Niemann-Pick type C (NPC) disease is an autosomal lipid storage disease resulting from mutations in either the NPC1 (95% of families) or NPC2 genes (1–3). It is characterized by progressive hepatosplenomegaly and neurodegeneration, leading to premature death (4, 5). The exact function of both proteins remains unknown, but there is much evidence that they facilitate the transport of lipids, primarily cholesterol, from late endosome to the Golgi apparatus, endoplasmic reticulum (ER), mitochondria, and plasma membrane (6, 7). Thus the storage involves the accumulation of unesterified cholesterol, sphingolipids, and other lipids within the endosomal/lysosomal compartments of cells in almost all body tissues (8). Impaired cholesterol traffic in NPC disease cells prevents the normal down-regulation of endogenous cholesterol synthesis and the low density lipoprotein (LDL) receptor (9, 10), through the interruption of normal cholesterol regulation by sterol regulatory element-binding protein at the ER and the interruption of generation of LDL cholesterol-derived oxysterols in the mitochondria (11).

Liver dysfunction in patients with NPC disease is more common than previously believed (12) and understanding the mechanism of cholesterol homeostasis in hepatocytes is most important for the treatment of this disease. In NPC1-null mice, biliary secretion is altered (13, 14) and hepatocyte plasma membrane and ER compartments accumulate large amounts of cholesterol (15, 16). The abnormal cholesterol homeostasis in NPC1-null hepatocytes leads to increased secretion of cholesterol-rich very low density lipoprotein (VLDL) (17). However, one report indicated that NPC1+/− subjects do not exhibit elevated apoB lipoproteins but rather low HDL-C (18). Because the liver is a major expression site for both apoA-I and Abca1 (19, 20) and a main contributor to maintaining plasma level of HDL (21, 22), we hypothesized that HDL secretion might be impaired by NPC1 deficiency. Here we studied the effect of NPC1 ablation on hepatocyte secretion of lipidated apoA-I and compared lipid efflux to apoA-I in NPC1−/− hepatocytes and macrophages. We show...
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that Npc1 deficiency strikingly increases efflux and HDL formation in hepatocytes, whereas it decreases efflux in macrophages. We have explored the regulatory mechanisms involved and demonstrate a post-transcriptional up-regulation of Abca1 in hepatocytes linked to increased Ctsd expression and Abca1 translation rate.

EXPERIMENTAL PROCEDURES

Materials—[1,2-3H]Cholesterol, [5-3H]mevalono-lactone-Rs, choline [methyl-3H]chloride, and [35S]methionine were obtained from PerkinElmer Life Sciences. Williams medium E, HepatoZYME-SFM, and antibiotic-antimycotic were purchased from Invitrogen. Rabbit polyclonal anti-human apoA-I antibody was purchased from Calbiochem. Monoclonal antibodies directed against human apoA-I (a combination of 4H1 (against the extreme N terminus) and 5F6 (against the central region)) were described previously (23) and biotinylated with sulfo-NHS-biotin from Pierce. Streptavidin-horseradish peroxidase conjugate and protein G-Sepharose were obtained from Amersham Biosciences.

Animals and Primary Hepatocyte Cultures—Breeder Npc1+/− mice were purchased from Jackson Laboratories and bred in our animal facility. All mice were maintained on a normal chow diet in a 12-h light/12-h dark schedule and used between the ages of 6 and 8 weeks. All experiments performed were in accordance with protocols approved by the University of Ottawa Animal Care Committee. Primary hepatocytes were isolated from these mice by liver collagenase perfusion according to established protocols (24). Briefly, the cells were plated in fibronectin-precoated (25 μg/well) 6-well plates at an initial density of 1.5 × 106 cells/well in Williams medium E containing penicillin (100 units/ml), streptomycin sulfate (100 units/ml), Fungizone (250 ng/ml), and 10% fetal bovine serum (25, 26).

Generation of Macrophages—Bone marrow-derived macrophages were flushed from mouse femurs. Macrophages were generated by incubating bone marrow cells (106 cells/ml) with DMEM of 10% FBS complemented with 15% L929 conditioned medium for 7 days (27).

