Effects of G6pc2 deletion on body weight and cholesterol in mice

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Abstract

Genome-wide association study (GWAS) data have linked the G6PC2 gene to variations in fasting blood glucose (FBG). G6PC2 encodes an islet-specific glucose-6-phosphatase catalytic subunit that forms a substrate cycle with the beta cell glucose sensor glucokinase. This cycle modulates the glucose sensitivity of insulin secretion and hence FBG. GWAS data have not linked G6PC2 to variations in body weight but we previously reported that female C57BL/6J G6pc2-knockout (KO) mice were lighter than wild-type littermates on both a chow and high-fat diet. The purpose of this study was to compare the effects of G6pc2 deletion on FBG and body weight in both chow-fed and high-fat-fed mice on two other genetic backgrounds. FBG was reduced in G6pc2 KO mice largely independent of gender, genetic background or diet. In contrast, the effect of G6pc2 deletion on body weight was markedly influenced by these variables. Deletion of G6pc2 conferred a marked protection against diet-induced obesity in male mixed genetic background mice, whereas in 129SvEv mice deletion of G6pc2 had no effect on body weight. G6pc2 deletion also reduced plasma cholesterol levels in a manner dependent on gender, genetic background and diet. An association between G6PC2 and plasma cholesterol was also observed in humans through electronic health record-derived phenotype analyses. These observations suggest that the action of G6PC2 on FBG is largely independent of the influences of environment, modifier genes or epigenetic events, whereas the action of G6PC2 on body weight and cholesterol are influenced by unknown variables.

Introduction

The glucose-6-phosphatase (G6Pase) enzyme system is located in the endoplasmic reticulum and catalyzes the hydrolysis of glucose-6-phosphate (G6P) to glucose and inorganic phosphate (Hutton & O'Brien 2009, O'Brien 2013). In addition to a catalytic subunit, which can be one of three isoforms, G6PC1, G6PC2 or G6PC3, the G6Pase system is composed of a glucose transporter and a G6P/Pi transporter, encoded by the SLC37A4 gene (Hutton & O'Brien 2009, O'Brien 2013). G6PC2 is thought to be expressed exclusively in pancreatic islet beta cells (Hutton & O'Brien 2009, O'Brien 2013). Experiments comparing wild-type (WT) and G6pc2-knockout (KO)
mouse islets suggest that G6pc2 opposes the action of the beta cell glucose sensor, glucokinase, which catalyzes the formation of G6P from glucose (Matschinsky 2005, Lymedjian 2009). In isolated G6pc2 KO islets, G6Pase activity (Pound et al. 2013) and glucose cycling (Wall et al. 2015) are abolished. This results in a leftward shift in the dose–response curve for glucose-stimulated insulin secretion (GSIS) (Pound et al. 2013). Under fasting conditions, where insulin levels are the same in WT and G6pc2 KO mice, this shift results in reduced fasting blood glucose (FBG) in KO mice (Wang et al. 2007, Pound et al. 2013, Boortz et al. 2016). In contrast, under stimulatory conditions using a sub-maximal concentration of glucose, this shift results in increased GSIS from G6pc2 KO relative to WT mouse islets (Pound et al. 2013). As predicted from a parallel shift in the dose–response curve for GSIS, under stimulatory conditions using a high concentration of glucose, this shift results no difference in GSIS between G6pc2 KO and WT as assessed in either isolated islets in situ (Pound et al. 2013) or mice in vivo using hyperglycemic clamps (Wang et al. 2007).

Consistent with these mouse studies, genome-wide association studies (GWAS) have linked the rs560887 single nucleotide polymorphism (SNP) in the G6PC2 gene to variations in FBG (Bouatia-Naji et al. 2008, Chen et al. 2008). Molecular studies have shown that the rs560887-G allele represents a gain of function that is associated with increased G6PC2 RNA splicing, which is predicted to lead to increased full-length G6PC2 protein expression and elevated glucose cycling (Baerenwald et al. 2013). As GWAS data show that the rs560887-G allele is associated with elevated FBG (Bouatia-Naji et al. 2008, Chen et al. 2008), the combination of these splicing (Baerenwald et al. 2013) and G6pc2 KO mouse (Wang et al. 2007, Pound et al. 2013) studies suggest that rs560887 is a potentially causative variant. The association between G6PC2 and FBG has been confirmed in multiple GWAS and in different populations (Prokopenko et al. 2008, Bouatia-Naji et al. 2009, Hu et al. 2009, 2010, Reiling et al. 2009, Dupuis et al. 2010, Tam et al. 2010, Wang et al. 2013).

