Cholesterol balance and metabolism in mice with loss of function of Niemann-Pick C protein

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1Department of Internal Medicine, The University of Texas Southwestern Medical Center at Dallas, Dallas, Texas 75235-8887; and 2Developmental and Metabolic Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20892

Xie, Chonglun, Stephen D. Turley, Peter G. Pentchev, and John M. Dietschy. Cholesterol balance and metabolism in mice with loss of function of Niemann-Pick C protein. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E336–E344, 1999.—Type C Niemann-Pick disease is due to a mutation in Niemann-Pick C (NPC) protein, a putative determinant of intracellular cholesterol transport. This study quantifies cholesterol balance in vivo across all tissues in mice with this defect. Cholesterol balance in the heterozygous animal is normal, but in the homozygous mouse the whole animal cholesterol pool expands continuously from birth, reaching 5,442 mg/kg at 7 wk. The size of this pool in each organ is proportional to the rate at which each tissue clears low-density lipoprotein-cholesterol. Despite this expansion, however, cholesterol synthesis is increased so that whole animal synthesis equals 180 mg·day−1·kg−1. Forcing additional cholesterol into the liver through the clathrin-coated pit pathway increases the hepatic cholesterol pool in control mice, all of which is esterified, while there is a much greater increase in this pool in mutant mice, all of which is unesterified. These findings are consistent with the view that there is a block in sterol movement from the lysosome to the sites of regulation in NPC disease and have important implications for understanding the function of the NPC protein in intracellular cholesterol metabolism, in general, and in the brain, in particular.

neurodegeneration; cholesterol synthesis; lysosome; endoplasmic reticulum; low-density lipoprotein receptor

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and may occur through an excretory process that involves the formation of 24-hydroxycholesterol by a sterol 24-hydroxylase that is uniquely expressed in the brain (20).

Sterol balance across the liver is more complex than in either of these two tissue compartments. Most of the LDL-C reaching the plasma is returned to the liver [78 and 80%, respectively, in the mouse and monkey (24, 39)]. This process involves binding the lipoprotein particle to the LDL receptor (LDLR) in clathrin-coated pits, acidification and hydrolysis of the cholesteryl ester in the lysosome, and transport of at least a portion of this unesterified cholesterol to the ER (5). This transfer process is postulated to be the step mediated by NPC1. Within the ER, the unesterified cholesterol can regulate LDLR activity and cholesterol synthesis in the cell and, in the liver, is the substrate for esterification through the acyl-CoA-cholesterol acyl transferase enzyme and for bile acid synthesis through the cholesterol 7α-hydroxylase enzyme. In addition, this cholesterol may become incorporated in detergent-insoluble glycosphingolipid-enriched complexes (DIGs) and caveolae and, in the liver, into nascent very low-density lipoprotein (VLDL) particles. The percentage numbers in each compartment refer to the relative contribution of each group of tissues to whole animal weight in species varying from the mouse to human.

**MATERIALS AND METHODS**

Animals and diets. BALB/c mice carrying the genetic mutation in the NPC protein (19) were transferred from the National Institutes of Health to the laboratories in Dallas and were used to generate animals that were normal controls (NPC+/+) or were either heterozygous (NPC+/-) or homozygous (NPC-/-) for this genetic defect. These animals were housed in groups in plastic colony cages in rooms with alternating periods of light and dark (24). After genotyping and weaning, all animals were fed ad libitum a low-cholesterol, pelleted diet (no. 7001; Harlan Teklad, Madison, Novatek Lab Supplies, St. Paul, MN).

**Fig. 1.** Comparison of the sources for the net movement of cholesterol through the cells of the liver, extrahepatic organs, and brain. This diagram illustrates in semiquantitative terms the relative importance of de novo cholesterol synthesis from acetyl-CoA and cholesterol uptake from the plasma through the low-density lipoprotein (LDL) receptor (LDLR) to net sterol flux in these three compartments. Uptake of cholesterol carried in LDL (LDL-C) occurs through the clathrin-coated pit pathway to the lysosome (LYS) where hydrolysis of the cholesteryl ester takes place.

