Control of Cholesterol Turnover in the Mouse*

Published, JBC Papers in Press, December 3, 2001, DOI 10.1074/jbc.R100057200

**John M. Dietschy** and **Stephen D. Turley**

From the Department of Internal Medicine,
University of Texas Southwestern Medical Center,
Dallas, Texas 75390

General Pathways for Net Cholesterol Flux through the Mouse

The mouse is now commonly used to explore the physiological changes that take place in sterol metabolism when a specific protein function involved in cholesterol turnover is either deleted or overexpressed. The major pathways for the net flow of cholesterol through the tissue compartments of this species are outlined in Fig. 1. This diagram separates the overall process of cholesterol turnover into those events that occur within the cells of the extrahepatic tissues (90% of body mass), that promote movement through the plasma space (4.6% of body mass), and that are involved in secretion from the liver (5% of body mass) into the intestine. As implied by the thickness of the arrows, most cholesterol is synthesized in the extrahepatic organs and moves from the ER1 to the bulk phase and specialized microdomains of the plasma membrane (1–3). Because cholesterol does not accumulate in these tissues, it must be continuously removed from the plasma membrane and bound to circulating apoA-I, a process recently postulated to be under the control of ABCA1 (4, 5). Within the plasma space, cholesterol is esterified to CE in the mature HDL particle utilizing the enzyme lecithin:cholesterol acyltransferase. This CE is then selectively removed from the lipoprotein particle by SR-BI primarily in the liver (and endocrine tissues) and hydrolyzed (6–8). The resultant cholesterol mixes with other sterols newly synthesized in the liver or absorbed from the diet. A portion of this pool is utilized as substrate for bile acid synthesis or for secretion into the bile and, ultimately, into the intestine. Alternatively, part of this sterol may be esterified by acyl-CoA:cholesterol acyltransferase, incorporated into the nascent VLDL particle, and secreted into the plasma space (9). During metabolism of this VLDL particle, a portion of the cholesterol reaches the LDL particle, and a small fraction (∼20%) of this lipoprotein is cleared by the cells of the extrahepatic tissues utilizing the LDLR clustered in clathrin-coated pits (10). After processing through the lysosomal pathway, this small amount of cholesterol joins the much larger pool of newly synthesized cholesterol destined for transport to the plasma membrane (Fig. 1).

This scheme emphasizes that most cholesterol is synthesized in the extrahepatic tissues and that the excretion of this sterol from the body largely takes place by secreting cholesterol itself from the liver into the feces or after first metabolizing the cholesterol molecule to bile acid and then secreting this acidic sterol into the feces (11). Significant amounts of cholesterol are also excreted from the body through the sloughing of skin cells and secretions and after conversion to steroid hormones, but these pathways are not shown in Fig. 1. Nevertheless, in the mouse, measuring the output of fecal neutral and acidic sterols always underestimates cholesterol turnover when compared with quantifying sterol input into the animal from dietary cholesterol intake and de novo synthesis.

Sterol Metabolism in the Mouse Compared with Other Mammalian Models

In many respects, cholesterol turnover in the mouse is both quantitatively and qualitatively different from other animal models, particularly primates. As shown in Table I, on a typical, cereal-based animal diet the mouse ingests about 30 mg of cholesterol/day/kg of body weight whereas other species, including humans, usually eat much less (column A). In different strains of mice, as well as in different humans, the amount of this dietary sterol load that is absorbed varies between 30 and 70%. Typically, the mouse synthesizes ∼160 mg/day/kg of cholesterol whereas the human makes only ∼10 mg/day/kg (column B). As in other rodents, the liver of the mouse is relatively more important as a site for this synthesis (∼40%) than is true in the primate (10–12%) (column C). Because the pool of cholesterol in the whole animal is similar in the rodent and human (∼2200 mg/kg) and does not change with age, the total input of sterol from the diet and synthesis in the mouse (∼190 mg/day/kg) is much greater than in the other species and, in particular, is 13-fold higher in the human (∼15 mg/day/kg). There is a similar, marked difference in the handling of cholesterol carried in circulating LDL. Although the liver is the primary site for the removal of LDL from the plasma in all species (column E), the rate of entry of cholesterol into the LDL pool in the mouse (∼50 mg/day/kg) is only 4-fold higher than in the human (∼13 mg/day/kg) (column D), but the rate of hepatic LDL clearance in this animal (500 ml/day/kg) is 40-fold greater than in the human (∼12 ml/day/kg) (12, 13). As a consequence, the steady-state concentration of cholesterol carried in LDL in the mouse is usually ∼7 mg/dl whereas in the human this value usually exceeds 100 mg/dl (column F). Despite these variations, however, most animals, including the mouse, ultimately excrete cholesterol in the feces in approximately equal amounts as neutral (column G) and acidic (column H) sterols.