Numerous GWAS have also examined the genes that are associated with variations in body weight, fat mass and fat distribution and have shown that greater than 160 loci are linked to these parameters (Lu et al. 2016). Although G6PC2 was not one of the loci identified (Lu et al. 2016), we previously observed that female C57BL/6j G6pc2 KO mice were lighter than wild-type (WT) littermates on both a chow-fed and high-fat-fed diet (Pound et al. 2013). This observation prompted us to examine whether genetic background influences the effect of G6pc2 deletion on body weight and the response to diet-induced obesity (DIO). The results show that the effect of G6pc2 deletion on FBG is largely independent of gender, genetic background and diet, whereas the effect of G6pc2 deletion on body weight is highly dependent on these variables. We also found that deletion of G6pc2 reduced plasma cholesterol levels in a manner dependent on gender, genetic background and diet. These observations suggest that the action of G6PC2 on FBG is largely unaffected by the influences of environment, gender, modifier genes or epigenetic events, whereas the action of G6PC2 on body weight and cholesterol are influenced by unknown variables.

Materials and methods

Animal care

The Vanderbilt University Medical Center Animal Care and Use Committee approved all protocols used. Mice were maintained on either a standard rodent chow diet (calorie contributions: 28% protein, 12% fat, 60% carbohydrate (14% disaccharides); LabDiet 5001; PMI Nutrition International) or a high-fat diet (calorie contributions: 15% protein, 59% fat, 26% carbohydrate (42% disaccharides); Mouse Diet F3282; BioServ). High-fat feeding studies were initiated at 8 weeks of age, and mice were maintained on the diet for 8–14 weeks as indicated. Food and water were provided ad libitum.

Generation of G6pc2-knockout (KO) mice

Previous studies have described the generation of G6pc2 KO mice on a mixed 129SvEv × C57BL/6j (Wang et al. 2007), C57BL/6j (Pound et al. 2013) and 129SvEv (Boortz et al. 2016) genetic background. The targeting vector used to generate the KO allele replaced exons 1–3 of the G6pc2 gene with a LacZ/Neo cassette leaving exons 4 and 5 intact (Wang et al. 2007). Exon 1 contains the translation initiation methionine (Ebert et al. 1999). As such, the design of the targeting vector completely abolishes G6pc2 expression (Wang et al. 2007). All the mice examined in these studies were littermates generated by interbreeding of heterozygous (HET) mice.

Intraperitoneal glucose tolerance tests

Intraperitoneal glucose tolerance tests (IPGTTs) were performed on –22-week-old male mice as previously described (Pound et al. 2012).
Phenotypic analysis of fasted G6pc2 KO mice

Mice were fasted for 5h and then weighed. After an additional hour of fasting, mice were anesthetized using isoflurane, and blood samples were isolated from the retro-orbital venous plexus. Glucose concentrations were measured in whole blood using a glucose monitor (Accu-Check Advantage; Roche). EDTA (5 µL; 0.5 M) was then added to blood samples prior to the isolation of plasma by centrifugation. Insulin samples were assayed using RIA (Morgan & Lazarow 1963) by the Vanderbilt Hormone Assay and Analytical Services Core. Cholesterol was assayed using a cholesterol reagent kit (Raichem, San Diego, CA, USA), whereas triacylglycerol was assayed using a serum triacylglycerol determination kit (Sigma). Body composition was assessed using an mq10 NMR analyzer (Bruker Optics).