Unesterified cholesterol is then transferred to the endoplasmic reticulum (ER) and plasma membrane. Mutant Niemann-Pick C (NPC) gene isolated from human and mouse (NPC1) presumably is involved in the transfer from the lysosome to the ER. Cholesterol entering the ER regulates the level of LDLR activity and cholesterol synthesis in the cell and, in the liver, is the substrate for esterification through the acyl-CoA-cholesterol acyl transferase enzyme and for bile acid synthesis through the cholesterol 7α-hydroxylase enzyme. In addition, this cholesterol may become incorporated in detergent-insoluble glycosphingolipid-enriched complexes (DIGs) and caveolae and, in the liver, into nascent very low-density lipoprotein (VLDL) particles. The percentage numbers in each compartment refer to the relative contribution of each group of tissues to whole animal weight in species varying from the mouse to human.
CAAGTA-3' and 5'-GATGGTCTGTTCTCCATG-3'). The PCR products were resolved on 1.2% (wt/vol) agarose gel electrophoresis. Genotype analysis of the Npc1 allele demonstrated a size alteration. The control mice had a 1,048-bp band, whereas the homozygous NPC animals showed a 1,209-bp band because of an insertion of a MaLR sequence. The heterozygous NPC mice manifested both of these bands.

Calculations. The data in all experiments are presented as mean values ± SE. The two-tailed, unpaired Student's t-test was used to compare the various sets of data. In Figs. 1–5 and Tables 1 and 2, a value that was significantly different (P < 0.05) from the appropriate control value is indicated by an asterisk.

RESULTS

Three preliminary studies were carried out to delineate the most appropriate ages and conditions under which to make detailed measurements of cholesterol balance in the NPC mice. The first experiment investigated whether it would be informative to utilize both the heterozygous (NPC+/−) and homozygous (NPC/−) animals in subsequent experiments. The NPC/− mice developed neurological deficits and began to lose weight at 7–8 wk of age. They also manifested biochemical evidence of a cellular defect in cholesterol transport, as summarized in Table 1. At 7 wk of age, for example, these homozygous mice had cholesterol concentrations in the liver, spleen, lung, and kidney that were 2.5- to 10-fold higher than those seen in control animals and rates of cholesterol synthesis in these same organs that were also elevated. In contrast, the NPC+/− mice did not develop neurological disease, did not lose weight, and did not have any alteration in tissue cholesterol metabolism compared with the NPC+/− controls (Table 1).

Finally, the plasma total cholesterol concentration was similar in the NPC+/− (95 ± 6 mg/dl), NPC+/− (93 ± 7 mg/dl), and NPC/− (116 ± 8 mg/dl) mice. Although this study indicated that only the homozygous animals would be informative, a second preliminary investigation was necessary to establish whether gender had a significant effect on these measurements. As illustrated in Table 2, the phenotype manifested in cellular cholesterol concentration and synthesis rate in the homoyzogous mice was identical in the males and females. Only the adrenal gland had a higher sterol concentration in the female (64.3 mg/g) than in the male (38.7 mg/g), as has been described previously (24). There was also a higher concentration of cholesterol in the ovary (13.1 mg/g) compared with the testis (4.9 mg/g). Because these three endocrine glands, however, accounted for

<table>
<thead>
<tr>
<th>Organ</th>
<th>Tissue Cholesterol Concentration, mg/g</th>
<th>NPC+/−</th>
<th>NPC+/−</th>
<th>NPC/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>2.4 ± 0.1</td>
<td>2.4 ± 0.1</td>
<td>24.3 ± 2.3*</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>3.8 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>17.4 ± 1.5*</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>5.5 ± 0.1</td>
<td>6.0 ± 0.2</td>
<td>13.8 ± 0.9*</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>4.4 ± 0.2</td>
<td>4.3 ± 0.2</td>
<td>10.8 ± 0.4*</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Tissue cholesterol concentration and rates of sterol synthesis in NPC+/+, NPC+/−, and NPC−/− mice

