Published in final edited form as:

Gene. 2013 September 15; 527(1): 65–70. doi:10.1016/j.gene.2013.05.080.

The C57BL/6J Niemann–Pick C1 mouse model with decreased gene dosage has impaired glucose tolerance independent of body weight

David Jelinek, Joseph J. Castillo, and William S. Garver*

Department of Biochemistry, The University of New Mexico Health Sciences Center, Albuquerque, NM, USA
Department of Molecular Biology, School of Medicine, The University of New Mexico Health Sciences Center, Albuquerque, NM, USA

William S. Garver: wgarver@salud.unm.edu

Abstract

The human Niemann–Pick C1 (*NPC1*) gene has been found to be associated with extreme (early-onset and morbid-adult) obesity and type 2 diabetes independent of body weight. We previously performed growth studies using BALB/cJ *Npc1* normal (*Npc1*^{+/+}) and *Npc1* heterozygous (*Npc1*^{+/-}) mice and determined that decreased *Npc1* gene dosage interacts with a high-fat diet to promote weight gain and adiposity. The present study was performed using both BALB/cJ and C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice to determine if decreased *Npc1* gene dosage predisposes to metabolic features associated with type 2 diabetes. The results indicated that C57BL/6J *Npc1*^{+/-} mice, but not BALB/cJ *Npc1*^{+/-} mice, have impaired glucose tolerance when fed a low-fat diet and independent of body weight. The results also suggest that an accumulation of liver free fatty acids and hepatic lipotoxicity marked by an elevation in the amount of plasma alanine amino-transferase (ALT) may be responsible for hepatic insulin resistance and impaired glucose tolerance. Finally, the peroxisome-proliferator activated receptor α (PPAR α) and sterol regulatory element-binding protein-1 (SREBP-1) pathways known to have a central role in regulating free fatty acid metabolism were downregulated in the livers, but not in the adipose or muscle, of C57BL/6J *Npc1*^{+/-} mice compared to C57BL/6J *Npc1*^{+/+} mice. Therefore, decreased *Npc1* gene dosage among two different mouse strains interacts with undefined modifying genes to manifest disparate yet often related metabolic diseases.

Keywords

Adiposity; Cholesterol; Free fatty acids; Niemann–Pick C1; Obesity; Type 2 diabetes

1. Introduction

The human Niemann–Pick C1 (*NPC1*) gene has been primarily investigated in relation to a rare autosomal-recessive lipid-storage disorder characterized by neonatal jaundice, hepatosplenomegaly, and progressive neurological degeneration (Garver et al., 2007a,

*Corresponding author. Tel.: +1 505 272 4790, fax: +1 505 272 6587.

Conflict of interest: The authors declare no conflict of interest.

2010). A number of studies performed using human NPC1 fibroblasts and the BALB/cJ *Npc1* mouse model have demonstrated that the encoded NPC1 protein has a central role in regulating the transport of lipoprotein-derived lipid (cholesterol and free fatty acids) from late endosomes/ lysosomes to other cellular compartments to maintain cellular, tissue, and whole body lipid homeostasis through feedback inhibition of the sterol regulatory element-binding protein (SREBP) pathway (Chen et al., 2005; Liscum et al., 1989; Pentchev et al., 1984, 1985, 1986; Xie et al., 1999a, 1999b).

Two genome-wide association studies (GWAS) and a population-based meta-analysis have determined that the human *NPC1* gene is associated with extreme (early-onset and morbid-adult) obesity and adiposity (Cotsapas et al., 2010; den Hoed et al., 2012; Meyre et al., 2009). The human *NPC1* gene non-synonymous single nucleotide polymorphisms (SNPs) used in these studies (rs1805081 representing 644A>G that encodes His215Arg and rs1805082 representing 2572A>G that encodes Ile858Val) indicate that the ancestral alleles (644A and 2572A) are in complete linkage-disequilibrium and associated with increase risk for extreme obesity. Whether these alleles correspond with an increased or decreased function of the encoded NPC1 protein was unknown when these studies were initially published. However, our subsequent growth studies using BALB/cJ *Npc1* normal (*Npc1*^{+/+}) and *Npc1* heterozygous (*Npc1*^{+/-}) mice determined that decreased *Npc1* gene dosage interacts with a high-fat diet to promote weight gain and adiposity, consistent with a loss-of-function for the encoded NPC1 protein predisposing to obesity (Jelinek et al., 2010a).