Hepatocyte Labeling and ApoA-I Lipidation—The hepatocytes following a 5-h attachment period with 10% FBS (1 ml/well) were washed in serum-free Williams medium E (3 × 2 ml) and incubated with 2 ml of HepatoZYME-SFM containing an antibiotic/antimycotic mixture, 10 μCi/ml [3H]mevalonate, or 5 μCi/ml [3H]cholesterol delivered with LDL (50 μg/ml) or [3H]choline (5 μCi/ml) for 40 h. For exogenous apoA-I-mediated efflux, cells were washed once and incubated with 1 ml/well DMEM containing 10 μg/ml human recombinant apoA-I (28). For endogenous apoA-I-mediated efflux assay, 24 h after labeling, the medium was removed, and the cells in serum-free Williams medium E were infected for 1 h with either the recombinant adenovector expressing human apoA-I (Ad5-Ad Al) or as control, adeno-luciferase (Ad5-Ad Luc) at a multiplicity of infection of 75:1 plaque-forming units/cell (25, 26). After a 1-h infection, hepatocytes were incubated for an additional 24 h with the original labeling medium, then washed and incubated for the indicated period with efflux medium as described above.

Immunoprecipitation of ApoA-I-associated [3H]Cholesterol or [3H]Choline-Phospholipid—ApoA-I was immunoprecipitated either directly from the efflux medium or from lipoprotein fractions separated by fast protein liquid chromatography with a polyclonal anti-human apoA-I antiserum and pulled down with protein G-Sepharose. Control immunoprecipitations were carried out with equal volumes of an anti-human apoB antiserum from sheep, which does not cross-react with murine apoB, or with the time 0 efflux medium immunoprecipitated with anti-human apoA-I antiserum. The immunoprecipitates were collected as described previously (25). Radioactivity of cholesterol and phospholipid associated with human apoA-I was quantified by scintillation counting.

Lipid Efflux in Macrophages—Unless indicated in legends, labeling conditions are as follows. Macrophages were washed three times with plain DMEM and then labeled with LDL or acetylated LDL (50 μg of protein/ml) that had been preincubated with 5 μCi/ml [3H]cholesterol in 1% FBS of DMEM for 24 h. The cells were equilibrated with 2 mg/ml bovine serum albumin overnight. For labeling with [3H]mevalonate (10 μCi/ml) or [3H]choline (5 μCi/ml), the cells were incubated for 40 h in DMEM with 1% FBS. Efflux to apoA-I (10 μg in 2 mg/ml bovine serum albumin medium) was monitored for 3–5 h (29). Efflux to mβ-CD (10 mM in 2 mg/ml bovine serum albumin DMEM) was carried out for 15 min at 37 or 4°C (27).

Lipid Analysis—Cellular lipids were extracted (30), separated by thin layer chromatography (TLC) using hexane:diethyl ether:acetic acid (105:45:1.5) as running solvent on Sil-G TLC plates (EMD Chemicals, Darmstadt). Lipid bands were detected by exposure to iodine vapors, scraped off the TLC plate, and radioactivity measured with a scintillation counter. For total cholesterol determination, cells were washed with cold phosphate-buffered saline, cholesterol was extracted by isopropyl alcohol, and measured by colorimetric assay (Wako Chemicals, Richmond, VA).