Analysis of gene expression in mouse pancreas

Pancreatic gene expression was analyzed as previously described (Boortz et al. 2016). The following mouse primer pairs were used for the analysis of gene expression:

- G6pc2 forward 5′-CCCTGATGGTGTTGGCTCTA-3′
- G6pc2 reverse 5′-GTCTGTTGGTGGAGCAGGAC-3′
- Ins2 forward 5′-CACCCAGGCTTTTGCTACGC-3′
- Ins2 reverse 5′-CCAGTGCCAAGGTCTGAGG-3′
- Mouse Ppia forward 5′-GGCCGATGACGAGCCC-3′
- Mouse Ppia reverse 5′-TGTCTTTGGAACTTTGTCTGCAA-3′

Electronic health record (EHR)-based phenotyping of human research subjects

EHR-based phenotyping was conducted using data on human subjects in the Vanderbilt University Medical Center (VUMC) BioVU DNA databank. Genotyping data in BioVU is linked to the synthetic derivative (SD), a de-identified version of the VUMC EHR repository. Detailed descriptions of program operations, ethical considerations and continuing oversight and patient engagement have been published (Roden et al. 2008, Pulley et al. 2010). For these studies, we used a previously genotyped cohort of 29,722 European descendants from VUMC with longitudinal medical care. Genotyping was performed on the Illumina Human Exome BeadChip platform. For this study, we specifically analyzed the intronic G6PC2 SNP rs560887. Lipid measurements utilized routine clinical laboratory testing values present in the EHR.

Statistical analyses

Other than IPGTTs, data were analyzed using a Student’s t-test: two sample assuming equal variance. The level of significance was as indicated (two-sided Student’s t-test). IPGTT data were analyzed using a two-way ANOVA assuming normal distribution and equal variance. A post hoc analysis was performed using the Bonferroni correction for multiple comparisons. The level of significance was as indicated.

To analyze genetic associations with lipids in BioVU, we used the median value for each individual. The associations between the genotypes and the aggregated laboratory values (as continuous variables) were performed on R with linear model, adjusted for age, sex and body mass index (BMI). We report beta values, 95% confidence intervals (CI) and P values. P<0.05 was considered to be significant. All tests assumed a two-tailed distribution.

Results

Analysis of the effect of G6pc2 deletion on body weight and composition in chow- and high-fat-fed 129SvEv mice

We have previously shown that 16-week-old chow-fed female, but not male, C57BL/6j G6pc2-knockout (KO) mice are slightly lighter than wild-type (WT) littermates and have reduced body fat (Pound et al. 2013). These differences were also observed following 12 weeks of high-fat feeding in female, but not male, C57BL/6j G6pc2 KO mice (Pound et al. 2013). In this study, we repeated these analyses with G6pc2 KO mice on a 129SvEv or mixed genetic background.

In 16-week-old chow-fed 129SvEv G6pc2 mice, no differences in weight or body fat were observed between female WT or KO mice (Table 1). However, male chow-fed 129SvEv G6pc2 KO mice were slightly lighter than WT littermates, and female chow-fed 129SvEv G6pc2 KO mice had slightly increased muscle mass (Table 1).

High-fat feeding is a standard nutritional challenge in the field of obesity and diabetes research that induces insulin resistance and is considered to model human disease (Young & Kirkland 2007). High-fat feeding of 129SvEv mice was started at 8 weeks of age and continued for 12 weeks. In contrast to C57BL/6j mice that markedly increase their body weight in response to high-fat feeding (Surwit et al. 1988, Winzell & Ahren 2004, Pound et al. 2013) almost no difference in body weight was observed between 16-week-old
chow-fed female and male 129SvEv mice (Table 1) vs 20-week-old high-fat-fed female and male 129SvEv mice (Table 2). Weekly measurements of body weight in non-fasted high-fat-fed mice during the 12 weeks of high-fat feeding showed no evidence for a biphasic change in weight, that would have been suggestive of a toxic effect of prolonged high-fat feeding, in either female (Fig. 1A) or male (Fig. 1B) WT and KO mice. These data are consistent with previous studies that have observed that 129SvEv mice are resistant to DIO (Almind & Kahn 2004).

Despite the lack of weight gain, high-fat-fed female and male 129SvEv mice showed a marked increase in body fat (%) relative to chow-fed mice (Fig. 1C). This was associated with a reduction in body muscle (%) in both high-fat-fed female and male 129SvEv mice (Table 2) relative to chow-fed female and male mice (Table 1) (*P < 0.05).