All values represent means ± SE. NPC, Niemann-Pick C. NPC+/+, NPC+/−, and NPC−/− groups of mice were 7 wk of age and included both male and female animals that had been maintained on the low-cholesterol basal diet since birth. *Values significantly different (P < 0.05) from the values in NPC+/+ animals.
The final preliminary study was undertaken to establish the age at which to quantitate sterol fluxes, since such measurements would be invalid unless food intake, weight gain, and other indicators of steady-state conditions had been established. A number of measurements, therefore, were carried out in male and female NPC+/− and NPC−/− mice between the ages of 1 day and 8 wk. Both the control and homozygous animals had similar food intakes and gained weight at similar rates through the first 7 wk of life (Fig. 2A). Beyond this time, the NPC−/− animals developed neurological findings, had poor food intake, and began to lose weight. During this same interval, the relative weight of the liver, spleen, and other organs in the mutant mice progressively increased (Fig. 2B), a finding reminiscent of the hepatosplenomegaly seen in young children with this disease. In contrast to all of the other organs, however, relative brain weight progressively decreased in the NPC−/− mice, reaching only 0.90 of the control weight at 7 wk. Most striking were the age-dependent changes in the whole animal sterol pool shown in Fig. 2C. At 1 day of age, this pool was already higher in the NPC−/− pups (2,377 ± 38 mg/kg body wt) than in the control mice (1,833 ± 21 mg/kg), and over the next 7 wk this pool expanded at a rate of 64 mg·day−1·kg−1, reaching 5,442 ± 324 mg/kg. In the NPC+/− mice, the rate of expansion was only one-ninth of this rate and equalled 7 mg·day−1·kg−1. As shown in Fig. 2D, this expansion was due to marked increases with age in the cholesterol content of most organs in the NPC−/− mice with one important exception. The cholesterol content of the brain in these mutant animals at 7 wk of age was only 0.76 of that found in the NPC+/− mice.

Based on these studies, it appeared that the NPC+/− and NPC−/− mice were in steady state with respect to food intake and weight gain at 7 wk, but not later. The NPC−/− mice, however, never reached steady state with respect to the cholesterol pool in the whole animal. Thus detailed measurements of cholesterol balance were undertaken using 7-wk-old male and female NPC+/− and NPC−/− mice. Under these conditions, the concentration of cholesterol was elevated in every tissue of the NPC−/− mice (Fig. 3A) with the exception of the brain where this concentration was significantly reduced (13.2 ± 0.3 vs. 15.7 ± 1.1 mg/g). Notably, these relative increases in tissue sterol concentration were greatest in the liver (10-fold), intermediate in organs like the spleen, lung, and portions of the gastrointestinal tract (2- to 5-fold), and small in tissues like the carcass, muscle, and fat (1.3- to 2-fold). This pattern very closely reflects the rates of LDL-C uptake in the mouse, which are very high in the liver, much lower in organs like the spleen and gastrointestinal tract, and virtually undetectable in muscle and adipose tissue (24). The absolute cholesterol pool in each organ of the mutants also was increased (Fig. 3B), although, because of their large size, the liver and carcass together accounted for most of the expanded pool found in the

Table 2. Tissue cholesterol concentration and rates of sterol synthesis in male and female NPC−/− mice

<table>
<thead>
<tr>
<th>Organs</th>
<th>Tissue Cholesterol Concentration, mg/g</th>
<th>Tissue Cholesterol Synthesis Rate, nmol·h−1·g−1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>Liver</td>
<td>23.1 ± 3.3</td>
<td>26.1 ± 4.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>17.8 ± 2.6</td>
<td>16.9 ± 1.7</td>
</tr>
<tr>
<td>Lung</td>
<td>13.1 ± 0.4</td>
<td>14.8 ± 2.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>10.7 ± 0.8</td>
<td>10.8 ± 0.1</td>
</tr>
<tr>
<td>Adrenal</td>
<td>38.7 ± 1.9</td>
<td>64.3 ± 6.3*</td>
</tr>
<tr>
<td>Testis/ovary</td>
<td>4.9 ± 0.4</td>
<td>13.1 ± 4.4*</td>
</tr>
</tbody>
</table>

All values represent means ± SE. All animals were NPC−/− mice that were 7 wk of age and had been maintained on the low-cholesterol basal diet since birth. *Values in females were significantly different (P < 0.05) from those in males.
whole animal. Thus, at 7 wk of age, the whole animal cholesterol pool was nearly threefold greater in the NPC\(^{2/2}\) mice (5,442 ± 324 mg/kg) than in the NPC\(^{1/1}\) animals (2,175 ± 40 mg/kg) and, furthermore, only trace amounts (<1%) of esterified cholesterol were detected in most of the major organs. Again, however, the brain was the exception in that the cholesterol content of the CNS was significantly reduced (253 ± 6 vs. 305 ± 16 mg/kg) in the mutant animals, but there were no noncholesterol sterols found in either genotype.