RT-PCR—For transcription analysis in ex vivo hepatocytes or macrophages, the cells were transferred immediately after isolation into a tube with ice-cold DMEM containing 10% FBS and total RNA was isolated. In some experiments, hepatocytes also were cultured in media with 5% lipoprotein-deficient serum or 10% FBS or in HepatoZYME for 2 to 5 days. Total RNA from bone marrow-derived macrophages was isolated under the efflux conditions. DNA was removed by incubation with DNase and cDNA was reverse transcribed by addition of 1 μl of Moloney murine leukemia virus reverse transcriptase (Invitrogen) in the buffer with 2 μl of decamers, 1 μl of dNTP (25 mM), 5 μl of H2O plus 5 μl (0.5 μg) of mRNA and incubated for 1 h at 42°C. The products of 0.5 μg of target gene were amplified in a PCR tube with a specific set of primers (for one or more target genes) and one pair of internal control glyceraldehyde-3-phosphate dehydrogenase primers (see supplementary materials Table S1). All primers were designed based on same annealing temperature (60°C) and the PCR product size for each gene was preset. The PCR was run for 30 cycles. Specificity of the primers of each target gene was tested by individual PCR before mixing with internal control primers or primers of other genes. The bands of final PCR products were analyzed with Quantity One.
These results contrast with those in macrophages, where the B-null hepatocytes compared with wild type (Fig. 2). Npc1 ids was significantly increased by 5- and 6-fold, respectively, in nouveaux apoA-I by newly synthesized cholesterol and phospholipids. Similarly, the lipidation of exogenously added LDL, respectively (Fig. 2). Lipidation of exogenous apoA-I in hepatocytes and macrophages, pre-labeled with exogenously added or de novo synthesized lipids. Compared with control, exogenous apoA-I lipidation by cholesterol was significantly increased by 2.6-, 3.2-, 4.8-, and 6.0-fold in NPC1-null hepatocytes labeled with [3H]cholesterol-LDL (A), [3H]mevalonate (B), or [3H]choline (C) in hepatocyte (5 μCi/ml) for 40 h. The cells were transfected with adenovirus encoding human apoA-I or with an adenovector encoding luciferase as a control during the last 24 h of the labeling period. The cells were then washed with plain Williams medium twice; 1 ml of fresh hepatocyte was added to each well and the cells incubated for 4 h. ApoA-I lipidation was determined after immunoprecipitation with anti-human apoA-I antibody. Cellular cholesterol labeling was not affected by infection with adenovirus apoA-I compared with control virus. The results are presented as apoA-I-associated counts adjusted for control virus. The results are presented as apoA-I-associated counts adjusted for control virus. The results are presented as apoA-I-associated counts adjusted for control virus. The results are presented as apoA-I-associated counts adjusted for control virus. The results are presented as apoA-I-associated counts adjusted for control virus.

Statistics—Significance was evaluated by Student’s t test.

RESULTS

Npc1 Deficiency Increases the Lipidation of Newly Synthesized ApoA-I in Hepatocytes—Whereas Npc1 inactivation is well known to impair intracellular cholesterol traffic and decrease cholesterol efflux to apoA-I in macrophages or fibroblasts (6, 18, 31, 32), its effect on the lipidation of apoA-I in liver, the major site of HDL synthesis, is unknown. Here, we evaluated the effect of Npc1 inactivation on the lipidation of endogenous apoA-I in hepatocytes that were labeled with either endogenously synthesized lipids or exogenous lipids delivered by lipoproteins. Surprisingly, the lipidation of endogenously synthesized apoA-I with LDL-derived cholesterol or de novo synthesized cholesterol or de novo synthesized phospholipids was decreased by 30% and 55%, respectively (Fig. 2). As well, exogenous apoA-I lipidation by newly synthesized cholesterol or phospholipids was decreased by 30 and 55%, respectively, in NPC1-null versus wild type macrophages (Fig. 2D). These observations indicate that lipid transport and homeostasis in hepatocytes and macrophages is differentially affected by Npc1 inactivation.

Cholesterol Traffic from Intracellular Compartments to the Plasma Membrane Is Similarly Impaired in Npc1-null Hepato-
and Chinese hamster ovary cell lines expressing mutant Npc1 is commonly ascribed to impaired cholesterol traffic of late endosomes to plasma membrane (1, 6, 33), where a pool of Abca1 is situated and where apoa-I-mediated lipid efflux occurs (34, 35). To test if cholesterol traffic from intracellular compartments to plasma membrane is differentially impaired in Npc1-null hepatocytes versus macrophages, cells were labeled with \[^{3}H\]cholesterol delivered by LDL (Fig. 3, A and C) or with \[^{3}H\]mevalonate (Fig. 3, B and D). Cholesterol efflux to mβ-CD at 4 °C, which measures labeled cholesterol associated with the plasma membrane (27), was significantly and similarly reduced by more than 30% in Npc1-null hepatocytes and macrophages. Similar results were obtained with hepatocytes and macrophages metabolically labeled with \[^{3}H\]mevalonate (Fig. 3, B and D), where cholesterol efflux to mβ-CD at 4 °C and at 37 °C was proportionately lower. Therefore, the similar impairment of cholesterol traffic in Npc1-null hepatocytes and macrophages cannot account for the difference in cholesterol efflux between Npc1-null hepatocytes and macrophages.