Cholesterol Balance in the Normal Control Mouse

Fig. 2 summarizes five aspects of cholesterol balance in mice in which the function of a specific protein either has been deleted or overexpressed. For comparison, column A gives values for these different processes in control mice of a mixed
genetic background. The pool of cholesterol in such whole animals averages 2175 mg/kg (A1), whereas that portion of this pool circulating in the plasma space is much smaller and averages only 45 mg/kg (at a concentration of 95 mg/dl) (A3). The net centripetal flux of cholesterol from the extrahepatic organs through the plasma space to the liver equals about 92 mg/day/kg (A2) where it mixes with additional amounts of cholesterol derived from hepatic synthesis and the diet. Ultimately, 60 mg/day/kg of cholesterol is excreted as fecal neutral sterols (A4), and 50 mg/day/kg is lost from the body as fecal acidic sterols (A5).

FIG. 1. Pathways for the net flow of cholesterol through the major tissue compartments of the mouse. This diagram illustrates the major pathways postulated to be involved in the net flow of cholesterol from the endoplasmic reticulum to the plasma membrane of the cells of the extrahepatic tissues (yellow arrows), through the plasma space to the liver (green arrows), and, finally, from the liver into the intestine and feces (red arrows). The specific proteins that may be involved in these pathways are shown in boxes, the ligands for the various transporters are in parentheses, and the anatomical structures within cells are italicized. The thickness of the lines showing net cholesterol flow is meant to have semiquantitative significance. This diagram does not show the smaller amounts of sterol that are excreted from the body through the sloughing of skin and cutaneous oils or after conversion to various steroid hormones. These latter pathways are relatively small in the human but relatively large in the mouse. This diagram has been modified from one appearing in Ref. 25. BA, bile acid; CE, cholesteryl ester; DIGs, detergent-insoluble glycosphingolipid-enriched complexes; LCAT, lecithin:cholesterol acyltransferase; IBAT, ileal bile acid transporter; Lyso, lysosome; CM, chylomicron; CT, cholesterol transporter.

FIG. 2. Cholesterol turnover in the mouse with either deletion or overexpression of the physiological activity of a specific protein. This diagram shows the size of the pool of cholesterol in the steady state in all of the tissues of the whole animal (line 1) and in the plasma compartment (line 2) expressed as mg of sterol/kg of body weight. Also illustrated are the rates of net cholesterol flux from the extrahepatic tissues to the liver (line 3) and from the whole animal into the feces as neutral (line 4) and acidic (line 5) sterols expressed as mg of cholesterol moving/day/kg of body weight. The symbol ++ implies that the protein was overexpressed, whereas the symbol −− implies complete loss of function of that protein in specific groups of animals. In column C the symbol ABCA1++ denotes a group of mice overexpressing ABCA1, as well as other proteins, after treatment with a liver X receptor agonist. The symbol +− denotes groups of mice where cholesterol absorption (column K) or bile acid absorption (column L) was partially inhibited by pharmacologic agents. There are important differences in some of these flux rates in various strains of mice, in the two genders of these strains and in animals of varying age. However, for the purposes of this figure, all of these values have been normalized to the same control group (A) represented by a mixed strain background (C57BL/6:129Sv) (24). All data in this figure come from animals 7 weeks to 3 months of age that were on low cholesterol, cereal-based diets that contributed about 30 mg/day/kg of body weight (∼20% of the daily sterol synthesis rate) to the whole animal. Some of these values were derived from unpublished observations in this laboratory, but most came from references (8, 10, 13, 16, 17, 23, 24, 26–32). There are no data available for three of these parameters in the apoE−− and CETP++ animals. IBAT, ileal bile acid transporter.
Cholesterol Balance in Mice with Defects in Intracellular Sterol Transport

