Centripetal cholesterol flux to the liver is dictated by events in the peripheral organs and not by the plasma high density lipoprotein or apolipoprotein A-I concentration

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Abstract **The major net flux of cholesterol in the intact animal or human is from the peripheral organs to the liver. This flux is made up of cholesterol that is either synthesized in these peripheral tissues or taken up as lipoprotein cholesterol. This study investigates whether it is the concentration of apolipoprotein (apo) A-I or high density lipoprotein in the plasma that determines the magnitude of this flux or, alternatively, whether events within the peripheral cells themselves regulate this important process. In mice that lack apoA-I and have very low concentrations of circulating high density lipoprotein, it was found that there was no accumulation of cholesterol in any peripheral organ so that the mean sterol concentration in these tissues was the same (2208 ± 29 mg/kg body weight) as in control mice (2176 ± 50 mg/kg). Furthermore, by measuring the rates of net cholesterol acquisition in the peripheral organs from de novo synthesis and uptake of low density lipoprotein, it was demonstrated that the magnitude of centripetal sterol movement from the peripheral organs to the liver was virtually identical in control animals (78 ± 5 mg/day per kg) and in those lacking apoA-I (72 ± 4 mg/day per kg). These studies indicate that the magnitude of net sterol flux through the body is not related to the concentration of high density lipoprotein or apolipoprotein A-I in the plasma, but is probably determined by intracellular processes in the peripheral organs that dictate the rate of movement of cholesterol from the endoplasmic reticulum to the plasma membrane.—**Jolley, C. D., L. A. Woollett, S. D. Turley, and J. M. Dietschy. **Centripetal cholesterol flux to the liver is dictated by events in the peripheral organs and not by the plasma high density lipoprotein or apolipoprotein A-I concentration.** ***J. Lipid. Res.*** **1998.** 39: **2143–2149.**

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Cholesterol homeostasis in the whole animal or human involves two entirely separate sets of regulatory processes. The first of these relates to those poorly understood mechanisms that maintain sterol balance within the cells of all of the extrahepatic organs. The second involves those processes that maintain sterol balance across the whole animal by facilitating the movement of excess cholesterol from these peripheral organs to the liver for excretion. The magnitude of the net cholesterol flux involved in these two processes is now known and varies inversely with animal size. In the mouse, for example, approximately 100 mg of cholesterol per kg body weight moves from the peripheral organs to the liver each day (1, 2). However, this flux is lower in the rat (~50 mg/day per kg), hamster (~30 mg/day per kg), rabbit (~30 mg/day per kg), monkey (~10 mg/day per kg), and human (~6 mg/day per kg) (3, 4). Furthermore, the sources of this sterol have been identified. All extrahepatic organs synthesize significant amounts of cholesterol, and the relative importance of each tissue to this synthetic activity is similar in all of the species that have been investigated (5). In contrast, with the exception of the endocrine glands, these extrahepatic organs take up little cholesterol carried in low density lipoprotein (LDL-C) (1, 3, 4). As a result, for example, 95% and 77%, respectively, of the sterol that moves from the peripheral organs to the liver in the mouse and monkey is newly synthesized (1, 4).

A number of recent observations have provided important insights into the processes that may dictate this flow of sterol, and these are shown diagrammatically in **Fig. 1**. The major contribution of unesterified cholesterol to the metabolically active pool in the endoplasmic reticulum (ER) is the synthesis of sterol from acetyl CoA. Only a small amount of cholesterol is taken up by these peripheral organs from the plasma as LDL-C, and this is processed through the lysosome and transferred by the Niemann-Pick protein (NPC1) to the ER (6, 7). There is no accumulation of excess sterol within the cell, however, as

Abbreviations: peripheral organs, all tissues except liver and endocrine glands; LDL-C, low density lipoprotein cholesterol; ER, endoplasmic reticulum; HDL, high density lipoprotein; apoA-I, apolipoprotein A-I; apoE, apolipoprotein E; LCAT, lecithin:cholesterol acyltransferase; CETP, cholesteryl ester transfer protein; LDLR, LDL receptor.

