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The C57BL/6J Niemann-Pick C1 mouse model with decreased gene dosage is susceptible to increased weight gain when fed a high-fat diet: Confirmation of a gene-diet interaction

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Dear Editor

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., 2007, 2010). A number of early studies performed using human *NPC1* fibroblasts and the BALB/cJ *Npc1* mouse model have determined that the *NPC1* protein has a central role in regulating the transport of lipoprotein-derived lipid (cholesterol and fatty acids) from late endosomes/lysosomes to other cellular compartments to maintain cellular, tissue, and whole body lipid homeostasis (Liscum et al., 1989; Pentchev et al., 1986).

Several years ago the first and second waves of genome-wide association studies (GWAS) revealed that common human *NPC1* gene risk variants (644A>G encoding His215Arg and 2572A>G encoding Ile858Val) were associated with extreme adult obesity and increased measures of adiposity (Cotsapas et al., 2010; den Hoed et al., 2012; Meyre et al., 2009). These findings were subsequently replicated in other populations and extended to include an additional *NPC1* gene risk variant (1926C>G encoding Ile642Met) associated with type 2

diabetes independent of body weight (Al-Daghri et al., 2012; Robiou-du-Pont et al., 2013; Sandholt et al., 2011). Consistent with these results, we have recently reported that the human *NPC1* gene risk variants reside in complete linkage-disequilibrium ($D' > 0.99$) among certain ethnic groups (non-Hispanic white, Hispanic, and Native American) and associated with maternal overweight or gestational diabetes independent of body weight in our local obstetric population (Garver et al., 2015).

Whether the human *NPC1* gene risk variants correspond to an increase or decrease of encoded NPC1 protein function was unknown when initial GWAS and replication studies were performed. To properly address this important question we performed a series of growth studies using different *Npc1* mouse models comprised of *Npc1* normal mice (*Npc1*^{+/+}) and *Npc1* heterozygous mice (*Npc1*^{+/-}) with decreased gene dosage resulting from a null mutation and hence proposed to genetically mimic human *NPC1* gene loss-of-function variants. As a result of our studies, we were able to clearly demonstrate that male and female pure BALB/cJ or hybrid BALB/cJ-C57BL/6J *Npc1*^{+/-} mice are susceptible to increased weight gain when fed a high-fat diet (45% kcal fat) but not when fed a low-fat diet (10% kcal fat), consistent with an *Npc1* gene-diet interaction responsible for weight gain (Jelinek et al., 2010a,b, 2012).

However, soon after our three studies were published an independent study reported that a C57BL/6J *Npc1* mouse model with a loss-of-function variant (3163A>G encoding Asp1005Gly) produced by the potent mutagen ethyl-nitrosourea (*Npc1*^{nmf164}) was instead susceptible to weight loss starting at approximately 20 weeks of age when fed a normal-fat diet (18% kcal fat) (Borbon et al., 2012). This study also indicated that weight loss occurred in male mice, but not female mice, when fed the normal-fat diet. To examine and clarify this apparent discrepancy, we had produced a novel C57BL/6J *Npc1* mouse model by backcrossing the BALB/cJ *Npc1* mouse model null mutation into wild-type C57BL/6J mice for 15 generations. We published our first results in this journal (Gene) indicating that C57BL/6J *Npc1*^{+/-} mice were susceptible to impaired glucose tolerance at 10 weeks of age compared to littermate *Npc1*^{+/+} mice when fed a low-fat diet (10% kcal fat) and independent of body weight (Jelinek et al., 2013). This result was highly significant because it confirmed a previous replication study indicating that an additional human *NPC1* gene risk variant (1926C>G encoding Ile642Met) predisposes to insulin resistance or type 2 diabetes independent of body weight in certain populations. Finally, we have more recently performed another growth study with our novel C57BL/6J *Npc1* mouse model and report results to this journal (Gene) that *Npc1*^{+/-} mice are predisposed to increased weight gain at approximately 10 weeks of age compared to *Npc1*^{+/+} mice when fed a high-fat diet, but not when fed a low-fat diet, thereby confirming our original studies indicating an *Npc1* gene-diet interaction responsible for increased weight gain (Fig. 1A). Moreover, our results also indicate that *Npc1*^{+/+} and *Npc1*^{+/-} mice have significantly impaired yet similar glucose tolerance when fed a high-fat diet compared to *Npc1*^{+/+} and *Npc1*^{+/-} mice fed a low-fat diet (Fig. 1B). Nonetheless, the *Npc1*^{+/-} mice fed a low-fat diet have glucose tolerance that is intermediate compared to *Npc1*^{+/+} mice fed the low-fat diet (normal glucose tolerance) and *Npc1*^{+/+} and *Npc1*^{+/-} mice fed a high-fat diet (impaired glucose tolerance).

In summary, our *Npc1* mouse model studies using different genetic backgrounds (pure BALB/cJ, hybrid BALB/cJ-C57BL/6J, and mostly pure C57BL/6J) indicate that decreased gene dosage predisposes to increased weight gain and impaired glucose tolerance when fed a high-fat diet. These results confirm an *Npc1* gene-diet interaction responsible for these common metabolic diseases. Moreover, our combined mouse model studies are consistent with GWAS and subsequent replication studies indicating that the human *NPC1* gene ancestral risk variants (644A>G, 1926C>G, and 2572A>G) reported to be in complete linkage-disequilibrium represent loss-of-function variants, or at least are genetically linked with undefined causal variants that impart metabolic thriftiness and therefore enhance energy storage. In contrast, although the C57BL/6J *Npc1^{nmf164}* homozygous affected mouse model may be appropriate for investigating classical human NPC1 disease, there are limitations for using this mouse model to investigate common human metabolic diseases (obesity and diabetes). Although speculative, these limitations may have resulted from the general methodology used to induce indiscriminate mutations throughout the mouse genome using ethyl-nitrosourea.

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Abbreviations

GWAS	genome-wide association study
NPC1	Niemann–Pick C1

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Fig. 1.

Growth curves and glucose tolerance test for the C57BL/6J *Npc1* mouse model fed a low-fat or high-fat diet. (A) The change in body weights for C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice fed a low-fat diet or high-fat diet and weighed on an approximate weekly basis. (B) The change in blood glucose concentrations for C57BL/6J *Npc1*^{+/+} and *Npc1*^{+/-} mice fed a low-fat diet or high-fat diet measured at 10 weeks of age. Values are represented as mean $\pm$ SE (n = 8–10 mice). An asterisk indicates a significant difference ($P < 0.05$). LFD = low-fat diet. HFD = high-fat diet.