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Genetic variation in the mouse model of Niemann Pick C1 affects female, as well as male, adiposity, and hepatic bile transporters but has indeterminate effects on caveolae

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Abstract

We have previously shown that male *Npc1* heterozygous mice (*Npc1*^{+/-}), as compared to homozygous wild-type mice (*Npc1*^{+/+}), both maintained on the —lean BALB/cJ genetic background, become obese on a high fat but not on a low fat diet. We have now extended this result for female heterozygous mice. When fed high-fat diet, the *Npc1*^{+/-} white adipose weight is also increased in females, therefore following the same trend as males. Bile transporters which had previously been found to be altered in *Npc1*^{-/-} mice on a high fat diet, showed related, but small, changes in mRNA levels but large changes in protein expression. We have addressed the possible role of caveolae in these differences. It has long been known that caveolin 1 is increased in the liver (sex not specified) of *Npc1*^{+/-} (compared to *Npc1*^{+/+} and *Npc1*^{-/-}) mice and in heterozygous cultured skin fibroblasts of *NPC1* carriers. We now find that caveolin 1 is increased in male, but not female liver and female, but not male adipose tissue. The caveolin 1 increase was not accompanied by changes in another caveolar protein, polymerase 1 and transcript release factor (Ptrf). The numbers of caveolae in female adipose cells could not be correlated with levels of caveolae. Thus, we conclude that *Npc1* affects female as well as male obesity and bile transporters but that effects on caveolin 1 are not discernible.

Keywords

obesity; white adipose tissue; caveolin 1; polymerase 1 and transcript release factor

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1. Introduction

Niemann-Pick disease type C (NPC) is a neurodegenerative disorder that usually presents in the first decade of life [Patterson, et al, 2001]. The classic presentation of NPC is a child of either sex developing coordination problems, dysarthria, and hepatosplenomegaly during early school-age years. The neurological progression of the disorder is relentless and is characterized by increasing severity of ataxia, developmental dystonia, and dementia, until death supervenes, usually during the second decade of life. Identification of the major gene responsible for the disorder, *NPC1/Npc1*, revealed one coding for a multipass transmembrane protein containing a sterol-sensing domain that shows homology to the genes for Patched, HMG CoA reductase, and SCAP (SREBP cleavage activating protein) [Carstea, et al, 1997; Loftus, et al, 1997]. Analysis of NPC1 protein function suggests that it is involved in late endosomal lipid sorting/vesicular trafficking [Garver, et al, 2000; Higgins, et al, 1999; Neufeld, et al, 1999]. The fundamental role of NPC1 in intracellular lipid transport, and the severe visceral disease sometimes causing death before the onset of neurodegeneration, established the idea that lipid accumulation is the primary defect in NPC and leads to secondary neurological impairment.

We have previously reported that mice homozygous recessive for *Npc1* develop severe liver disease, with altered levels of bile transporters, when fed a high fat, 1% cholesterol diet [Erickson, et al, 2005]. We did not study the effects of heterozygosity for *Npc1* at that time. Our interest in heterozygous effects was stimulated by Meyre, et al's finding [2009] that common variants in the human gene were associated with early onset and morbid obesity. It was not known whether these variants increased or decreased NPC1 function. We found that heterozygous *Npc1*^{+/-} male mice, when fed a high fat diet, deposited more fat and were heavier than their wild-type siblings [Jelinek, et al, 2010].

Previously we had also shown that caveolin 1 was increased in NPC1 variants, both man and mouse, mostly in heterozygotes, but to a lesser degree in homozygotes. There are multiple lines of evidence implicating caveolin 1 and caveolae in the regulation of lipid metabolism. The mouse knockout (KO) for caveolin 1 has a lean phenotype [Razinié, et al, 2002] and caveolin-1 is upregulated in adipose tissue from obese individuals [Catalan, et al, 2008]. Mechanisms for this relationship have been postulated. The adipocyte cell surface insulin receptor is found in caveolae [Gustavsson, et al, 1999]. Not only is the insulin receptor located in caveolae, but insulin-induced translocation of glucose transporter proteins (GLUT4) from intracellular stores to the plasma membrane leads to their location in caveolae [Scherer, et al, 1994]. Caveolin 1 deficient mice become insulin resistant [Cohen, et al, 2003]. The finding that hexokinase localizes with glucose transporters in caveolae suggests the possibility that glucose can also be metabolized in caveolae [Rauch, et al, 2006]. Another major class of metabolites, well-documented to be taken up by cells through caveolae, are fatty acids. Caveolin 1 itself is a fatty acid binding protein [Trigatti, et al, 1999]. The fatty acid transport proteins 1, 4 and fatty acid translocase are other proteins that bind fatty acids in caveolae [Rauch, et al, 2006; Ortegren, et al, 2006 Ring, et al, 2006]. Thus, we have also explored the possibility that increases in caveolin 1 found in heterozygous female adipose tissue could be causal for the adiposity phenotype by increasing the number of caveolae.