Columns B and C, respectively, show the values of these parameters in two animal models with altered activity of proteins that are thought to function in the movement of sterol through the cell and across the plasma membrane. NPC1 is expressed in virtually every tissue and is involved in the movement of cholesterol, derived from the uptake of apoB-containing lipoproteins, from the lysosomal compartment to the pool of newly synthesized sterol destined for insertion into the plasma membrane (Fig. 1) (14, 15). When this translocating activity is abrogated, cholesterol accumulates in late endosomes and lysosomes in the cells of every organ, including the brain, in proportion to the amount of sterol normally taken up by that tissue through the coated pit pathway (13, 16). As a result, the cholesterol pool in the whole mouse progressively expands with age, reaching 2.5 times the normal value at 7 weeks (B1) (17). However, functional loss of this sequestered sterol is compensated for by an increase in the rate of sterol synthesis in every organ so that the flux of cholesterol from the extrahepatic tissues to the liver (B2) and the excretion of this sterol in the feces (B4, B5) continues at essentially normal, or even elevated, rates. Thus, loss of function of NPC1 results in an unusual phenotype in the mouse (and child) in which the pool of cellular cholesterol continuously expands with age whereas the centripetal flow of newly synthesized sterol from the extrahepatic tissues to the liver and from the liver into the feces is essentially normal.

By administration of the liver X receptor agonist, T0901317, increased expression of several sterol transporters (ABCA1, ABCG1, ABCG5, and ABCG8) was achieved in a second mouse model. ABCA1 is expressed in most tissues and, particularly, in placenta, endocrine glands, intestine, and liver. Recent data suggest that it may play a role in the specific transfer of cholesterol across the plasma membrane of cells to lipid-poor apoA-I particles (18–20). Certainly, this appears to be the case for cells such as macrophages. However, the question remains as to its role in the steady-state movement of cholesterol from the organs of the extrahepatic compartment to the liver. If this protein were rate-limiting for the translocation of cholesterol between the intracellular pools and the plasma space, then overexpression of ABCA1 should increase the sterol turnover of the extrahepatic tissues of the mouse whereas deletion of this activity should markedly suppress extrahepatic cholesterol synthesis and fecal sterol output. The limited data currently available, however, do not support either of these predictions. When ABCA1 is overexpressed pharmacologically, the whole animal sterol pool (C1) and the flux of cholesterol from the extrahepatic tissues to the liver (C2) remain essentially unchanged. There is, however, a 5-fold increase in the rate of hepatic cholesterol synthesis and a corresponding increase in the excretion of fecal neutral, but not acidic, steroids (C4, C5). Similarly, when ABCA1 activity is deleted, the whole mouse cholesterol pool remains constant, and the movement of sterol from the extrahepatic tissues through the liver to the feces also remains essentially unchanged (21, 22). Thus, whereas such balance data are incomplete, those that are available suggest that ABCA1 may be important for the removal of cholesterol from cells like macrophages but not from the majority of the cells making up the tissues of the extrahepatic compartment.

Cholesterol Balance in Mice with Defects in the Metabolism of Plasma Lipoproteins

Abrogation of the function of specific transport proteins markedly lowers the rate of clearance of cholesterol carried in specific lipoprotein fractions in vivo, as it does in isolated cells studied in vitro. For example, in the LDLR−/− mouse, the clearance of cholesterol carried in LDL by the liver is reduced from a normal value of ~400 ml/day/kg to only 25 ml/day/kg. Similarly, the hepatic clearance of sterol carried in HDL is reduced from 60 ml/day/kg to only 6 ml/day/kg in the SR-BI−/− animal. However, under these in vivo conditions, the concentration of these two respective lipoproteins increases until the amount of cholesterol removed from the plasma by non-receptor-mediated clearance mechanisms returns to essentially normal, or even elevated, values (10).