A recent population-based study performed using more than 17,000 individuals has indicated yet a third human *NPC1* gene non-synonymous SNP (rs1788799 representing 1926C>G that encodes Ile642Met) associated with type 2 diabetes independent of body weight (Al-Daghri et al., 2012). In this case, the risk allele (1926G) for type 2 diabetes is non-ancestral. The present study was performed using both BALB/cJ and C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice to determine if decreased *Npc1* gene dosage predisposes to metabolic features associated with type 2 diabetes. The results indicated that C57BL/6J *Npc1*^{+/-} mice, but not BALB/cJ *Npc1*^{+/-} mice, have impaired glucose tolerance when fed a low-fat diet and independent of body weight. The results also suggested that an accumulation of liver free fatty acids and hepatic lipotoxicity marked by an elevation in the amount of plasma alanine aminotransferase (ALT) may be responsible for hepatic insulin resistance and impaired glucose tolerance. Finally, the peroxisome-proliferator activated receptor α (PPAR α) and sterol regulatory element-binding protein-1 (SREBP-1) pathways known to have a central role in regulating free fatty acid metabolism were downregulated in the livers, but not in the adipose or muscle, of C57BL/6J *Npc1*^{+/-} mice compared to C57BL/6J *Npc1*^{+/+} mice. Therefore, decreased *Npc1* gene dosage among two different mouse strains interacts with undefined modifying genes to manifest disparate yet often related metabolic diseases.

2. Materials and Methods

2.1. The *Npc1* mouse models

A male BALB/cJ *Npc1* heterozygous mouse (BALB/cJ *Npc1*^{+/-}) and a female C57BL/6J *Npc1* normal or wild-type mouse (C57BL/6J *Npc1*^{+/+}) were obtained from The Jackson

Laboratory (Bar Harbor, ME). These mice were interbred and the *Npc1* gene was backcrossed onto the C57BL/6J background for 15 generations to produce the C57BL/6J *Npc1* mouse model. For the present study, 10 breeding pairs of both the BALB/cJ and C57BL/6J *Npc1* mouse model were used to produce BALB/cJ and C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} sibling mice. The *Npc1*^{+/+} mice from each background were used as controls for investigating physical and metabolic phenotypes of *Npc1*^{+/-} mice with decreased *Npc1* gene dosage. These mice were maintained at The University of New Mexico Health Sciences Animal Resources Facility in accordance with the regulations mandated by the Institutional Animal Care and Use Committee.

2.2. Genotype analysis

The sibling mice were weaned at 21 days of age and male BALB/cJ and C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice were genetically identified at the *Npc1* locus using PCR as previously described (Loftus et al., 1997). In brief, PCR amplification buffer was used with 20–40 ng of tail-tip DNA added at 85 °C, amplification was performed for 35 cycles of 30 s at 95 °C, 30 s at 61 °C, and 1 min at 72 °C, with a terminal extension of 10 min at 72 °C. The PCR products were separated using a 1.2% agarose gel and visualized with an UV light.

2.3. Study protocol

A week after weaning and genotype analysis (28 days of age), the male BALB/cJ and C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice were weighed and placed on a low-fat diet (10% kcal fat or 4% gram fat, Diet-07021301) produced by Research Diets. The male mice of similar background were housed together with an equivalent number of *Npc1*^{+/+} and *Npc1*^{+/-} mice (2–4 mice per cage) to ensure identical environments. The mice were weighed at the time of weaning and weighed on a weekly basis until 77 days of age. An intraperitoneal glucose tolerance test (IPGTT) was performed at 70 days of age and an intraperitoneal insulin tolerance test (IPITT) was performed at 77 days of age as previously described (Jelinek et al., 2012). The mice were fasted (5 h) and a blood sample was collected from the mandibular vein at 85 days of age after which mice were sacrificed using CO₂ asphyxiation. The liver, gonadal white adipose tissue, and thigh muscle (hereafter referred to as liver, adipose, and muscle) were collected, weighed, snap-frozen into liquid N₂, and stored at –80 °C for molecular and biochemical analyses.