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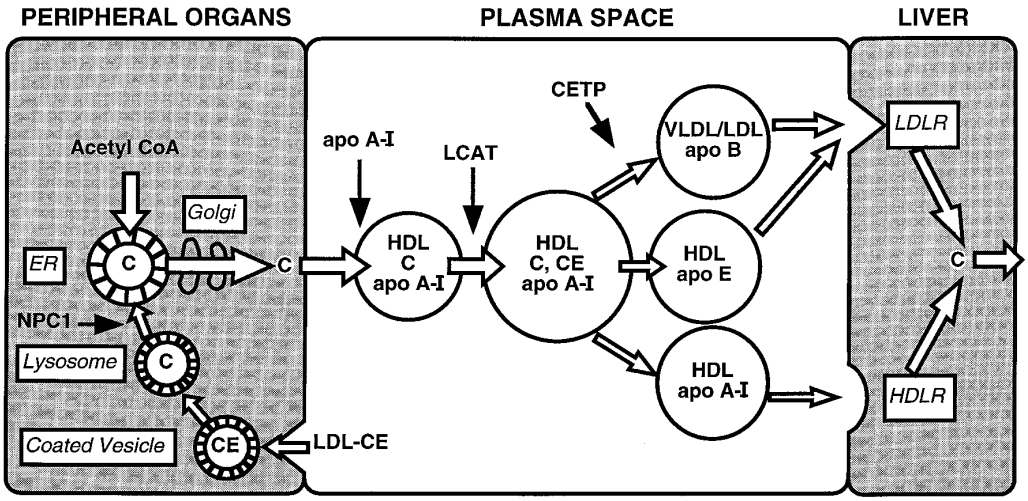

Fig. 1. A model showing the major, potentially rate-limiting steps for the daily net movement of sterol from all of the peripheral organs to the liver where excretion takes place. The cells of these peripheral organs constantly acquire cholesterol (C) from de novo synthesis in the endoplasmic reticulum (ER) and from uptake of cholesterol carried in low density lipoproteins (LDL-CE). This cholesterol then moves to the plasma membrane, is excreted from the cell, and transported through the plasma space to the liver by a series of interactions that may involve apoA-I, high density lipoprotein (HDL), lecithin:cholesterol acyltransferase (LCAT), cholesteryl ester (CE) transfer protein (CETP), very low density lipoprotein (VLDL), and two hepatic receptors, the low density lipoprotein receptor (LDLR) and a high density lipoprotein receptor (HDLR).

this cholesterol is apparently continuously transferred outward through the Golgi stack and becomes incorporated into structures like caveolae and the plasma membrane (8–10). In the steady state, cholesterol must be lost from the cell at the same rate that it is transferred up to the plasma membrane.

Once the sterol leaves the peripheral cells, the pathways involved in its delivery to the liver are better understood and are also shown in Fig. 1 (for reviews see references 11–14). The unesterified cholesterol is probably taken up from the plasma membrane by a lipid-poor high density lipoprotein (HDL) containing apolipoprotein A-I (apoA-I) with pre-β-mobility (15), although an alternative lipoprotein acceptor containing only apolipoprotein E (apoE) (γ-LpE) has also been described (16). A portion of this sterol is then converted to cholesteryl ester by the enzyme lecithin:cholesterol acyltransferase (LCAT) and a larger HDL particle is formed. While the cholesteryl ester in this lipoprotein can be taken up by endocrine tissues (17), most, presumably, is delivered to the liver for excretion (18). At least three pathways may account for this net uptake process. First, a portion of the cholesteryl ester may be exchanged by cholesteryl ester transfer protein (CETP) into an apoB-containing lipoprotein particle which, in turn, is cleared into the liver by the LDL receptor (LDLR). Second, any HDL that accumulates apoE may similarly be taken up into the liver by the LDLR. Finally, there is selective uptake of cholesteryl esters from the HDL particle through intervention of a high density lipoprotein receptor (HDLR) located on the liver that is probably the scavenger receptor, SR-B1 (18–20). Undoubtedly, the relative importance of each of these pathways varies in different species (18, 21).