Even though these intracellular pools of unesterified cholesterol were markedly expanded, rates of cholesterol synthesis in nearly every organ of the NPC\(^{2/2}\) mice were increased (Fig. 4A). As a consequence, the rates of cholesterol synthesis in the whole animal increased from 120 ± 12 mg·day\(^{-1}\)·kg\(^{-1}\) in the NPC\(^{1/1}\) mice to 182 ± 21 mg·day\(^{-1}\)·kg\(^{-1}\) in the NPC\(^{2/2}\) animals (Fig. 4B). As is also apparent in Fig. 4, most of the increase in whole animal synthesis could be attributed to enhanced synthesis in the small intestine and liver, although other organs also manifested significant increases. However, the brain was once again exceptional in that mean sterol synthesis was lower in the mutant animals (40 ± 6 nmol/h) than in the control mice (58 ± 4 nmol/h).

To further establish that the defect in cellular sterol transport in the mutant animals resided in the clathrin-coated pit pathway (Fig. 1), a final study was done in which additional cholesterol was selectively forced into the liver through this pathway. Six-week-old mice of both genotypes were placed on either the low-cholesterol basal diet or this same diet supplemented with 0.2% cholesterol for 7 days. The animals receiving the higher cholesterol diet had a net increase in cholesterol entry in the gastrointestinal tract of ~4.5 mg/day (~32 mg over the 7-day experiment). Because 40 to 55% of this load is absorbed in the mouse (unpublished observations), from 13 to 18 mg should have reached the liver in 7 days. As shown in Fig. 5, at the termination of this study, cholesterol feeding altered the tissue cholesterol concentrations only in the liver of the NPC\(^{2/2}\) and NPC\(^{-/-}\) animals, a finding consistent with the view

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**Fig. 3.** Tissue cholesterol concentration and organ and whole animal cholesterol content in NPC\(^{1/1}\) and NPC\(^{-/-}\) mice at 7 wk of age. These animals were all maintained on the low-cholesterol basal diet and included nearly equal numbers of males and females. A: concentration of cholesterol in the various organs; B: content of cholesterol in the whole organs. Inset, content of cholesterol in the whole animals, normalized to a constant body weight of 1 kg. Mean values ± SE are shown for 6–7 animals in each group. *Value in NPC\(^{-/-}\) mice was significantly different (P < 0.05) from that in control animals.

**Fig. 4.** Rates of cholesterol synthesis in vivo in NPC\(^{1/1}\) and NPC\(^{-/-}\) animals at 7 wk of age. These animals were all maintained on the low-cholesterol basal diet and included nearly equal numbers of males and females. A: rates of synthesis per g of tissue; B: rates in whole organs. Inset: rates of whole animal synthesis expressed as mg of cholesterol synthesized per day per kg body weight. Mean values ± SE are shown for 6–7 animals in each group. *Value in NPC\(^{-/-}\) mice was significantly different (P < 0.05) from that in control animals.
that the liver primarily clears chylomicron remnants utilizing both the LDLR and LRP (14, 30). In the control mice, the concentration of hepatic total cholesterol only increased from 2.4 ± 0.1 to 4.6 ± 0.4 mg/g, the increase in the ester pool fully accounted for this rise, and the rate of cholesterol synthesis was suppressed 94% (to 51 ± 6 nmol·h⁻¹·g⁻¹). In contrast, in the NPC⁻/⁻ mice, the concentration of total cholesterol rose from 24.3 ± 2.3 to 38.0 ± 1.7 mg/g, the cholesteryl ester fraction remained at near-undetectable levels, and sterol synthesis was suppressed only 60% (to 480 ± 104 nmol·h⁻¹·g⁻¹). This change in hepatic sterol concentration accounted for an absolute increase in cholesteryl ester in the whole liver of ~15 mg. Thus, remarkably, the liver of the NPC⁻/⁻ mouse had captured and retained essentially all of the excess cholesterol reaching it during the 7-day experiment yet was unable to esterify or excrete this sterol or to fully suppress de novo synthesis. In contrast, the liver of the control mouse was able to excrete most of the additional load of sterol, presumably through the bile or after conversion to bile acids, and could fully esterify the remaining excess cholesterol in the hepatocyte and markedly suppress synthesis. As a result of these different responses, the rate of cholesterol synthesis in the whole animal remained much higher in the NPC⁻/⁻ mice (148 ± 15 mg·day⁻¹·kg⁻¹) than in the NPC⁺/+ animals (72 ± 5 mg·day⁻¹·kg⁻¹) even in the face of this influx of exogenous sterol.