2.4. Plasma analysis

The concentration of plasma glucose, total cholesterol (cholesterol and cholesteryl ester), triacylglycerol, and liver enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was determined using colorimetric enzymatic kits (Thermo Scientific). The concentration of plasma free fatty acids was determined using the EnzyChrom Free Fatty Acid Assay Kit (BioAssay Systems). The concentration of plasma insulin was determined using the Insulin ELISA kit (ALPCO Immunoassays). The concentration of plasma glucose and insulin was used to calculate the homeostasis model assessment-insulin resistance index (HOMA-IR), homeostasis model assessment-β cell function percentile (HOMA-β%), and quantitative insulin sensitivity check index (QUICKIE) as previously described (Katz et al., 2000; Matthews et al., 1985).

2.5. Concentration of liver lipids

The total liver lipids were extracted using hexane/isopropanol (3:2, v/v) as previously described (Garver et al., 2007b). The dried lipid extract was suspended in water containing 2% Triton X-100 and the concentration of total cholesterol (cholesterol and cholesteryl ester), triacylglycerol, and free fatty acids was determined using the colorimetric enzymatic kits as previously described (Scullion et al., 2012).

2.6. RNA preparation and qRT-PCR

Total RNA was extracted from the mouse livers, adipose, and muscle using the RNeasy Mini Kit (Qiagen) and treated with RNase-free DNase to remove contaminating DNA. The relative amounts of mRNA transcribed from genes for liver X receptor α (*Lxra*, Mm00443454_m1), peroxisome proliferator-activated receptor α (*Ppara*, Mm00440939_m1), sterol regulatory element-binding proteins 1 and 2 (*Srebp1*, Mm01138338_g1 and *Srebp2*, Mm01306292_m1) were determined using qRT-PCR. Reverse transcription was performed using 2.5 μ mol/L random hexamers, 4.0 mmol/L dNTPs, 15 mmol/L MgCl₂, 50 U reverse transcriptase, and 100 U RNA inhibitor to produce cDNA, followed by performing qRT-PCR using a TaqMan Gene Expression Assay containing pre-developed PCR primers and MGB probes (FAM dye-labeled) with an ABI-PRISM Sequence Detection System (Applied Biosystems). The relative amount of mRNA was normalized to 18S rRNA (internal control).

2.7. Statistics

Quantitative data is represented as the mean $\pm$ standard error (SE) using 10 *Npc1*^{+/+} mice and 10 *Npc1*^{+/-} mice within each group. The F-test was used to determine whether samples have significantly different variances (confirmed by Levene's test). Thereafter, appropriate two-tailed Student's t-test assuming equal/unequal variance was used to determine significant differences ($P < 0.05$) between these two groups of mice.

3. Results

The change in body weight growth curves indicated no significant difference in weight gain between BALB/cJ *Npc1*^{+/+} and *Npc1*^{+/-} mice (Fig. 1A) and weight gain between C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice (Fig. 1B) when fed a low-fat diet. However, the average change in the body weight of C57BL/6J mice (*Npc1*^{+/+} and *Npc1*^{+/-}) was significantly increased (18%) compared to the average change in the body weight of BALB/cJ mice (*Npc1*^{+/+} and *Npc1*^{+/-}) at 77 days of age. With respect to the IPGTT, the results indicated no significant difference in glucose tolerance between BALB/cJ *Npc1*^{+/+} and *Npc1*^{+/-} mice at 70 days of age (Fig. 1C). In contrast, C57BL/6J *Npc1*^{+/-} mice had significantly increased concentrations of blood glucose at 15, 30, and 60 min during the glucose tolerance test compared to C57BL/6J *Npc1*^{+/+} mice at 70 days of age (Fig. 1D). These IPGTT results for BALB/cJ *Npc1*^{+/+} and *Npc1*^{+/-} mice (Fig. 1E) and C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice (Fig. 1F) were also analyzed by measuring area under the curve (AUC). The results again indicated no significant difference in glucose tolerance between BALB/cJ *Npc1*^{+/+} and *Npc1*^{+/-} mice but a significant difference between C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice. With respect to the IPITT, the results indicated no significant difference in insulin tolerance between BALB/cJ

Npc1^{+/+} and *Npc1*^{+/-} mice or between C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice at 77 days of age (data not shown).