2. Materials and Methods

2.1 Mice

BALB/cJ *Npc1*^{inh} heterozygous (*Npc1*^{+/-}) mice were bred to generate *Npc1* wild-type (*Npc1*^{+/+}) mice and *Npc1* heterozygous (*Npc1*^{+/-}). The mice were maintained at the

University of Arizona Animal Care Facility, with 12 hr light, 12 hr dark cycles and water *ad libitum*.

2.2 Genotype analysis

At the time of weaning (21 days), the *Npc1* mice were genetically identified using PCR at the *Npc1* locus as previously described [Loftus, et al, 1997]. In brief, PCR amplification was used with 20–40 ng of DNA (prepared from tail-tips) with amplification 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 for 10 min at 72 °C. The PCR products were separated using a 1.5% agarose gel and visualized with a UV light.

2.3 Study protocol

One week after weaning (28 days of age), the female *Npc1*^{+/+} and *Npc1*^{+/-} mice were weighed and placed on either a regular/low-fat diet (18% kcal [6.2% wt.] fat, NIH-301) or high-fat diet (45% kcal [24% wt.] fat, Diet-07021302) produced by Research Diets (New Brunswick, NJ). As previously reported, compared to *Npc1*^{+/+} mice, the *Npc1*^{+/-} mice express one-half the normal amounts of functional Npc1 protein, thereby providing a model to investigate decreased Npc1 gene dosage in the absence of disease [Garver, et al, 2000; Garver, et al, 2007]. The *Npc1*^{+/+} and *Npc1*^{+/-} mice were weighed every week from 5 to 25 weeks of age. At about 30 weeks of age, the *Npc1*^{+/+} and *Npc1*^{+/-} mice fed either the regular or the high-fat diet were killed and the liver weights and the ovarian fat pad weights were determined. Livers were snap frozen in liquid N₂.

2.4 Bile transporter gene expression

Multidrug resistance protein (Mrp) 1, Mrp2, Mrp 3, Mrp5, Na⁺-taurocholic co-transporting polypeptide (Ntcp), Bile salt export pump (Bsep), and Organic anion transport protein 2 (Oatp2) were measured by the branched DNA signal amplification test as previously described [Erickson, et al 2005]. Briefly, total RNA was prepared from snap-frozen liver using RNazol B reagent (Tel-Test, Friendswood, TX). The quality of the RNA samples was judged by gel electrophoresis, monitoring the integrity and ratio of 28S and 18S bands. Using QuantiGene Discover kit (Genospectra, Fremont, CA) reagents, 10 ug of total RNA was hybridized overnight at 37 °C in 96-well plates with blocker, capture and label probes for each transporter—sequences are provided in Erickson, et al [2005] and references therein. After post-hybridization washes, luminescence was measured with the Quantiplex 320 branched DNA Luminometer (Bayer Diagnostics) interfaced with the Quantiplex Data Management Software version 5.02 (Bayer Diagnostics).