**Abca1 and Abcg1 Expression Is Up-regulated by Npc1 Inactivation in Hepatocytes but Not in Macrophages**—The similar defect in cholesterol traffic in hepatocytes and macrophages suggested that the difference observed for apoa-I lipidation between these cells could be related to the regulation of transporters. Under basal conditions in complete medium, Abca1 protein was greatly up-regulated (3–7-fold) in Npc1-null hepatocytes compared with control (Fig. 4), and addition of LDL further increased Abca1 expression independently of Npc1 expression (data not shown). This increase in Abca1 protein was independent of the hepatocyte culture con-
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To test if the regulation of these Abc transporters by Npc1 also occurred transcriptionally, mRNA levels of Abca1 and Abcg1 were determined by RT-PCR. Whereas Abcg1 mRNA was markedly and significantly increased in Npc1-null hepatocytes, Abca1 mRNA was only modestly but significantly increased (Fig. 4 and Table 1). In contrast, transcriptional expression of Abca1 and Abcg1 in macrophages was slightly reduced. These results suggested that unlike Abcg1 regulation, the up-regulation of Abca1 observed in Npc-null hepatocytes was mostly post-transcriptional.

The Increased Abca1 Transcription in Npc1-null Hepatocytes Is Independent of Regulation by Lxrα, Lxrβ, and Retinoid X Receptor α—To elucidate the origin of the differential regulation of Abca1 and Abcg1 expression, the expression of their known transcriptional regulatory factors (38, 39) and of other major genes for lipid homeostasis were determined by RT-PCR. The expression of Lxrα, Lxrβ, and retinoid X receptor α were not significantly altered in Npc1-null hepatocytes compared with wild type, and furthermore, the expression of Lxrα target genes, such as Abcg5/8, sterol regulatory element-binding protein-1c (Srebp), steroyl-CoA desaturase 1 (Scd1), Cyp7a, and Cyp27a, were not up- but down-regulated (Table 1), again suggesting that Lxrα was not up-regulated and that the increased expression of Abca1 in Npc1-null hepatocytes was not linked to the transcriptional up-regulation by Lxrα activation.

The Differential Expression of Genes Regulating Lipid Transport and Homeostasis in Hepatocytes and Macrophages Indicates a Tissue-specific Regulation of Abca1 Expression—The observation that Abca1 mRNA is not up-regulated as Abca1 protein indicates that post-transcriptional regulation of Abca1 may be involved. Recently, we identified a lysosomal protease, cathepsin D (Ctsd), whose expression positively regulated Abca1 and Abcg1 protein levels in wild type macrophages as expected (36), it had no effect on Abca1 in Npc1-null macrophages. Abcg1 is a transporter that mediates efflux to HDL and is highly expressed in both hepatocytes and macrophages (37). We evaluated whether its expression would be differentially regulated by Npc1 in these cells. Like Abca1, Abcg1 protein was highly significantly increased in Npc1-null hepatocytes but not in macrophages (Fig. 4).

$$\text{Abca1}$$
$$\text{Abcg1}$$
$$\text{Ctsd}$$
$$\text{GAPDH}$$
$$\text{RT-PCR}$$

**FIGURE 5.** Ctsd mRNA and protein are up-regulated by Npc1 deficiency in hepatocytes but not in macrophages. Ctsd mRNA was measured by RT-PCR with the mRNA isolated from WT and Npc1-null hepatocytes or macrophages (top panel). Ctsd protein was measured by Western blot of hepatocyte cell lysates (bottom panel). These results are representative of two separate experiments. The number below each band indicates the -fold change against control. **WT**, Western blot. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Table 1

<table>
<thead>
<tr>
<th>Genes</th>
<th>Hepatocytes</th>
<th>Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abca1</td>
<td>1.30 ± 0.20a</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>Abcg1</td>
<td>8.51 ± 0.05a</td>
<td>8.85 ± 0.02</td>
</tr>
<tr>
<td>Cystatin D</td>
<td>4.94 ± 0.15a</td>
<td>1.04 ± 0.03</td>
</tr>
<tr>
<td>Creb</td>
<td>3.32 ± 0.05a</td>
<td>0.53 ± 0.09a</td>
</tr>
<tr>
<td>Neutral CE hydrolase</td>
<td>1.57 ± 0.28a</td>
<td>0.69 ± 0.01a</td>
</tr>
<tr>
<td>Scd1</td>
<td>0.39 ± 0.04a</td>
<td>1.65 ± 0.08</td>
</tr>
<tr>
<td>Cyp27α</td>
<td>0.57 ± 0.09a</td>
<td>0.92 ± 0.11</td>
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<td>Ppar γ</td>
<td>7.24 ± 0.30a</td>
<td>1.49 ± 0.13</td>
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<td>Fabp-4</td>
<td>2.70 ± 0.08a</td>
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<tr>
<td>Acid CE hydrolase</td>
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<tr>
<td>Cavelin-1</td>
<td>2.43 ± 0.18a</td>
<td>1.68 ± 0.16a</td>
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<tr>
<td>Ldlr</td>
<td>1.35 ± 0.14a</td>
<td>1.27 ± 0.12a</td>
</tr>
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</table>