Since the C57BL/6J *Npc1*^{+/-} mice have impaired glucose tolerance in the absence of a change in body weight, further characterization of C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice was performed to define the possible mechanism. The additional results indicated no significant difference in the body weights (Fig. 2A), liver weights (Fig. 2B), or adipose weights (Fig. 2C) between *Npc1*^{+/+} and *Npc1*^{+/-} mice at 84 days of age after sacrifice. Moreover, as would be expected for no change in body weight, there was no significant difference in liver weights or adipose weights when normalized to respective body weights (data not shown). The liver lipid analysis of C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice indicated no significant difference in the concentration of liver total cholesterol (cholesterol and cholesteryl ester) (Fig. 2D) or triacylglycerol (Fig. 2E), although there was a significant increase (35%) in the concentration of liver free fatty acids (Fig. 2F) among *Npc1*^{+/-} mice compared to *Npc1*^{+/+} mice.

The plasma obtained from C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice indicated no significant difference in the concentration of glucose or insulin at 84 days of age (Table 1). These concentrations of plasma glucose and insulin were used to calculate the measures of insulin resistance (HOMA-IR, HOMA-β%, and QUICKIE) which were found not to be significantly different. Moreover, there was no significant difference in the concentration of plasma lipids (total cholesterol, triacylglycerol, and free fatty acids) between C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice. Although the concentration of plasma AST was not significantly different between C57BL/6J *Npc1*^{+/-} and *Npc1*^{+/+} mice, the concentration of plasma ALT was significantly increased (100%) in *Npc1*^{+/-} mice compared to *Npc1*^{+/+} mice. This particular result suggests that the increased concentration of liver free fatty acids may be responsible for hepatic lipotoxicity and promoting hepatic insulin resistance.

It is generally accepted that the NPC1 protein has a central role in regulating the transport of lipids (cholesterol and free fatty acids) from late endosomes/lysosomes to other cellular compartments to maintain cellular, tissue, and whole body lipid homeostasis. The primary nuclear receptors and transcription factors influenced by these lipids at the transcriptional, posttranscriptional, and functional level include the LXRα, PPARα, SREBP-1, and SREBP-2 proteins. Therefore, the relative amounts of *Lxra*, *Ppara*, *Srebp-1*, and *Srebp-2* mRNA were determined in the liver, adipose, and muscle of C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice. The results indicated no significant difference in the relative amounts of *Lxra* mRNA in the tissues of C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice (Fig. 3A). In contrast, C57BL/6J *Npc1*^{+/-} mice had significantly decreased amounts (65%) of *Ppara* mRNA in the livers, but not in the adipose or muscle, compared to *Npc1*^{+/+} mice (Fig. 3B). The results also indicated that C57BL/6J *Npc1*^{+/-} mice had significantly decreased amounts (56%) of *Srebp-1* mRNA in the liver, but not in the adipose or muscle, compared to C57BL/6J *Npc1*^{+/+} mice (Fig. 3C). Finally, although somewhat decreased, the results indicated no significant difference in the relative amounts of *Srebp-2* mRNA in the tissues of C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice (Fig. 3D).