2.5 Western blotting

Levels of bile transporters Ntcp, OatP2, and Mrp2 were determined in liver, caveolin 1 was determined in liver and white adipose tissue, and Ptrf only in white adipose tissue, of the *Npc1*^{+/-} and *Npc1*^{+/+} females and *Npc1*^{+/+} males at 30 weeks of age, by Western blots. Whole cell lysate preparations of mouse liver tissue were prepared from tissue homogenized in NP-40 buffer (20 mM Tris HCl, 137 mM NaCl, 10% glycerol, 1% nonidet P-40, and 2 mM EDTA with 1 Protease Inhibitor Cocktail Tablet (Roche, Indianapolis, IN) per 50mL) at 4°C. Homogenized tissue was then agitated at 4°C for 2 hours, centrifuged at 10,000 x g for 30 minutes, and the supernatant transferred to a clean collection tube. The relative amounts of protein were determined using immunoblot analysis. Protein samples were separated using 7% (transporters) or 14% (caveolin 1 and Ptrf) SDS-PAGE under reduced conditions and then transferred to a PVDF (transporters) or nitrocellulose (caveolin 1 and Ptrf) membrane. In brief, blocking buffer (10mM sodium phosphate pH 7.4, 150 mM NaCl, 0.05% Tween 20, and 5% non-fat dry milk) was used to block non-specific sites on the

nitrocellulose membrane (2h). The membranes were then incubated in blocking buffer containing the appropriate dilution of primary antibody (4°C for 16 hr). The membranes were rinsed with blocking buffer (3 x 10 min) to remove residual primary antibody and then incubated in blocking buffer containing the appropriate dilution of peroxidase-conjugated secondary antibody (90 min). The membranes were rinsed with blocking buffer (3 x 10 min) to remove residual secondary antibody and enhanced chemiluminescence (ECL) was performed to obtain autoradiograms. The relative amounts of caveolin 1 and Ptrf protein and endogenous control protein (β -actin) were quantified within the linear range of film using a BioRad Model GS-700 Imaging Densitometer. The antibody for caveolin 1 (clone pAb) was purchased from BD Biosciences (San Jose, CA) and the antibody for Ptrf was from Abcam (Cambridge, MA). For bile transporters, the following antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) and used to determine relative protein levels: Mrp2 (H-17, 1:1000), Ntcp (M-130, 1:5000), and Oatp2 (N-16, 1:1000). Quantification of relative protein expression was determined using image processing and analysis with Image J software (NIH, Bethesda, MD) and normalized to Gapdh (1:7000, Santa Cruz Biotechnology, Santa Cruz, CA).

2.6 Transmission electron microscopy

Portions of the adipose tissue samples from 30 week old females were minced to several mm² cubes and fixed in Karnovsky's. After overnight fixation, they were rinsed in Karnovsky's storage buffer. The specimens were flat embedded in eponate resin. Areas of interest were cut and mounted on cylinders for further sectioning and imaging. Numbers of caveolae were determined by manual counts per areas ranging from 1,850 – 2,100 μ M². They were expressed per 670 μ M².

3. Results

3.1 Weight gain in female mice

No significant differences in weight curves were found between female *Npc1*^{+/-} and *Npc1*^{+/+} mice when fed a regular diet (18% of calories from fat) although there was a trend for them to be lighter at 19–23 weeks of age (Fig 1A). However, when placed on the high fat diet (45% of calories from fat), female *Npc1*^{+/-} were heavier than female *Npc1*^{+/+} mice from week 9 onwards and the difference had become significant from 18 weeks onwards (Fig 1B). At 30 weeks of age, the *Npc1*^{+/-} mice on the high fat diet had a significantly increased (23.3%, $p = 0.0022$) body weight compared to *Npc1*^{+/+} mice fed the same high-fat diet. This was associated with increased white adipose tissue weight but not liver weight (Table 1). The females on the regular diet did not show differences in these parameters.

3.2 Bile transporter expression

Of the 7 bile transporter mRNAs quantified (Mrp1, Mrp2, Mrp3, Mrp5, Ntcp, Bsep, Oatp2), only 3 showed significant differences (Mrp2, Ntcp, Oatp2) (Fig 2). Mrp2 mRNA, as previously reported, had been elevated in homozygous affected (*Npc1*^{-/-}) mice [Erickson, et al, 2005], more so with a high-fat diet. There was a slight, but significant, decrease in Mrp2 mRNA in *Npc1*^{+/+} females fed a high-fat diet compared to *Npc1*^{+/+} females fed a regular diet. When compared to males on a regular diet, Mrp2 mRNA was also decreased in *Npc1*^{+/+} females fed a high-fat diet but the difference was not significant ($p = 0.08$) (Fig 2A). At the protein level, Mrp2 was markedly decreased in females of either genotype fed the high fat diet (Fig 3A). Moreover, Mrp2 protein was decreased in both female genotypes fed the high fat diet and *Npc1*^{+/-} females fed a regular diet as compared to males on a regular diet (Fig 3A). Ntcp mRNA had also been elevated in *Npc1*^{-/-} on regular diet and decreased with the high-fat diet in *Npc1*^{-/-} females [Erickson, et al, 2005]. In the current work, the *Npc1*^{+/+} females fed a regular diet showed a significant increase in Ntcp mRNA