The concentration of cholesterol carried in HDL (HDL-C) can be markedly altered by changing the level of expression of apoA-I, LCAT, or CETP (2, 22–24). There are now abundant data from both epidemiological studies and experiments in genetically altered mice and rabbits that the severity of the pathological process of atheroma formation is inversely related to the steady-state concentration of apoA-I or HDL-C. In contrast, there is little support for the concept that in the live animal the rate of the physiological process of net cholesterol flux from the peripheral organs to the liver is controlled by the concentration of any component of this plasma HDL transport system. The present studies, therefore, were undertaken to test the hypothesis that the movement of cholesterol from the ER of every peripheral tissue to the liver is controlled by events within the plasma space, i.e., the levels of apoA-I and HDL, rather than by events within the cells themselves, i.e., the vesicular movement of cholesterol through the Golgi to the plasma membrane. If this were the case, then in the absence of apoA-I and HDL there should be accumulation of cholesterol in the peripheral organs and suppression of the rates of both cholesterol synthesis and LDL-C uptake in these organs. This question is of fundamental importance to understanding sterol homeostasis in the live animal.

MATERIALS AND METHODS

Animals and diets

The control animals (apoA-I$^{+/+}$) were male C57BL/6 animals while the experimental group consisted of male C57BL/6 mice that were homozygous for apoA-I deletion (Jackson Laborato-

ries, Bar Harbor, ME) (24). The animals were purchased at 2 months of age and were then housed in colony cages (1). The mice were subjected to 12 h of light and 12 h of darkness, and all measurements were carried out at the mid-dark phase of the light cycle. In addition, during this 4-week equilibration period, the mice had free access to water and ground rodent diet (No. 8604, Harlan Teklad, Madison, WI) which, by direct analysis, contained 0.24 mg of cholesterol and 42 mg of total lipid per g of diet. Experimental measurements were carried out when the mice were 3 months of age. All mice were in the fed state at the time of study. Experiments were approved by the Institutional Animal Care and Research Advisory Committee.

Plasma and tissue cholesterol concentrations

Blood was obtained by exsanguination and the concentration of cholesterol in the various plasma lipoprotein fractions was determined as described (25). The major organs were removed from the animals, weighed, and saponified. The remaining carcass, which was composed primarily of skeleton, muscle, and adipose tissue, was also saponified. The cholesterol content of these tissue samples was determined (1, 2, 26). The term "peripheral organs" refers to all organs except the liver, adrenal gland, and testis.

Cholesterol synthesis in the extrahepatic organs in vivo

Rates of cholesterol synthesis were measured in vivo at the mid-dark phase of the light cycle. Each animal was injected intraperitoneally with 40 mCi of [^{3}H]water contained in 100 μl of isotonic saline solution (2). One hour later the animals were anesthetized and 800 μl of blood was aspirated from the inferior vena cava. The organs were removed and saponified, and the digitonin-precipitable sterols were isolated as described (5, 27). The rates of sterol synthesis in each of the organs were calculated and are presented as the nmol of [^{3}H]water incorporated into digitonin-precipitable sterols per h per g of tissue or per whole organ (nmol/h per g or organ). The rate of incorporation of [^{3}H]water into sterols by the whole body was also converted to an equivalent mg quantity of cholesterol assuming 0.69 ^{3}H atoms were incorporated into the sterol molecule per carbon atom entering the biosynthetic pathway as acetyl CoA (27, 28).

Isolation and radiolabeling of lipoprotein fractions

In the studies designed to measure the rate of LDL-C transport in vivo, lipoproteins were isolated from both male and female mice homozygous for deletion of the LDLR that had been maintained on the same low cholesterol ground rodent diet (1). The LDL fraction was isolated in the density range of 1.020–1.055 g/ml by preparative ultracentrifugation. Aliquots of this fraction were then labeled with either ^{125}I-labeled tyramine cellobiose or ^{131}I (2, 4). The labeled LDL fraction was next passed through a heparin Sepharose column (Pharmacia Biotech, Uppsala, Sweden) to remove the small amount of apoE-containing HDL that contaminated this preparation. The purified and labeled LDL fraction was then dialyzed extensively against 0.9% NaCl solution and passed through a 0.45-μm Millex-HA filter (Millipore Products, Bedford, MA) immediately prior to injection into the experimental animals. All fractions were used within 24 h of preparation.