4. Discussion

In terms of whole body physiology, BALB/cJ *Npc1*^{+/-} mice have historically been considered to be “normal” in contrast to BALB/cJ *Npc1*^{-/-} mice which accumulate lipoprotein-derived cholesterol in a time dependent manner (Pentchev et al., 1984, 1986; Xie et al., 1999a, 1999b). However, one of our earlier studies demonstrated that BALB/cJ *Npc1*^{+/-} mice have a significantly increased concentration (~50%) of liver triacylglycerol, but not cholesterol or other lipids including free fatty acids, compared to either BALB/cJ *Npc1*^{+/+} or *Npc1*^{-/-} mice when fed a low-fat diet (Garver et al., 2007b). This increase in liver triacylglycerol was present without differences in the concentration of liver enzymes suggesting normal liver function. In support of these results, previous studies have reported that BALB/cJ mice are inherently predisposed to an accumulation of liver lipid in the absence of liver pathology compared to mice from other genetic backgrounds, including the C57BL/6J mouse (Nishikawa et al., 2007, 2012). Therefore, although the mechanism remains undefined, the results clearly indicate that BALB/cJ *Npc1*^{+/-} mice are inherently predisposed to an accumulation of liver triacylglycerol. Our earlier studies have also demonstrated that BALB/cJ *Npc1*^{+/-} mice have an unexplained activation of the liver SREBP-1 pathway marked by significantly increased amounts of precursor and mature SREBP-1 protein compared to BALB/cJ *Npc1*^{+/+} mice, which may serve to increase expression of key genes involved in hepatic lipogenesis and provide an explanation for the development of hepatic steatosis (Garver et al., 2007b).

The C57BL/6J *Npc1* mouse model was produced by backcrossing the BALB/cJ *Npc1* null mutant allele onto the C57BL/6J normal or wild-type background for 15 generations. Similar to our previous results using the BALB/cJ *Npc1* mouse model, the results indicated no significant difference in body weight, liver weight, or adipose weight when this mouse model was fed a low-fat diet. However, in contrast to BALB/cJ *Npc1*^{+/-} mice, the C57BL/6J *Npc1*^{+/-} mice had significantly increased concentrations of blood glucose at 15, 30, and 60 min during the glucose tolerance test compared to C57BL/6J *Npc1*^{+/+} mice. Consistent with this result, we previously reported impaired glucose tolerance among hybrid BALB/cJ-C57BL/6J *Npc1*^{+/-} mice compared to BALB/cJ-C57BL/6J *Npc1*^{+/+} mice when fed either a low-fat or high-fat diet, but not in the absence of other physical and metabolic features that usually accompany this phenotype (Jelinek et al., 2010b). The only other metabolic abnormalities identified among C57BL/6J *Npc1*^{+/-} mice compared to C57BL/6J *Npc1*^{+/+} mice were an accumulation of liver free fatty acids and hepatic lipotoxicity marked by elevated ALT that has previously been shown to be associated with increased hepatic glucose production and impaired glucose tolerance (Lam et al., 2003; Larter et al., 2008). Although unexplained and hypothesized to be responsible for the different metabolic phenotypes, the C57BL/6J *Npc1*^{+/-} mice liver displays an SREBP-1 pathway marked by significantly decreased amounts of *Srebp-1* mRNA, consistent with the significantly increased concentration of liver free fatty acid known to inhibit LXR α , which serves as a transcription factor for the *Srebp-1* gene (Ou et al., 2001; Yoshikawa et al., 2002). In addition to sterols (cholesterol and oxysterol) that inhibit the SREBP cleavage activating protein (SCAP) and insulin-induced gene (INSIG) protein, free fatty acids have been shown to inhibit the UBXD8 protein (a fatty acid-sensing protein that prevents degradation of

INSIG) to potentiate feedback inhibition at the post-translational level and further decrease production of liver SREBP-1 protein (Lee et al., 2010; Shimomura et al., 1997). Together, the resulting decreased amounts of SREBP-1 protein may therefore serve as a possible mechanism for decreasing transcription of the *Srebp-1* gene and respective target genes through an “autoloop regulatory circuit” (Amemiya-Kudo et al., 2000; Takeuchi et al., 2010). The results also indicated significantly decreased amounts of *Ppara* mRNA in the liver, the respective gene of which encodes the PPAR α protein which serves as a master regulator of liver lipid metabolism, including transcription factor for the *Srebp-1* gene (Rakhshandehroo et al., 2010). Moreover, since PPAR α regulates the expression of liver genes involved in mitochondrial and/or peroxisomal fatty acid β -oxidation, decreased expression of *Ppara* gene in the livers of C57BL/6J *Npc1*^{+/-} mice provides a plausible explanation for the accumulation of free fatty acids. In this regard, it is important to emphasize that our earlier study indicated no significant difference in the relative amounts of LXRA or PPAR α protein between BALB/cJ *Npc1*^{+/+} and *Npc1*^{+/-} mice when fed a low-fat diet (Garver et al., 2007b).