compared to all studied groups. There was a significant decrease in *Ntcp* mRNA in both *Npc1*^{+/+} and *Npc1*^{+/-} females when fed the high-fat diet (Fig 2B). Additionally, *Ntcp* protein levels were remarkably decreased in females of both genotypes when fed the high fat diet, and *Ntcp* protein levels were greatly diminished in all females compared to males (Fig 3B). *Oatp2*, which had the same levels of mRNA expression as *Ntcp* in *Npc1*^{-/-} [Erickson, et al, 2005], showed no differences in the pattern of gene expression between the heterozygous and homozygous wild-typed females on either diet while all four female groups showed a significant increase in mRNA compared to regular diet fed *Npc1*^{+/+} males (Fig 2C). Similar to *Ntcp*, *Oatp2* protein levels were markedly decreased in females of both genotypes fed the high fat diet (Fig 3C). As well, females of both genotypes fed the high fat diet exhibited decreased protein levels of *Oatp2* compared to males (Fig 3C).

3.3 Caveolin 1 and *Ptrf* levels in liver and adipose tissue

The studies performed more than a decade ago, and for which laboratory records are no longer available, which reported increased caveolin 1 levels in *Npc1*^{+/-} mouse liver (Introduction) did not state the sex of the mice. They were likely to be males since males are not affected by estrous cycle which alters many aspects of metabolism. We now find that caveolin 1 is significantly elevated in *Npc1*^{+/-} male liver, but not *Npc1*^{+/-} female liver when compared to the *Npc1*^{+/+} same sex animals fed the same regular diet (Fig 4A). Caveolin 1 levels in female liver for both *Npc1*^{+/-} and *Npc1*^{+/+} are comparable to male *Npc1*^{+/-} levels. On the other hand, the female *Npc1*^{+/-} caveolin 1 levels in white adipose tissue are significantly higher when compared to those of *Npc1*^{+/+} females. We did not detect any difference between *Npc1*^{+/+} and *Npc1*^{+/-} males for white adipose tissue levels of caveolin 1 (Fig 4B). Polymerase 1 and transcript release factor (*Ptrf*, another caveolae marker) levels were not different between the sexes or genotypes in white adipose tissue (levels were below our limits of detection in liver) (Fig 4C).

3.4 Caveolar numbers

As seen in Fig 5, caveolae are much more abundant in adipose tissue than in liver. Thus caveolar numbers and diameters were compared for the adipose tissue in which elevated levels of caveolin 1 were found in heterozygotes, i.e. in female white adipose tissue. There were no significant differences in number of caveolae or their diameter (Table 2) but the numbers were highly variable.

4. Discussion

4.1 NPC1 and obesity

We originally studied the effects of a high fat diet on homozygous recessive *Npc1*^{-/-} mice which have liver disease as well as the neurodegenerative disease. This work was stimulated since some cases of NPC1 may be missed because of death from cholestatic liver disease before development of neurological disease [Kelly, et al, 1993]. In fact, one study concluded that NPC is the second most common genetic cause of liver disease in infancy in the United Kingdom [Mieli-Vergani, et al, 1991]. A recent survey found that NPC explained 27% of idiopathic neonatal cholestasis and 8% of all infants evaluated for cholestasis [Yerushalmi, et al 2002].

Our interest in the effects of heterozygosity for *Npc1* on obesity followed the findings of a Genome Wide Association Study (GWAS) implicating variation in NPC1 with early-onset (<6 yrs.) and morbid (BMI >40) adult obesity [Meyre, et al, 2009]. The authors performed a genome-wide association study on nearly 1,400 obese Europeans compared to a similar number of age matched, normal-weight controls. The results were then confirmed with 2,100 obese and 2,400 non-obese individuals. The positively associated non-synonymous

single nucleotide polymorphism (SNP) variant (rs1805081) results in the replacement of an arginine for a histidine at amino acid 215 (H215R). Histidine 215 is the more common allele and is the allele associated with lower risks of early onset morbid obesity [Meyre, et al, 2009].