**DISCUSSION**

These studies provide the first description in the live animal of the unique alterations in cholesterol metabolism that result from the mutational inactivation of NPC1, and these findings support the concept that this protein plays a critical role in the metabolism of sterol entering the cell through the clathrin-coated pit pathway. One of the most remarkable findings was that the cholesterol pool in the mutant animal began expanding in utero (Fig. 2C) and continued during the 7 wk after birth at a rate of 64 mg·day⁻¹·kg⁻¹ (Fig. 3B). Because the amount of cholesterol absorbed from the diet during this time was small and constant (~7 mg·day⁻¹·kg⁻¹), this rate of expansion was fully accounted for by the higher rate of cholesterol synthesis in the NPC⁻/⁻ mice (182 mg·day⁻¹·kg⁻¹) compared with the control animals (120 mg·day⁻¹·kg⁻¹). This increased amount of newly synthesized cholesterol came from many organs (Fig. 4), was presumably carried to the liver as HDL-C, and was then recirculated as LDL-C. As a consequence, the amount of cholesterol that accumulated in the various organs and that accounted for this expansion of the whole animal sterol pool was proportional to the magnitude of LDL-C uptake in each organ system (Fig. 3; see Ref. 24). Clearly, the liver was the overwhelmingly important organ in this regard and showed the highest relative and absolute increase in cholesterol accumulation (Fig. 3). A similar conclusion was reached in the animals challenged with additional dietary cholesterol. Over this 7-day experiment, the mice absorbed an additional 80–90 mg·day⁻¹·kg⁻¹ of sterol, and the pool of cholesterol in the NPC⁻/⁻ animals expanded at a rate of ~80 mg·day⁻¹·kg⁻¹. In this case, however, expansion of the whole animal pool was associated exclusively with cholesterol accumulation in the liver (Fig. 5), since the dietary sterol was selectively taken up by the hepatocytes using apoprotein E (apoE) on the chylomicrons as the ligand with which to interact with hepatic LDLR and LRP (14, 30). Thus, taken together, these findings strongly support the concept that, in NPC disease, lipoprotein-cholesterol of either endogenous or exogenous origin enters cells normally through the clathrin-coated pit pathway, and the cholesteryl ester is hydrolyzed, but the unesterified cholesterol is trapped in the lysosomal compartment (4, 17, 26). Furthermore, these studies demonstrate that this trapping is remarkably complete in that, over the first 7 wk of life, virtually all excess cholesterol entering the

**Fig. 5. Effect of increased delivery of cholesterol through clathrin-coated pit pathway to the liver.** Groups of both NPC⁺/+ and NPC⁻/⁻ mice were switched from the low-cholesterol basal diet to the same diet supplemented with 0.2% cholesterol at 6 wk of age. Two other groups remained on the same low-cholesterol basal diet. After 1 additional wk, concentrations of unesterified cholesterol and cholesteryl ester (filled portion of each bar) were measured in different tissues taken from 4 groups of animals [liver (A), spleen (B), lung (C), kidney (D), and carcass (E)]. Mean values ± SE are shown for 6–7 animals in each group. *Values for concentration of total cholesterol in mice on 0.2% cholesterol diet were significantly different (P < 0.05) from those animals of the same genotype on low-cholesterol diet.
whole body pool in the NPC−/− mouse, whether of exogenous or endogenous origin, is retained in the various tissues.