In conclusion, the human *NPC1* gene has now been reported to be associated with three different metabolic diseases (NPC1 disease, obesity, and type 2 diabetes) depending upon the specific *NPC1* gene variant. We have now used two different *Npc1* mouse models characterized with decreased *Npc1* gene dosage that represent a loss-of-function for the encoded NPC1 protein to demonstrate that the *Npc1* gene interacts with undefined nutritional components (high-fat diet) and/or modifying genes to substantiate these disparate human metabolic diseases. It is becoming increasingly clear that our understanding of the NPC1 protein in maintaining cellular, tissue, and whole body lipid homeostasis and predisposition to human metabolic diseases remains undefined and therefore further studies are necessary to determine the mechanism for disparate phenotypes.

Acknowledgments

This project was supported in part by the Diabetes Research Center at the University of Washington through grant number P30 DK017047 as an institutional affiliate, and a grant from the Tohono O'odham Nation, and private donations for the investigation of childhood genetic and metabolic diseases.

References

- Al-Daghri NM, et al. Mammalian *NPC1* genes undergo positive selection and human polymorphisms associate with type 2 diabetes. BMC Med. 2012; 10:140. [PubMed: 23153210]
- Amemiya-Kudo M, et al. Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. J Biol Chem. 2000; 275:31078–31085. [PubMed: 10918064]
- Chen FW, Gordon RE, Ioannou YA. NPC1 late endosomes contain elevated levels of non-esterified ('free') fatty acids and an abnormally glycosylated form of the NPC2 protein. Biochem J. 2005; 390:549–561. [PubMed: 15896196]
- Cotsapas C, et al. Common body mass index-associated variants confer risk of extreme obesity. Hum Mol Genet. 2010; 19:3690–3691.
- den Hoed M, et al. Evaluation of common genetic variants identified by GWAS for early onset and morbid obesity in population-based samples. Int J Obes. 2012; 34:1–6.
- Garver WS, et al. The National Niemann-Pick C1 disease database: report of clinical features and health problems. Am J Med Genet. 2007a; 143A:1204–1211. [PubMed: 17497724]