Two other SNPs in NPC1 were also correlated with this finding. Ikonen's group [Uronen, et al, 2010] found that intronic and 3' SNPs in NPC1 significantly influenced serum triglyceride levels. Previous data had shown that heterozygous NPC1 mice had a significant accumulation of triglyceride [Garver, et al 2007]. This was a somewhat surprising finding since the homozygous recessive mice with severe liver disease had normal triglyceride levels [Garver, et al, 2007] while others reported lower than normal levels [Uronen, et al, 2003]. We had not previously characterized weight gain and general adiposity in heterozygotes. When we did, we found that male *Npc1*^{+/-} mice, which express one-half the normal amount of functional Npc1 protein, had no significant difference in growth rate or weight gain when fed a low-fat diet but had a significantly increased growth rate and weight gain when fed a high fat diet [Jelinek, et al, 2010]. The mice also had a significantly increased liver weight and epididymal pad adipose weight. These studies were performed on the Balb/cJ genetic background. We now show similar results for female mice which may have differed because there are many sex effects on weight gain [Louet, et al, 2004; Gao & Horvath, 2008].

We have previously studied the liver disease in the *Npc1*^{-/-} mice. Serum alanine aminotransferase was elevated in *Npc1*^{-/-} mice on a regular diet and became markedly elevated with a high fat diet [Erickson, et al, 2005]. Non-zone dependent diffuse fibrosis was found in mice surviving the high fat diet (18% fat, 1% cholesterol) suggestive of non-alcoholic fatty liver disease (NAFLD). Bile duct function is altered in NAFLD [Fisher, et al, 2009] and was altered in *Npc1*^{-/-} with increases in gamma-glutamyl transferase and alterations in bile transporters: Mrps 1–5 were elevated in *Npc1*^{-/-} liver and became more elevated with the high fat diet; Ntcp, Bsep, and Oatp2 were also elevated in *Npc1*^{-/-} liver but were suppressed by the high fat diet [Erickson, et al, 2005]. This previous work has been confirmed and amplified. The cholesterol accumulation with high fat diet in *Npc1*^{-/-} mice correlated with cell death [Beltroy, et al, 2007] and could be partially prevented with ezetimibe therapy [Beltroy, et al, 2007; Zheng, et al, 2008].

4.3 NPC1 and NAFLD

NAFLD is rapidly becoming a major health problem [Barshop, et al, 2008], and the liver disease in *Npc1*^{-/-} mice provides a partial model. Since bile duct transporters were altered in the homozygous recessive mice and are known to be altered in NAFLD, we studied them in the heterozygotes. We now find that Mrp2 mRNA showed significant differences between high fat versus normal diet in *Npc1*^{+/+} females (p<0.05) but were not as greatly elevated as in *Npc1*^{-/-} on a high fat diet. However, the protein levels determined by Western blots were markedly depressed in the females of both genotypes fed the high fat diet. The mRNA for Ntcp, which transports bile acids from portal blood into hepatocytes, was also elevated in *Npc1*^{-/-} on a regular diet [Erickson, et al, 2005] but decreased with high fat diet as it does in female *Npc1*^{+/+} liver. In our current work, high fat diet caused a marked decrease in the protein level of this transporter in females of both genotypes. Oatp2 mRNA had shown a pattern similar to that of Ntcp in *Npc1*^{-/-} on a high fat diet [Erickson, et al, 2005] while it is now found to be consistently elevated in females compared to males regardless of the diet. At the protein level, it, too, was markedly lowered by the high fat diet treatment. Overall, the changes do not suggest a consistent pattern related to the increased weight gain of *Npc1*^{+/-} compared to *Npc1*^{+/+} mice since both genotypes shared small changes in mRNA levels and large changes in protein levels induced by the high fat diet. The effects of similar changes in transporter function with a high fat diet (non-alcoholic fatty liver disease model) on

bromosulphophthalein disposition have been well established in animal models [Fisher, et al, 2009].