**a** p < 0.05 versus wild type cells. Genes were grouped as a function of their differential or similar expression in hepatocytes and macrophages upon Npc1 inactivation.

$^a$ Creb, cAMP element binding protein; Ppar, peroxisome proliferator activated receptor γ; Fabp-4, fatty acid-binding protein 4.

Conditions and was also observed in media with 10% FBS, 5% LPDS or synthetic medium (Hepatocyte; lacking cholesterol containing serum) and in culture for 1–3 days (data not shown). In contrast, Abca1 protein was decreased in Npc1-null macrophages compared with wild type (Fig. 4 and Table 1) and, while loading the cells with acetylated LDL increased Abca1 protein in wild type macrophages as expected (36), it had no effect on Abca1 in Npc1-null macrophages. Abcg1 is a transporter that mediates efflux to HDL and is highly expressed in both hepatocytes and macrophages (37). We evaluated whether its expression would be differentially regulated by Npc1 in these cells. Like Abca1, Abcg1 protein was highly significantly increased in Npc1-null hepatocytes but not in macrophages (Fig. 4).
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tin A inhibits Abca1 in WT and Npc1-null hepatocytes (Fig. 6) in a dose-dependent fashion. These results indicate that Ctsd is involved in Abca1 up-regulation. Interestingly, even at the highest dose of pepstatin A, the suppression of Abca1 protein expression in Npc1-null hepatocytes was not as effective as in WT hepatocytes, indicating other mechanisms may also be involved in the up-regulation.

Because the mRNA level of Abca1 is modestly increased in Npc1-null hepatocytes, unlike the protein level, which is increased several folds, the rate of Abca1 translation was measured by incubation of the hepatocytes with [3S]methionine/cystine. Surprisingly, the Abca1 translation rate was also significantly increased in Npc1-null hepatocytes (Fig. 7). The profile of radioactivity distribution in the full size of Abca1 band (Fig. 7A) and the cleaved Abca1 bands (Fig. 7, B–D) was similar in Npc1-null and WT, suggesting that Abca1 degradation rates were unaffected by Npc1 activity. This conclusion was further confirmed by a cycloheximide chase experiment, where Abca1 translation in Npc1-null or WT hepatocytes was blocked with cycloheximide. After 1 and 3 h, the residual Abca1 band was about 60 and 35% of the time 0 value in either WT or Npc1-null hepatocytes. These results suggest that the increased Abca1 expression in Npc1-null hepatocytes is contributed by increased translation rate and increased Ctsd activity, but not by an increased half-life of the Abca1.

We also screened by RT-PCR the expression of a number of lipid metabolism-related genes (Table 1 and data not shown). Many genes, measured in several replicate experiments, display a differential expression profile in Npc1-null hepatocytes compared with Npc1-null macrophages, and others are similarly up- or down-regulated, but with differing magnitude in the two cell types (Table 1). These data further support the conclusion that the regulation of Abca1 is tissue-specific and differentially responsive to the inactivation of Npc1 in hepatocytes and macrophages.