- Garver WS, et al. Characterization of liver disease and lipid metabolism in the Niemann–Pick C1 mouse. *J Cell Biochem.* 2007b; 101:498–516. [PubMed: 17216601]
- Garver WS, et al. The National Niemann–Pick Type C1 disease database: correlation of lipid profiles, mutations, and biochemical phenotypes. *J Lipid Res.* 2010; 51:406–415. [PubMed: 19744920]
- Jelinek D, Heidenreich RA, Erickson RP, Garver WS. Decreased *Npc1* gene dosage in mice is associated with weight gain. *Obesity.* 2010a; 18:1457–1459. [PubMed: 19910939]
- Jelinek D, Millward V, Birdi A, Trouard TP, Heidenreich RA, Garver WS. *Npc1* haploinsufficiency promotes weight gain and metabolic features associated with insulin resistance. *Hum Mol Genet.* 2010b; 20:312–321. [PubMed: 21036943]
- Jelinek D, Castillo JJ, Richardson LM, Luo L, Heidenreich RA, Garver WS. The Niemann–Pick C1 gene is downregulated in livers of C57BL/6J mice by dietary fatty acids, but not dietary cholesterol, through feedback inhibition of the SREBP pathway. *J Nutr.* 2012; 142:1935–1942. [PubMed: 22990467]
- Katz A, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab.* 2000; 85:2402–2410. [PubMed: 10902785]
- Lam TKT, Carpentier A, Lewis GF, van de Werve G, Fantus IG, Giacca A. Mechanisms of the free fatty acid-induced increase in hepatic glucose production. *Am J Physiol Endocrinol Metab.* 2003; 284:E863–E873. [PubMed: 12676648]
- Larter CZ, et al. Hepatic free fatty acids accumulate in experimental steatohepatitis: role of adaptive pathways. *J Hepatol.* 2008; 48:638–647. [PubMed: 18280001]
- Lee JN, Kim H, Yao H, Chen Y, Weng K, Ye J. Identification of Ubx8 protein as a sensor for unsaturated fatty acids and regulator of triglyceride synthesis. *Proc Natl Acad Sci U S A.* 2010; 107:21424–21429. [PubMed: 21115839]
- Liscum L, Ruggiero RM, Faust JR. The intracellular transport of low density lipoprotein-derived cholesterol is defective in Niemann–Pick type C fibroblasts. *J Cell Biol.* 1989; 108:1625–1636. [PubMed: 2715172]
- Loftus SK, et al. Murine model of Niemann–Pick C disease: mutation in a cholesterol homeostasis gene. *Science.* 1997; 277:232–235. [PubMed: 9211850]
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985; 28:412–419. [PubMed: 3899825]
- Meyre D, et al. Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat Genet.* 2009; 41:157–159. [PubMed: 19151714]
- Nishikawa S, Yasoshima A, Doi K, Nakagawa H, Uetsuka K. Involvement of sex, strain and age factors in high fat diet-induced obesity in C57BL/6J and BALB/c mice. *Exp Anim.* 2007; 56:263–272. [PubMed: 17660680]
- Nishikawa S, Sugimoto J, Okada M, Sakairi T, Takagi S. Gene expression in livers of BALB/c and C57BL/6J mice fed a high-fat diet. *Toxicol Pathol.* 2012; 40:71–82. [PubMed: 22105644]
- Ou J, et al. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of LXR. *Proc Natl Acad Sci U S A.* 2001; 98:6027–6032. [PubMed: 11371634]
- Pentchev PG, Boothe AD, Kruth HS, Weintraub H, Stivers J, Brady RO. A genetic storage disorder in BALB/c mice with a metabolic block in esterification of exogenous cholesterol. *J Biol Chem.* 1984; 259:5784–5791. [PubMed: 6325448]
- Pentchev PG, et al. A defect in cholesterol esterification in Niemann–Pick disease (type C) patients. *Proc Natl Acad Sci U S A.* 1985; 82:8247–8251. [PubMed: 3865225]
- Pentchev PG, Comly ME, Kruth HS, Patel S, Proestel M, Weintraub H. The cholesterol storage disorder of the mutant BALB/c mouse: a primary genetic lesion closely linked to defective esterification of exogenously derived cholesterol and its relationship to human type C Niemann–Pick disease. *J Biol Chem.* 1986; 261:2772–2777. [PubMed: 3949747]
- Rakhshandehroo M, Knoch B, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. *PPAR Res.* 2010; 2010:612089. [PubMed: 20936127]

- Scullion P, Edwards D, McKinnon H, Miller S, Watson D, MacIntyre L. Analysis of changes in triacylglycerol ratios in mouse liver and plasma in response to a liver X receptor agonist. *Metabolomics*. 2012; 8:74–85.
- Shimomura I, Bashmakov Y, Shimano H, Horton JD, Goldstein JL, Brown MS. Cholesterol feeding reduces nuclear forms of sterol regulatory element-binding proteins in hamster liver. *Proc Natl Acad Sci U S A*. 1997; 94:12354–12359. [PubMed: 9356453]
- Takeuchi Y, et al. Polyunsaturated fatty acids selectively suppress sterol regulatory element-binding protein-1 through proteolytic processing and autoloop regulatory circuit. *J Biol Chem*. 2010; 2010:11681–11691. [PubMed: 20145241]
- Xie C, Turley SD, Dietschy JM. Cholesterol accumulation in tissues of the Niemann–Pick type C mouse is determined by the rate of lipoprotein-cholesterol uptake through the coated-pit pathway in each organ. *Proc Natl Acad Sci U S A*. 1999a; 96:11992–11997. [PubMed: 10518564]
- Xie C, Turley SD, Pentchev PG, Dietschy JM. Cholesterol balance and metabolism in mice with loss of function of Niemann–Pick C protein. *Am J Physiol*. 1999b; 276:E336–E344. [PubMed: 9950794]
- Yoshikawa T, et al. Polyunsaturated fatty acids suppress sterol regulatory element-binding protein 1c promoter activity by inhibition of liver X receptor (LXR) binding to LXR response elements. *J Biol Chem*. 2002; 277:1706–1711.