In 20-week-old high-fat-fed 129SvEv G6pc2 mice, no differences in body fat were observed between female or male WT vs KO mice (Table 2). However, male high-fat-fed 129SvEv G6pc2 KO mice were slightly heavier than WT littermates, and free fluid was reduced in female high-fat-fed 129SvEv G6pc2 KO mice (Table 2).

Analysis of the effect of G6pc2 deletion on fasting blood glucose (FBG) and fasting plasma insulin (FPI) in high-fat-fed 129SvEv mice

We have previously shown that male 129SvEv G6pc2 KO mice have reduced FBG but no change in FPI relative to WT littermates (Boortz et al. 2016). When this analysis was repeated using female 129SvEv chow-fed mice, this same reduction in FBG was observed in G6pc2 KO mice (Fig. 1D) with no change in FPI (Fig. 1E).

We next analyzed the effect of high-fat feeding on FBG and FPI in 129SvEv mice. Despite 13 weeks of high-fat feeding, a comparison between female (Fig. 1D) and male (Boortz et al. 2016) chow fed with female (Fig. 1F) and male (Fig. 1G) high-fat fed 129SvEv WT mice revealed surprisingly no increase in FBG. Similarly, a comparison between female (Fig. 1E) and male (Boortz et al. 2016) chow-fed with female

Table 1 NMR analysis of chow-fed 129SvEv G6pc2 KO mouse body composition.

<table>
<thead>
<tr>
<th>Gender and genotype</th>
<th>Body weight (g)</th>
<th>Fat (g)</th>
<th>Muscle (g)</th>
<th>Free fluid (g)</th>
<th>Fat (%)</th>
<th>Muscle (%)</th>
<th>Free fluid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female WT</td>
<td>22.23±0.56 (15)</td>
<td>2.76±0.26 (11)</td>
<td>13.94±0.27 (11)</td>
<td>0.58±0.05 (11)</td>
<td>13.04±1.15 (11)</td>
<td>66.32±0.94 (11)</td>
<td>2.78±0.26 (11)</td>
</tr>
<tr>
<td>Female KO</td>
<td>22.11±0.54 (14)</td>
<td>3.09±0.46 (9)</td>
<td>14.69±0.25 (9)*</td>
<td>0.60±0.03 (9)</td>
<td>13.48±1.62 (9)</td>
<td>66.05±1.34 (9)</td>
<td>2.71±0.15 (9)</td>
</tr>
<tr>
<td>Male WT</td>
<td>28.47±0.34 (16)</td>
<td>2.02±0.35 (11)</td>
<td>19.23±0.37 (11)</td>
<td>0.82±0.10 (11)</td>
<td>7.40±1.29 (11)</td>
<td>69.90±0.91 (11)</td>
<td>2.96±0.25 (11)</td>
</tr>
<tr>
<td>Male KO</td>
<td>26.93±0.54 (12)*</td>
<td>1.51±0.25 (9)</td>
<td>18.21±0.38 (9)</td>
<td>0.75±0.12 (9)</td>
<td>6.03±1.00 (9)</td>
<td>72.45±0.96 (9)</td>
<td>3.01±0.51 (9)</td>
</tr>
</tbody>
</table>

Body composition of 6-h fasted, 16-week-old animals was assessed using a mq10 NMR analyzer. Results are means±S.E.M. obtained from the number of animals indicated in parentheses. Weight: *P<0.009 male WT vs KO; Muscle: *P<0.04 female WT vs KO.

KO, knockout; WT, wild type.

Table 2 NMR analysis of high-fat-fed 129SvEv G6pc2 KO mouse body composition.