LDL-C transport into the extrahepatic organs in vivo

Rates of tissue LDL-C clearance were determined in vivo using a primed-continuous infusion of ^{125}I-labeled tyramine cellobiose-labeled LDL through a catheter secured in an external jugular vein (1, 4). Each animal was then given a bolus injection of the radiolabeled LDL preparation followed by a continuous infusion of the same preparation at a rate calculated to maintain a constant specific activity in the plasma throughout the 4-h infusion period. Ten minutes before terminating the 4-h infusion, a bolus of ^{131}I-labeled LDL was administered through the same catheter (2, 4). Exactly 10 min after the second bolus was injected, and 4 h after beginning the infusion, the animals were again anesthetized and blood was drawn from the inferior vena cava. The individual organs were removed and processed as previously described (2). These clearance rates are expressed as the μl of plasma cleared of its LDL-C content per h per g of tissue or per whole organ (μl/h per g or organ). The rate of LDL-C clearance in the whole animal was calculated as the sum of the rates of clearance in all of the organs.

Bile acid excretion rates

Quantitative stool collections over 3 days were carried out in both groups of mice and bile acid output was measured as previously described (29). These measurements are expressed as mg of bile acid excreted each day per kg body weight (mg/d per kg).

Calculations

These experimental measurements provided the means to calculate a number of rate constants. The rates of [^{3}H]water incorporation into sterols were used to calculate the absolute rate of sterol synthesis expressed as the mg of cholesterol synthesized per day per kg of body weight (mg/d per kg) (27, 28). Using the rates of tissue LDL-C clearance and the plasma LDL-C concentration, it was also possible to calculate the mg of cholesterol that were taken up into each organ through the clearance of this lipoprotein fraction (2). The data are presented as mean values ± 1 SEM. The Student's unpaired *t*-test was used to compare the various sets of data. In the figures, an asterisk indicates a value found in the apoA-I$^{-/-}$ animals that was significantly different ($P < 0.05$) from that in the control animals.

RESULTS

All of the control and apoA-I$^{-/-}$ animals were males and were studied at 3 months of age. At the time of study the body weights of the apoA-I$^{+/+}$ mice (~25–33 g) were slightly higher than those of the apoA-I$^{-/-}$ animals (23–30 g), as were the weights of the livers in each group. The plasma total cholesterol concentration, however, in the mice with the disrupted apoA-I gene was only 24% (17 ± 1 mg/dl) of that found in the control mice (72 ± 3 mg/dl). The most striking difference was seen in the concentration of lipoprotein cholesterol with a density >1.063 g/ml which in the apoA-I$^{+/+}$ animals equalled 55 ± 5 mg/dl but only 9 ± 1 mg/dl in the apoA-I$^{-/-}$ mice. Furthermore, no apoA-I could be detected in the plasma of the apoA-I$^{-/-}$ animals using both gradient gel electrophoresis and immunoblotting with mouse anti-apoA-I. These findings, therefore, were very similar to those reported in the original description of this animal model (24).

The first study was designed to measure the steady-state concentration of cholesterol in all of the extrahepatic organs in the two types of mice under conditions where for 1 month the dietary intake of cholesterol in both groups was kept constant at ~0.8 mg/day. As shown in **Fig. 2A**, the concentration of sterol in the control animals varied from 38 ± 3 mg/g in the adrenal gland to only 1.5 ± 0.1 mg/g in the heart. In the absence of apoA-I, there was no significant difference in the sterol concentration in any of