Abbreviations

ALT	alanine amino-transferase
AST	aspartate aminotransferase
GWAS	genome-wide association study
HOMA-β%	homeostasis model assessment-β, cell function percentile
HOMA-IR	homeostasis model assessment-insulin resistance index
INSIG	insulin-induced gene
IPGTT	intraperitoneal glucose tolerance test
IPITT	intraperitoneal insulin tolerance test
LXRα	liver X receptor α
NPC1	Niemann–Pick C1
PCR	polymerase chain reaction
PPARα	, peroxisome-proliferator activated receptor α
QUICKIE	quantitative insulin sensitivity check index
SCAP	SREBP cleavage activating protein
SNP	single nucleotide polymorphism
SREBP	sterol regulatory element-binding protein

Fig. 1.

Change in body weight growth curves and glucose tolerance tests for the BALB/cJ and C57BL/6J *Npc1* mouse models fed a low-fat diet. The change in body weight for the BALB/cJ *Npc1*^{+/+} and *Npc1*^{+/-} mice (A) and C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice (B) weighed on a weekly basis from 28 to 77 days of age. The glucose tolerance tests for the BALB/cJ *Npc1*^{+/+} and *Npc1*^{+/-} mice (C) and C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice (D) at 70 days of age using the concentration of blood glucose (D). The glucose tolerance tests for the BALB/cJ *Npc1*^{+/+} and *Npc1*^{+/-} mice (E) and C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice (F) at 70 days of age using area under the curve (AUC). Values are represented as mean $\pm$ SE ($n = 10$). An asterisk indicates a significant difference ($P < 0.05$).

Fig. 2.

Body weight, liver weight, adipose weight, and concentration of liver lipids for the C57BL/6J *Npc1* mouse model fed a low-fat diet. The body weight (A), liver weight (B), and adipose weight (C) for the C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice weighed at 84 days of age after sacrifice. The concentration of liver total cholesterol (cholesterol and cholesteryl ester) (D), triacylglycerol (E), and free fatty acids (F) for the C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice. Values are represented as mean $\pm$ SE ($n = 10$). An asterisk indicates a significant difference ($P < 0.05$). TC, total cholesterol; TAG, triacylglycerol; FFA, free fatty acids.

Fig. 3.

Relative amounts of nuclear receptor and transcription factor mRNA in the liver, adipose, and muscle of C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice fed a low-fat diet. The relative amounts of *Lxr* mRNA (A), *Ppara* mRNA (B), *Srebp-1* mRNA (C), and *Srebp-2* mRNA (D) in the liver, adipose, and muscle of C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice. Values are represented as mean $\pm$ SE ($n = 10$). An asterisk indicates a significant difference ($P < 0.05$).

Table 1Plasma profile of the C57BL/6J *Npc1* mouse model.

<i>Npc1</i> genotype	+/+	+/-
Glucose (mmol/L)	8.72 ± 0.39	9.18 ± 0.34
Insulin (nmol/L)	0.13 ± 0.01	0.15 ± 0.02
HOMA-IR	0.25 ± 0.03	0.31 ± 0.05
HOMA-β, %	2.48 ± 0.13	2.57 ± 0.28
QUICKIE	1.43 ± 0.11	1.32 ± 0.11
Total cholesterol (mmol/L)	2.27 ± 0.09	2.38 ± 0.07
Triacylglycerol (mmol/L)	0.84 ± 0.03	0.94 ± 0.07
Free fatty acids (mmol/L)	0.75 ± 0.06	0.74 ± 0.07
ALT (U/L)	24.3 ± 2.2	48.7 ± 8.9*
AST (U/L)	109 ± 8.4	113 ± 7.3

Values are represented as mean ± SE (*n* = 10) for mice at 84 days of age. An asterisk indicates a significant difference (*P* < 0.05). ALT, alanine aminotransferase. AST, aspartate aminotransferase.