DISCUSSION

Here, we have demonstrated that both endogenous apoA-I lipidation and exogenous apoA-I-mediated lipid efflux are markedly increased by Npc1 inactivation in hepatocytes, in contrast to macrophages where apoA-I-mediated efflux is decreased. The difference in apoA-I lipidation is independent of lipid accumulation, because cultured Npc1-null hepatocytes and bone marrow-derived macrophages similarly accumulated lipids, including free cholesterol and sphingomyelin (data not shown). However, cholesteryl esters accumulated in Npc1-null hepatocytes as reported by others (17), but decreased in Npc1-null macrophages compared with wild type. The increased apoA-I lipidation in Npc1-null hepatocytes is linked primarily to the increased Abca1 protein level, which is only partly explained by increased Abca1 mRNA expression. Effectively, the translation rate of Abca1 in Npc1-null hepatocytes is significantly increased (Fig. 7) and largely explains the increased Abca1 expression in Npc1-null hepatocytes. We have also shown that Npc1 inactivation increases Ctsd expression both transcriptionally and post-transcriptionally in Npc1-null hepatocytes, whereas no such effect is seen in macrophages. Ctsd, an aspartic protease involved in the degradation of proteins in the lysosome-late endosome compartment, has been recently identified by our laboratory as a positive enhancer of Abca1 late endosome compartment, degradation of proteins in the lysosome-late endosome compartment. Ctsd, an aspartic protease involved in the degradation of proteins in the lysosome-late endosome compartment, has been recently identified by our laboratory as a positive enhancer of Abca1 late endosome compartment, degradation of proteins in the lysosome-late endosome compartment. Ctsd, an aspartic protease involved in the degradation of proteins in the lysosome-late endosome compartment, has been recently identified by our laboratory as a positive enhancer of Abca1 late endosome compartment, degradation of proteins in the lysosome-late endosome compartment.
The mechanism of action of Ctsd includes the activation of prosaposins and its maturation into saposins, which regulates transport of glycosphingolipids and cholesterol through the late endosomes, which in turn regulates Abca1 expression and activity (40). Here we demonstrated that Ctsd activity was also responsible for the increased Abca1 observed in Npc1-null hepatocytes (Fig. 6). Whereas Ctsd activity can by itself suffice to increase Abca1 levels, we also observed that ablation of Npc1 elicits distinct transcriptome profiles in hepatocytes and macrophages (Table 1), which supports the specific regulation of Abca1 and Abcg1 in these two cell types. In addition to the increase of Ctsd and Abca1 protein levels, the down-regulation of stearoyl-CoA desaturase 1 in Npc1-null hepatocytes but not in macrophages may change the membrane cholesterol organization supporting Abca1-mediated cholesterol efflux. The enhanced neutral cholesterol hydrolase activity may contribute to the mobilization of cytoplasmic cholesteryl esters and increase the availability of free cholesterol for efflux via Abca1 (Table 1) (42, 43). Ctsd regulation by Npc1 is clearly cell specific: whereas its basal level is very low in control hepatocytes, it is markedly increased by both transcriptional and post-transcriptional mechanisms by Npc1 inactivation. In contrast, Ctsd is highly expressed in macrophages but unaffected by Npc1 expression (Fig. 5). Why Ctsd is activated in Npc1-null hepatocytes but not in macrophages is not clear. The ceramide produced by acid sphingomyelinase directly binds and activates Ctsd (44), which up-regulates Abca1, possibly contributing to its downstream effect on Abca1 presentation to plasma membrane (45). However, the reduction of acid sphingomyelinase mRNA expression measured by RT-PCR in both Npc1-null hepatocytes and macrophages does not favor such a pathway (data not shown). In further studies, it will be of interest to evaluate if the varied causes of late endosomal cholesterol accumulation, such as progesterone treatment (46, 47), Npc2 inactivation (6), Gulp expression (48), or Rab7 inactivation (49), equally stimulate Ctsd expression.

Protein kinases, notably in the PKA pathway, do not appear to be major contributors to the increased Abca1 in Npc1-null hepatocytes. The major inducer of these kinases is the interaction of apoA-I and Abca1 at the plasma membrane (50), but the increased Abca1 expression in Npc1-null hepatocytes observed in this study is independent of the addition of exogenous apoA-I, and the levels of endogenous apoA-I secretion or expression in hepatocytes in vitro and in vivo are similar in both Npc1-null and wild type mice (13). Furthermore, the potent PKA stimulator, cAMP, failed to increase apoA-I lipidation in hepatocytes (data not shown). Interestingly, recent evidence showed that intracellular adaptors containing PDZ domains, α1β1-syntrophins, modulate the stabilization of Abca1 proteins in hepatic cells, macrophages, and transfected cell lines via binding to the KESYV motif at the C terminus of Abca1 (51, 52). The mechanisms by which Abca1 interacts with these adaptor proteins and the subsequent reduction of Abca1 protein degradation are unknown. Further studies will be required to identify the factors that bridge the gap between the lysosome protease Ctsd up-regulation and the binding of those adaptor proteins to Abca1 in Npc1-null hepatocytes.