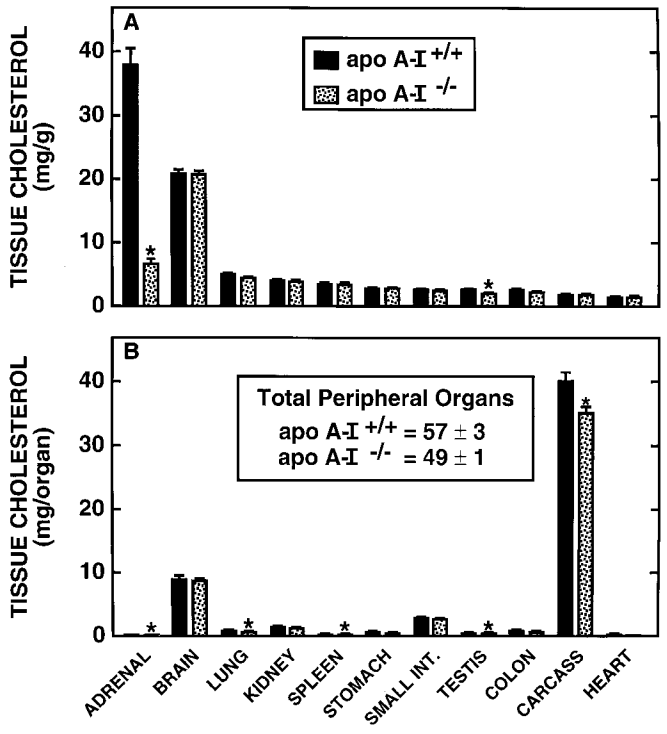

Fig. 2. Tissue cholesterol concentration and content in apoA-I$^{+/+}$ and apoA-I$^{-/-}$ mice. Panel A shows the concentration of cholesterol, expressed as mg/g of tissue, in all of the extrahepatic organs while panel B illustrates the content of cholesterol in each whole tissue, expressed as mg/organ. The insert gives the sum of the cholesterol contents in all of the peripheral organs, i.e., all tissues except the liver, adrenal gland, and testis. All values represent means ± 1 SEM for 10 animals in each group. The asterisk indicates the values found in the apoA-I$^{-/-}$ animals that were significantly different ($P < 0.05$) from those found in the control mice.

these extrahepatic tissues with the exception of the adrenal gland and testis. This difference in the latter tissues has also been reported in other mouse models with reduced concentrations of circulating HDL-C (2) and reflects the fact that the endocrine glands of the rodent preferentially utilize cholesterol from HDL to maintain tissue sterol pools (17). While not shown in Fig. 2, the concentration of hepatic cholesterol was also the same (2.6 mg/g) in the two genotypes. The absolute distribution of cholesterol in the various organs of these animals is shown in panel B. Most cholesterol in the body was contained in the tissues of the carcass (muscle, bone marrow, skin, and fat), brain, gastrointestinal tract, and kidneys.

While the liver, adrenal gland, and testis actually utilize and excrete sterol from the body, the remaining organs that account for ~95% of body mass in the mouse (designated the peripheral organs) must be the source for the major flux of cholesterol that is delivered daily to the liver for excretion from the body. The total content of cholesterol in these peripheral organs (Fig. 2B) equalled 57 ± 3 mg and 49 ± 1 mg in the apoA-I$^{+/+}$ and apoA-I$^{-/-}$ animals, respectively. After correction for small differences in body weight in the two groups, these values corresponded to a cholesterol content of 2176 ± 50 mg/kg body weight and 2208 ± 29 mg/kg, respectively, in the control and apoA-I-deficient mice. Clearly, in the steady state there was no accumulation of sterol in the peripheral organs of the apoA-I$^{-/-}$ animals. This implies either that sterol turnover in the periphery is suppressed in the absence of apoA-I or that circulating HDL and apoA-I are not rate limiting for centripetal cholesterol movement to the liver.

In the second study the rates of sterol acquisition from de novo synthesis in the peripheral organs were quantitated. As seen in **Fig. 3A**, in control animals the highest rates of synthesis per g of tissue in the extrahepatic organs were found in the adrenal gland and various portions of the gastrointestinal tract. While the rates of synthesis were essentially the same in the control animals as in most of the tissues of the apoA-I$^{-/-}$ mice, the rates of sterol production were nearly twice as high in the adrenal gland and testis, two organs where the previous study had shown a significant reduction in tissue cholesterol pools (Fig. 2A). The distribution of this synthetic activity in the whole organs is shown in Fig. 3B. The major sites for sterol synthesis are the cells of the tissues of the carcass and various areas of the gastrointestinal tract. The mean rate of [^{3}H] water incorporation into sterols by all of the peripheral or-