There are many post-translational modifications of bile transporters: Ntcp by phosphorylation [Anwer, et al, 2005] and ubiquitination, which leads to proteosomal degradation [Kuhlkamp, et al, 2005]; Mrp2 by SUMOylation, which confers stability to the protein [Minami, et al, 2009]; and Mrp2 by glycosylation which, if incomplete, prevents routing of the protein to the membrane and may lead to degradation [Zhang, et al, 2005]. Genetic translational regulation is only well studied for Mrp2 [Zhang, et al, 2007; Zhang, et al, 2010]. This was first found following PXR stimulation with pregnenolone-16 α -carbonitrile (PCN [Johnson, et al, 2002]) and was found to be due to *de novo* protein synthesis without changes in mRNA levels [Jones, et al, 2005].

4.4 NPC1, caveolin 1, and possible links to obesity

Using the BALB/c murine model for NPC1 disease, we previously found that the expression of caveolin 1 in total liver homogenates from *Npc1*^{+/-} and *Npc1*^{-/-} animals was altered [Garver, et al 1997a]. Immunoblot analysis of liver homogenates from *Npc1*^{+/-} mice revealed that caveolin 1 was significantly ($p<0.005$) elevated, 4.9 fold, compared to normal mice. Total liver homogenates from *Npc1*^{-/-} mice also had a significant ($p<0.05$) increase in caveolin 1 expression, 1.6 fold, compared to normal *Npc1*^{+/+} mice. Immunohistochemical staining of liver cross-sections revealed that the increased caveolin 1 was localized to sinusoidal endothelial cells in heterozygous mice [Garver, et al, 1997a]. The Triton insoluble floating fraction (TIFF) i.e. lipid rafts, was isolated using liver from each genotype and analyzed for caveolin 1 expression. Caveolin 1 in the TIFF from *Npc1*^{+/-} mice was significantly ($p<0.01$) elevated, 1.8 fold, compared to *Npc1*^{+/+} and *Npc1*^{-/-} animals; while *Npc1*^{+/+} and *Npc1*^{-/-} animals, were not significantly different from each other.

We also studied caveolin 1 levels in cultured human skin fibroblasts [Garver, et al, 1997b]. Caveolin 1 in heterozygous fibroblasts was significantly elevated, as much as 10-fold compared to caveolin 1 in normal and homozygous affected fibroblasts. Homozygous NPC fibroblasts expressed caveolin 1 levels similar to those in normal fibroblasts, while the expression of caveolin 1 in homozygous Niemann-Pick type D (NPD, a milder form due to a hypomorphic mutation in *NPC1*) fibroblasts was slightly elevated. Northern analyses indicated that normal fibroblasts and NPC heterozygous fibroblasts have similar amounts of caveolin 1 mRNA, while NPC homozygous fibroblasts have significantly less caveolin 1 mRNA. In contrast, heterozygous and homozygous NPD fibroblasts exhibit increased levels of caveolin 1 mRNA.

Thus, because caveolae have a major role on fat metabolism (see Introduction) we tested the hypothesis that the increase in caveolin 1 would be reflected in an increased number of caveolae. Although the variation was extremely large, there were no significant differences in the numbers of caveolae in the *Npc1*^{+/-} and *Npc1*^{+/+} female white adipose tissue. There was also no difference in Ptrf levels which are another marker for caveolae. We have not studied muscle where similar metabolic changes are found in the sarcolemma, in which caveolin 3 is the form of caveolin predominantly associated with caveolae [Parton, et al, 1997].

In conclusion, we have shown that reduced *Npc1* levels are associated with increased adiposity in both sexes when the mice are fed a high fat diet. The adiposity is associated with changes in liver bile transporters but not with detectable changes in caveolar number or Ptrf levels.

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List of abbreviations

BSEP	bile salt export pump
ECL	enhanced chemiluminescence
GLUT4	glucose transporter 4
KO	knockout
Mrp	multidrug resistant protein
NAFLD	non-alcoholic fatty liver disease
Npc1	mouse Niemann-Pick C1 <i>gene</i> or protein
NPC1	Niemann-Pick C1 type disease
NPD	a milder form of NPC1
Ntcp	Na ⁺ -taurocholate co-transporter polypeptide
Oat	organic ion transport protein
PAGE	polyacrylamide gel electrophoresis
PCN	pregnenolone-16- α carbonitrile
Ptfr	polymerase and transcript release factor
PXR	pregnane X receptor
SDS	sodium dodecyl sulfate
SNP	single nucleotide polymorphism
TIFF	triton insoluble floating fraction