It is noteworthy and unexpected that Abcg1 and Abca1 regulation differs in Npc1-null hepatocytes compared with wild type cells (Table 1). The synthetic Lxr ligand T091713 stimulates the expression of both Abc transporters in these hepatocytes (data not shown) in agreement with other studies (53–56). The up-regulation of Abcg1 expression in Npc1-null hepatocytes is mainly transcriptional, whereas Abca1 expression is largely dependent on post-transcriptional mechanisms. A differential regulation was also observed in human macrophages (57), in which the advanced glycation end products of bovine serum albumin decreased Abcg1 expression and cholesterol efflux to HDL, but did not affect Abca1 expression and apoA-I-mediated efflux. We also observed that lipopolysaccharide treatment reduces Abcg1 mRNA expression, but not Abca1 mRNA in mouse hepatocytes, whereas TNFα treatment reduces the mRNA levels of both transporters.3 It is clear therefore that the transcriptional factors regulating expression of these transporters are not identical, particularly in the liver.

The LDL receptor pathway, through complex regulated steps, integrates the cellular homeostasis of exogenous and endogenous cholesterol (58). Free cholesterol normally released from the late endosome-lysosome is transported to the ER and down-regulates cholesterol synthesis, but this process is impaired in NPC disease. Cholesterol homeostasis in the macrophage as a function of Npc1 activity is consistent with this paradigm. However, in the Npc1-null hepatocyte, free cholesterol elicits a regulatory response that suggests leakage from the lysosome by an unknown mechanism. As reported by others (17), we observed that cholesterol secretion into the media of cultured Npc1-null hepatocytes was increased (data not shown). This increase reflects the increase in apoB synthesis and secretion of cholesterol-rich VLDL (17). Here, we showed that apoA-I lipidation and HDL secretion are also increased in Npc-1 hepatocytes, in keeping with the increased HDL cholesterol levels and formation of larger HDL particles determined by fast protein liquid chromatography in Npc1-null mice (8, 13). In contrast, human NPC homozygote subjects apparently exhibit a moderate decrease in plasma HDL levels (18). It is unclear whether this discrepancy reflects the effects of chronic illness, a species difference, or methodologies for lipoprotein quantification. In the latter study, HDL levels were measured not by fast protein liquid chromatography but by routine clinical laboratory methods. In the murine system, all evidence is consistent with increased HDL-C. Indeed, HDL-C levels and HDL size are further increased in Npc1-null mice fed a high fat diet (13) and in Npc1-null mice crossed with LDL receptor null mice (8). Amigo and colleagues (13) also showed that Abca1 expression was up-regulated in Npc1-null liver homogenates in agreement with our results in cultured hepatocytes. Because the secretion of cholesterol-rich VLDL by Npc1-null hepatocytes is accompanied by up-regulation of apoB synthesis (17), we considered that the increased lipidation of apoA-I might be associated with up-regulation of endogenous apoA-I and apoE. However, the mRNA levels of apoA-I and apoE are not altered in Npc1-null hepatocytes (data not shown), in agreement with a

3 M.-D. Wang, V. Franklin, M. Sundaram, R. S. Kiss, K. Ho, M. Gallant, and Y. L. Marcel, unpublished data.
previous report (13). Thus, the major effect of Npc1 inactivation in hepatocytes on the HDL secretion is a stimulation of the apoA-I lipidation pathway.

Taken together, our study demonstrates that regulation of Abca1 expression is tissue specific. Inactivation of Npc1 in hepatocytes up-regulates Abca1 expression, largely by post-transcriptional mechanisms, including up-regulation of Ctsd, increasing Abca1 translation rate, and down-regulation of stearyl-CoA desaturase 1, but independent of the Lxα-related pathway. Because plasma HDL-C levels are predominantly regulated by the expression of hepatic apoA-I and Abca1, the observation that Npc1 activity regulates hepatic apoA-I lipidation and HDL secretion in a cell-specific manner is of major importance. The specific regulation of hepatic Ctsd is one such mechanism, but the association of Abca1 with syntrophins (51, 52), which can control Abca1 localization and transport in a cell-specific manner, might also play a role.

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Npc1 Regulation of Hepatocyte Abca1


Differential Regulation of ATP Binding Cassette Protein A1 Expression and ApoA-I Lipidation by Niemann-Pick Type C1 in Murine Hepatocytes and Macrophages

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