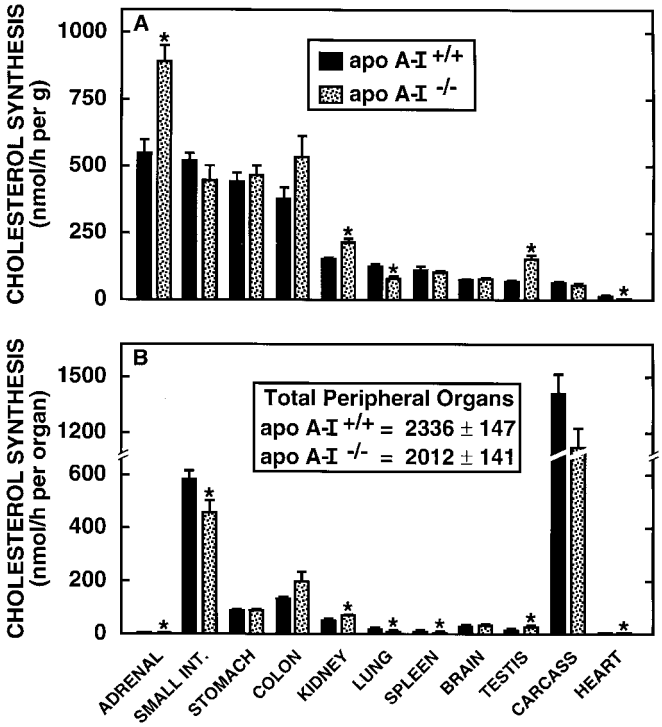

Fig. 3. Rates of cholesterol synthesis in the extrahepatic organs of apoA-I$^{+/+}$ and apoA-I$^{-/-}$ mice. Panel A shows the rates of cholesterol synthesis measured in vivo and expressed as the nmol of [^{3}H]water incorporated into sterols per h per g of each tissue. Panel B illustrates the rate of cholesterol synthesis found in each whole tissue, expressed as nmol/h per organ. The insert gives the sum of the rates of synthesis in all of the peripheral organs. All values represent means ± 1 SEM for 10 animals in each group. The asterisk indicates the values found in the apoA-I$^{-/-}$ animals that were significantly different ($P < 0.05$) from those in the control mice.

gans equalled 2336 ± 147 nmol/h in the apoA-I$^{+/+}$ mice and 2012 ± 141 nmol/h in the apoA-I$^{-/-}$ animals. From these incorporation rates, the weights of the various animals, and the known value of the ^{3}H/C incorporation ratio (27, 28), it could be further calculated that the absolute rate of cholesterol synthesis equalled 72 ± 4 mg/day per kg body weight in the control animals and 67 ± 5 mg/day per kg in the apoA-I$^{-/-}$ mice. Clearly, there was no suppression of cholesterol synthesis in any of the peripheral organs in the animals that lacked circulating apo A-I.

The final experiment quantitated the rate of cholesterol acquisition through uptake of LDL-C by the peripheral organs. As shown in **Fig. 4A**, the highest clearance rate in the extrahepatic tissues of the control animals was found in the adrenal gland (429 ± 48 μl/h per g) while uptake in the remaining organs was very low and <50 μl/h per g. Only in the adrenal gland was there a marked increase in LDL-C clearance (1198 ± 107 μl/h per g) when there was little circulating HDL-C. In the whole organs (B) only the carcass and small intestine manifested modest rates of LDL-C uptake, and these rates were essentially identical in the apoA-I$^{+/+}$ and apoA-I$^{-/-}$ mice. Thus, in all of the peripheral organs LDL-C clearance equalled 89 ± 10 μl/h and 120 ± 17 μl/h, respectively, in the control and apoA-I-deficient animals. These rates of clearance