Thus, there is a marked increase in the plasma cholesterol pool with deletion of function of the LDLR (105 mg/kg, D3), SR-BI (118 mg/kg, E3), and apoE (226 mg/kg, F3) that is attributable to an increase in the concentration of sterol carried in LDL, HDL, and remnant particles, respectively. Despite these changes in the plasma compartment, however, there are virtually no accompanying alterations in the pool of cholesterol in the tissues of the whole animal (D–F1) nor, importantly, is the flux of sterol from the extrahepatic compartment to the liver (D–F2) significantly affected. That net cholesterol balance in these animals is largely unaltered is also supported by the finding that in the mouse (and human) with these deletions, fecal excretion of neutral (D–E4) and acidic (D–F5) sterols is the same as in control animals. Similar findings have been reported with other manipulations affecting the concentration of HDL in the plasma space. The plasma cholesterol pool can be varied 11-fold, from 11 mg/kg in the apoA-I−/− mouse (G3) to 22 mg/kg in the CETP−/− mouse (H3) and to 118 mg/kg in the SR-BI−/− mouse (E2). Yet, in all of these situations, there is little alteration in the tissue cholesterol pool (E1, G–H1) nor is the rate of sterol movement from the extrahepatic compartment to the liver (E2, G–H2) significantly altered.
Thus, in general, deletion or overexpression of the function of proteins involved in the metabolism or transport of specific lipoproteins in the plasma space results in a phenotype with a markedly altered steady-state concentration of these respective lipoproteins in the circulation. However, these changes in the plasma cholesterol pool have little effect on the net flux of sterol from the extrahepatic tissues to the liver or from the liver into the fecal neutral and acidic sterols.

**Cholesterol Balance in Mice with Changes in Hepatic Neutral and Acidic Sterol Secretion**

When the rate of bile acid synthesis is significantly reduced by deletion of the activity of CYP7A1 (I) or CYP27 (J), fecal acidic steroid excretion decreases to 40% of the control value (I–J5). Because there is an even greater fall in the size of the pool of bile acid in the small intestine, cholesterol absorption decreases to essentially zero. As a result, there is a marked increase in the loss of fecal neutral sterols (I–J4) that is compensated for by an increase in hepatic synthesis (23, 24). Thus, whereas suppression of bile acid synthesis might be expected to decrease cholesterol turnover in the mouse, paradoxically, because of this secondary effect on absorption, cholesterol turnover is actually increased. Similar increases are seen when pharmacologic agents are used to partially inhibit the absorption of cholesterol (K) or bile acid (L) from the intestine. Inhibition of cholesterol absorption is associated with an increase in fecal neutral (K4) but not acidic (K5) sterols, whereas suppression of bile acid absorption is associated with an increase in acidic (L5) but not neutral (L4) fecal sterols.

Thus, in all four of these situations, the hepatocyte has responded to a change in net sterol balance across the liver with an appropriate alteration in cholesterol or bile acid synthesis. In the primate, such changes may be associated with a parallel alteration in hepatic LDLR activity and a corresponding change in the plasma cholesterol pool. In the mouse, however, these adjustments are so perfect that there is usually no associated change in hepatic LDLR activity, and there is no alteration in the pools of cholesterol in the whole animal (I–I1) or plasma (I–I3), and the flux of sterol from the extrahepatic tissues to the liver (I–I2) is unchanged.

**Conclusions**

In summary, there are significant quantitative and qualitative differences in the characteristics of cholesterol balance in the mouse, compared with the primate. Nonetheless, deletion or overexpression of specific protein functions often results in phenotypic changes in sterol balance that reflect similar changes in humans with mutations or pharmacologic treatments affecting these same proteins. In general, loss of function of proteins such as NPC1 (or NPC2) that alter intracellular cholesterol movement results in expansion of the whole animal sterol pool but no significant change in the rate of cholesterol movement from the peripheral tissues to the liver or from the liver into the feces. In contrast, alteration of function of proteins involved in the metabolism of plasma lipoproteins routinely results in significant changes in the plasma cholesterol pool but not in the level of cholesterol in the individual tissues or in the rate of flow of sterol into the feces. Finally, functional changes in the proteins affecting the enterohepatic circulation of cholesterol or bile acid profoundly alter the rates of fecal sterol excretion without changing the pools of cholesterol in the whole animal or plasma.

There is one important caveat concerning these data that should be emphasized. Because of the brevity of this review, it was not possible to describe cholesterol turnover in individual tissues in these various experimental models. In some cases, these tissues behave differently from the whole animal. In the endocrine glands, for example, changes in the concentration in cholesterol carried in HDL or the level of SR-BI activity clearly alters the pools of sterol. Similarly, changes in the plasma pool of cholesterol carried in LDL or HDL undoubtedly affect the pool of sterol in the endothelial lining cells of the arterial system. In both cases, however, the magnitude of the cholesterol fluxes involved are very small and are obscured by the much larger sterol pools and flux rates involved in overall sterol balance in the whole mouse.
