differences in bile acid pool size and composition and biliary lipid secretion could potentially account for some inter- and intraspecies differences in cholesterol absorption, in the case of the two strains of mice studied here such genetic influences appear to be exerted on another step in the absorption process, and this step presumably involves the uptake and processing of cholesterol by the jejunal absorptive cell. There are multiple proteins that are involved in the transcellular movement and processing of cholesterol that potentially could be genetically regulated. These include caveolin, the scavenger receptor SR-B1, and the sterol carrier protein SCP-2, which facilitate the move-
ment of unesterified cholesterol across cell membranes and through the cytosol, and the enzyme ACAT, which esterifies cholesterol before its incorporation into nascent chylomicron particles (7, 12, 27, 34, 36). Recent studies carried out in vitro showed that SR-B1 functions in facilitating the movement of cholesterol and other lipids across the brush-border membrane (8). It is also now known that there are at least two forms of ACAT, one of which, ACAT-2, is located principally in the small intestine (1, 4, 19). Measurement of the expression and activity of these various types of proteins in C57BL/6 and 129/Sv mice will potentially yield new insights into the mechanism(s) responsible for the marked difference in cholesterol absorption between these two strains.

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REFERENCES