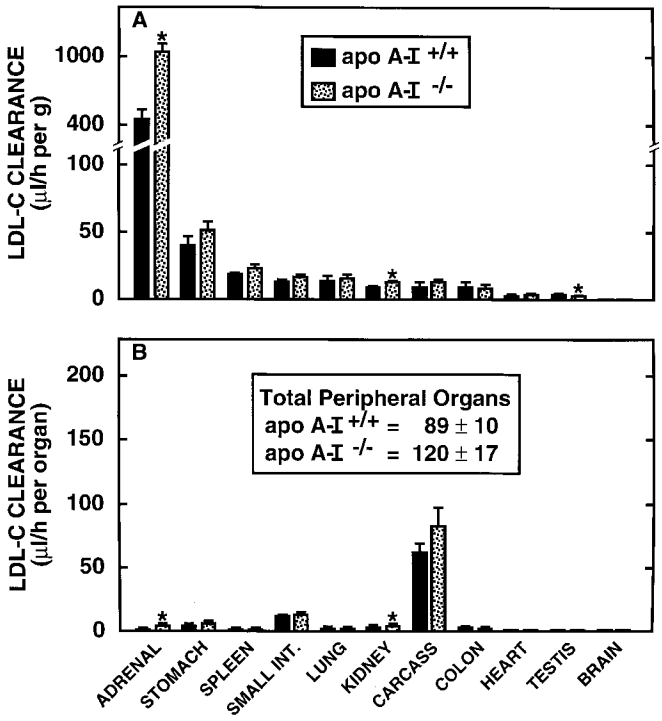

Fig. 4. Rates of LDL-C clearance into all of the extrahepatic organs in the apoA-I$^{+/+}$ and apoA-I$^{-/-}$ mice. Panel A shows the μl of plasma cleared of its LDL-C content per h per g of tissue. Panel B illustrates the LDL-C cleared each h by the whole organs. The insert shows the sum of the clearance rates by all of the peripheral organs in the two groups of animals. These values represent the means ± 1 SEM for data in 10 mice in each group. The asterisk indicates those values in the apoA-I$^{-/-}$ animals that were significantly different ($P < 0.05$) from those in the control mice.

represented only ~20% of the whole animal LDL degradation rates as the liver of these same two groups cleared LDL at rates of 385 ± 44 μl/h and 425 ± 39 μl/h, respectively. From these clearance values, the weights of the various organs, and the concentration of LDL-C in the plasma, it could be calculated that the absolute rate of cholesterol acquisition through LDL-C uptake in the peripheral organs equalled 6 ± 1 mg/day per kg in the apoA-I$^{+/+}$ mice and 5 ± 1 mg/day per kg in the apoA-I$^{-/-}$ animals. Clearly, the acquisition of cholesterol by the peripheral organs through the uptake of LDL-C was trivial compared to that derived from de novo sterol synthesis and, further, this process occurred at similar rates in the presence and absence of apoA-I.

Finally, three critical additional rate constants were measured in these studies. First, under circumstances where there was no change in the cholesterol pools in the peripheral organs (Fig. 2), the rate of net centripetal cholesterol flux from the periphery to the liver must have equalled the sum of the rates of net sterol acquisition by these organs from synthesis and LDL-C uptake. These rates equalled 78 ± 5 mg/day per kg in the apo A-I$^{+/+}$ animals and 72 ± 5 mg/day per kg in those animals lacking apoA-I. Second, from these values it can be calculated that each day 3.6% of the cholesterol pool in the peripheral organs turned over in the apoA-I$^{+/+}$ mice and 3.3% turned over in the apoA-I$^{-/-}$ animals. Third, by direct measurement, fecal bile acid output equalled 35 ± 3 mg/day per kg and 38 ± 2 mg/day per kg, respectively, in the apoA-I$^{+/+}$ and A-I$^{-/-}$ mice. Thus, in the absence of apoA-I and most HDL, there was no detectable difference in the rate of cholesterol turnover in the peripheral organs, the rate of net centripetal cholesterol flow from the periphery to the liver, or the rate of bile acid synthesis.

DISCUSSION

These studies provide direct evidence that in the mouse, the concentrations of HDL-C and apoA-I in the plasma play no role in maintenance of cholesterol homeostasis in any peripheral organ and do not regulate the rate of net centripetal flux of sterol from these tissues to the liver. This is in contrast to the adrenal gland and testis which, in the rodent, are known to take up HDL-C by a concentration-dependent transport process for hormone synthesis (17, 19, 30). Thus, in the apoA-I$^{-/-}$ animals with no apoA-I-containing HDL-C, the steady-state concentration of cholesterol in the adrenal gland and testis was significantly lower than in the control animals (Fig. 2), and in these same organs there were compensatory increases in the rates of both sterol synthesis (Fig. 3) and LDL-C uptake (Fig. 4). However, in all of the remaining extrahepatic organs that account for ~95% of body mass, the rate of cholesterol turnover (3.6% and 3.3% per day, respectively) and the magnitude of centripetal sterol flux (78 mg/day per kg and 72 mg/day per kg, respectively) were not significantly different in the apoA-I$^{+/+}$ and apoA-I$^{-/-}$ animals.