Importantly, these studies also provide both direct and indirect data suggesting that this pool of sequestered unesterified cholesterol cannot reach the sites within the cell where regulation of sterol balance and cholesterol degradation take place. First, for example, despite the marked increase in unesterified cholesterol concentration in most tissues (Fig. 3A), nearly all of these organs manifested increased rates of sterol synthesis (Fig. 4A). Even when excess dietary cholesterol was forced in the liver in sufficient amounts to nearly completely inhibit synthesis in the NPC+/+ mice (to 51 nmol·h−1·g−1), hepatic synthesis was only partially inhibited in the NPC−/− animals (to 480 nmol·h−1·g−1). This finding indicates that unesterified cholesterol in the mutant animals could not effectively reach the ER, block the release of transcriptionally active SREBP, and suppress cholesterol synthesis (31). Second, this unesterified cholesterol also did not have access to the sites of esterification on the ER of the NPC−/− mice. ACAT, the enzyme that normally esterifies sterol, is unregulated, and the rate of this reaction appears to be driven by the appearance of excess cholesterol in the ER once the rate of sterol synthesis has been suppressed nearly to zero (7, 29, 33, 41). Thus, even though the pool of unesterified cholesterol was greatly expanded in the liver and other organs by either endogenously or exogenously derived cholesterol (Figs. 3A and 5A), virtually no cholesteryl ester formation could be detected in the tissues of the mutant animals. Third, the NPC−/− mice also were apparently unable to respond to an increased load of dietary cholesterol by elevating bile acid synthesis. Whereas the normal mouse can increase bile acid synthesis two- to threefold (38), the mutant animal simply accumulated this excess sterol in the liver when challenged with additional dietary cholesterol (Fig. 5). Thus the unesterified cholesterol in the tissues either could not reach the nuclear oxysterol receptor LXRα (25) or could not enter the pools of cholesterol that are the substrate for the cholesterol 7α-hydroxylase or 27-hydroxylase pathways. Finally, even though the concentration of unesterified cholesterol was very high in the liver of the mutant animals, LDLR activity apparently was not suppressed. The plasma cholesterol concentration in these animals remained at ~115 mg/dl, whereas values of 200–300 mg/dl are expected in mice with suppression of LDLR function (24). Thus, in the NPC−/− mice, the expanded pool of unesterified cholesterol was sequestered in a compartment where it could not regulate the rates of sterol synthesis or LDLR activity or act as a substrate for esterification or degradation.

In contrast to virtually all other organs in the body, the brain of the mutant mouse had a significantly lower cholesterol concentration (Fig. 3A) and content (Fig. 3B) and, in addition, exhibited a lower rate of sterol synthesis (Fig. 4). Many features of sterol metabolism in the CNS are known to be different from those in the remaining organs of the body. Most, or perhaps all, cholesterol in the brain and spinal cord comes from local synthesis while virtually no LDL-C is taken up from the plasma across the blood-brain barrier, even in the fetus (15, 35–37). Nevertheless, two lipoprotein receptors, LDLR and LRP (28), and one receptor ligand, apoE (9), have been identified in various regions of the CNS. It has been postulated, therefore, that the integrity of the myelin sheath and neuron may depend on an “internal” recycling of cholesterol among the cells of the brain that depends on apoE to carry cholesterol in the interstitial fluid and LDLR or LRP to mediate cellular uptake. Certainly, neurite growth in vitro appears to require a source of cholesterol and the common isoform of apoE, i.e., apoE 3 (10, 23). Nevertheless, the abnormalities in cholesterol metabolism found in all organs, including the brain, are far more profound in the NPC−/− mouse than in animals lacking either LDLR or apoE. In both the LDLR−/− and apoE−/− mouse, for example, there is a marked increase in the plasma cholesterol level, no change in tissue cholesterol concentrations, and no gross neurological abnormalities, and repair of damaged peripheral nerves proceeds normally, at least in the case of the apoE−/− mouse (11). In contrast, in these NPC−/− animals, plasma cholesterol levels are normal, there are marked abnormalities in tissue cholesterol content, severe neurological abnormalities develop, and repair of damaged peripheral nerves is impaired (12). Thus these findings further suggest that NPC1 is critically involved in this sterol recycling process within the CNS and that progressive neurological death and demyelination may account for the paradoxical decrease in brain cholesterol content observed in these studies.

In any event, these findings emphasize the critical importance of further elucidating this cholesterol recycling process in the different regions of the mature brain. The observations that patients with apoE 4 are susceptible to the development of Alzheimer’s disease, that apoE 4 cannot support neurite growth (23), that both NPC and Alzheimer’s disease manifest neurofibrillary tangles, and that cholesterol depletion in neurons alters β-amyloid production (32) raise the likelihood that understanding cholesterol movement through the clathrin-coated pit pathway is of critical importance to elucidating the pathogenesis of NPC and, possibly, other degenerative neurological diseases.

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REFERENCES