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Highlights

Female Npc1 heterozygotes, but not wild-type, mice become obese on a high fat diet

High fat diet up-regulated some bile acid transporters in female Npc1 heterozygotes

Changes in caveolar proteins or numbers did not explain the Npc1 obesity effects

Fig 1. Growth curves for female *Npc1*^{+/+} and *Npc1*^{+/-} mice from 5 to 25 weeks of age when fed a regular (18% kcal fat) diet (A) and a high-fat (45% kcal fat) diet (B). The groups were represented with 6-13 mice. * $p \leq 0.05$ when compared to *Npc1*^{+/+} mice fed the same diet.

Fig 2.

Relative amounts of Mrp2, Ntcp, and Oatp2 mRNA. Messenger RNA abundance for liver transporters Mrp2 (A) Ntcp (B) and Oatp2 (C) of female *Npc1*^{+/+} and *Npc1*^{+/-} fed either regular or high-fat diet along with regular diet fed *Npc1*^{+/+} male are presented. * $p \leq 0.05$ compared to regular diet fed *Npc1*^{+/+} male, ** $p \leq 0.05$ when compared to regular diet fed *Npc1*^{+/+} female. (n = 3), RLU, relative luminescence unit.

Fig 3.

Relative amounts of Mrp2, Ntcp, and Oatp2 protein as determined by Western blotting.

Relative protein abundance compared to Gapdh in livers for Mrp2 (A) Ntcp (B) and OatP2 (C) of female *Npc1*^{+/+} and *Npc1*^{+/-} fed either regular or high-fat diet along with regular diet fed *Npc1*^{+/+} male are presented. * $p \leq 0.05$ compared to regular diet fed *Npc1*^{+/+} male, ** $p \leq 0.05$ when compared to regular diet fed *Npc1*^{+/+} female. (n = 3)

Fig 4.

Relative amounts of caveolin-1 protein in 3 week old male and female *Npc1*^{+/+} and *Npc1*^{+/-} liver and caveolin-1 and Ptrf protein in white adipose tissue. The average amounts (mean $\pm$ SE) of caveolin-1 protein in liver (A), caveolin-1 protein in white adipose tissue (B), and Ptrf protein in white adipose tissue (C), for mice fed a regular diet are presented. The amounts of caveolin-1 and Ptrf protein were normalized to the corresponding amounts of β -actin (internal control). A representative immunoblot for the respective proteins was incorporated into graphs. * $p \leq 0.05$ when compared to *Npc1*^{+/+} same sex animals. (n = 3)

Fig 5. Electron microscopy of caveolae in liver (A, B) and gonadal white adipose tissue (C, D). A representative image of liver caveolae of *Npc1*^{+/+} (A) and *Npc1*^{+/-} mice fed a regular diet (B), and white adipose tissue of *Npc1*^{+/+} (C) and *Npc1*^{+/-} mice fed a high fat diet (D) are presented. Arrows indicate plasma membranes while arrowheads indicate representative caveolae. Dashed bar = 1μM.

Table 1

Diet	Npc1	Weights (g)			
		Body (BW)	Liver (LW)	LW / BW	Adipose (AW)
Low-fat	+/, N=3	21.44 ± 1.54	0.98 ± 0.04	0.046	0.45 ± 0.08
	+/-, N=3	22.39 ± 0.36	0.98 ± 0.08	0.044	0.45 ± 0.08
High-fat	+/, N=7	31.22 ± 1.33	1.52 ± 0.09	0.0486	1.32 ± 0.13
	+/-, N=7	37.34 ± 1.24*	1.77 ± 0.08	0.0474	2.01 ± 0.19*
					0.0533*

* p<0.05 compared to +/+

Table 2

Genotype	# caveolae / 670 μ M ²	Diameter (μ M)
<i>Npc1</i> ^{+/+}	(3) 18.21 $\pm$ 11.1	(3) 318.4 $\pm$ 21.4
<i>Npc1</i> ^{+/-}	(4) 15.46 $\pm$ 4.6	(4) 330.3 $\pm$ 31.3

[†]
(n) average $\pm$ standard error