These findings are consistent with several previous reports and support the view that changes in resistance to cholesterol flow through the plasma space brought about by alterations in the levels of CETP, LCAT, or apoA-I (Fig. 1) profoundly affect the steady-state concentration of HDL-C but have virtually no effect on the magnitude of net sterol movement through this pathway. For example, in an earlier study, reducing the resistance to cholesterol flow by overexpressing CETP in the mouse lowered the HDL-C concentration from 59 to 15 mg/dl, yet the rate of centripetal cholesterol movement from the peripheral organs remained constant at about 89 mg/day per kg (2). Similarly, the HDL-C concentration was increased from about 28 to 121 mg/dl when LCAT was overexpressed in the rabbit (31). While centripetal cholesterol flux was not measured directly in this particular study, the net flux of apoA-I through the plasma did remain essentially unchanged (8.1 $\pm$ 0.6 mg/day per kg versus 10.8 $\pm$ 1.4 mg/day per kg) despite this 4-fold increase in circulating HDL-C. In hamsters, the concentration of cholesteryl esters carried in HDL was nearly twice as high in animals fed a Western-style diet as in those receiving psyllium in the diet, yet the absolute flux of these sterol esters to the liver was essentially the same (32). Finally, in humans, as in other primates, the great majority of cholesterol fluxing through the body comes from de novo synthesis in the peripheral organs (3, 4). If the concentration of apoA-I dictated the rate of movement of sterol from these tissues to the liver, then the rate of synthesis in humans would necessarily be higher in those individuals with higher HDL-C concentrations. However, in 32 individuals subjected to external sterol balance, the rate of whole body cholesterol synthesis remained remarkably constant in the face of a 4-fold variation in the plasma HDL-C concentration (33, 34).

Taken together, these four sets of experimental observations carried out in the mouse, rabbit, hamster and human strongly support the conclusion that net sterol balance across the cells of the peripheral organs is tightly regulated by processes within these cells and not by the concentration of circulating HDL-C or apoA-I. The concentration of HDL-C clearly can be varied over a wide range by altering the activities of LCAT or CETP or the rate of synthesis of apoA-I (Fig. 1), yet in all of these situations the net flux of cholesterol through the plasma remains essentially constant.

This situation is in contrast to what happens when one or more steps in the intracellular regulation of cholesterol homeostasis is disrupted (Fig. 1). Mutations that affect the NPC1 protein, for example, lead to marked changes in net sterol flux from the peripheral organs to the liver (35). These alterations, in turn, are associated with progressive expansion of the cholesterol pools in the peripheral organs and redistribution of these pools into the lysosomal and Golgi compartments (36). These profound changes in sterol homeostasis in the periphery occur under circumstances where there is virtually no change in the steady-state concentration of cholesterol in the plasma (C. Xie, S. D. Turley and J. M. Dietschy, unpublished observations). In general, the concept is evolving that changes in receptors (e.g., the LDL receptor), apolipoproteins (e.g., apoE and apoA-I), and other plasma enzymes (e.g., CETP and LCAT) that primarily function to move cholesterol through the vascular space profoundly affect the concentration of cholesterol in the blood, but these changes have little effect on sterol homeostasis in the peripheral organs (1, 2). In contrast, alterations in proteins that regulate intracellular sterol movement have little influence on plasma cholesterol levels, but profoundly alter cholesterol pools within the major peripheral organs (Fig. 1).

These conclusions do not negate the concept that HDL is the major lipoprotein fraction moving cholesterol from the peripheral organs to the liver and endocrine glands under normal circumstances. These findings do, however, indicate that the magnitude of this flux is determined by processes within the cells of the peripheral organs and not by the concentration of HDL or apoA-I in the blood. In the absence of apoA-I, there is clearly a decrease in the flux of cholesteryl esters through the HDL pathway (37) so that other lipoproteins presumably must take over this important task. Thus, in this situation, there is no accumulation of cholesterol in the peripheral organs, no change in the net flux of cholesterol to the liver, and no change in the excretion of this sterol as neutral fecal sterols or as bile acids.

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