<table>
<thead>
<tr>
<th>Gender and genotype</th>
<th>Body weight (g)</th>
<th>Fat (g)</th>
<th>Muscle (g)</th>
<th>Free fluid (g)</th>
<th>Fat (%)</th>
<th>Muscle (%)</th>
<th>Free fluid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female WT</td>
<td>23.06±0.69 (11)</td>
<td>4.46±0.34 (11)</td>
<td>14.80±0.32 (11)</td>
<td>0.58±0.06 (11)</td>
<td>19.26±1.23 (11)</td>
<td>64.46±1.44 (11)</td>
<td>2.48±0.23 (11)</td>
</tr>
<tr>
<td>Female KO</td>
<td>22.65±0.58 (11)</td>
<td>4.30±0.32 (11)</td>
<td>14.37±0.37 (11)</td>
<td>0.50±0.04 (11)</td>
<td>18.88±1.11 (11)</td>
<td>63.55±1.02 (11)</td>
<td>2.17±0.15 (11)*</td>
</tr>
<tr>
<td>Male WT</td>
<td>26.80±0.93 (10)</td>
<td>5.00±0.78 (10)</td>
<td>16.72±0.50 (10)</td>
<td>0.55±0.07 (10)</td>
<td>18.18±2.34 (10)</td>
<td>62.68±1.63 (10)</td>
<td>2.02±0.19 (10)</td>
</tr>
<tr>
<td>Male KO</td>
<td>30.20±0.82 (13)*</td>
<td>6.98±0.65 (13)</td>
<td>17.51±0.35 (13)</td>
<td>0.60±0.03 (13)</td>
<td>22.67±1.75 (13)</td>
<td>58.35±1.58 (13)</td>
<td>2.00±0.09 (13)</td>
</tr>
</tbody>
</table>

Body composition of 6-h fasted, 20-week-old animals after 12 weeks of high-fat feeding was assessed using a mq10 NMR analyzer. Results are means±S.E.M. obtained from the number of animals indicated in parentheses. Weight: *P<0.0124 WT vs KO.

KO, knockout; WT, wild type.
Effect of G6pc2 deletion on body weight, composition and metabolic parameters in chow-fed and high-fat-fed 129SvEv mice. Panels A and B: Starting at 8 weeks of age, female (Panel A) and male (Panel B) mice were fed a high-fat diet with non-fasting body weights measured weekly. Results are the mean±s.e.m. of data from the following number of animals: female WT n = 12; female KO n = 13; male WT n = 10; male KO n = 13.

Panel C: Body composition was assessed in chow-fed 129SvEv mice at 16 weeks of age and in high-fat-fed 129SvEv mice at 20 weeks of age after 12 weeks of high-fat feeding. Mice were fasted for 5 h and then weighed. One hour later, body fat was determined by NMR. Results are the mean±s.e.m. of data with the genotype, gender and number of animals indicated. WT, wild type; F, female; M, male. *P < 0.05 high-fat-fed vs chow-fed females; *P < 0.05 high-fat-fed vs chow-fed males. Panels D and E: At 17 weeks of age, chow-fed mice were fasted for 5 h and then weighed. One hour later, mice were anesthetized and blood was isolated. Blood glucose (Panel D) and plasma insulin (Panel E) were determined as described in the ‘Materials and methods’ section. Results are the mean±s.e.m. of data with the genotype, gender and number of animals indicated. WT, wild type; KO, knockout; F, female. *P < 0.014 WT vs KO (Panel D). Panels F, G, H and I: Metabolic parameters were assessed in high-fat-fed 129SvEv mice at 21 weeks of age after 13 weeks of high-fat feeding. Mice were fasted for 5 h and then weighed. One hour later, mice were anesthetized and blood was isolated. Blood glucose (Panels F and G) and plasma insulin (Panels H and I) were determined as described in the ‘Materials and methods’ section. Results are the mean±s.e.m. of data with the genotype, gender and number of animals indicated. WT, wild type; KO, knockout; F, female; M, male. *P < 0.001 female WT vs KO (Panel F); *P < 0.037 male WT vs KO (Panel G). Panel J: Glucose tolerance was assessed in chow-fed 129SvEv at ~22 weeks of age and in high-fat-fed 129SvEv mice at 22 weeks of age after 13 weeks of high fat feeding. IPGTTs using 2.0 g/kg glucose were performed on 6-h fasted, conscious, chow- or high-fat-fed wild-type (WT) and G6pc2-knockout (KO) male mice as described in the ‘Materials and methods’ section. The results show the mean glucose concentrations±s.e.m.

(Fig. 1H) and male (Fig. 1I) high-fat-fed 129SvEv WT mice revealed surprisingly no increase in FPI.

After 13 weeks of high-fat feeding, a reduction in FBG was observed in both female (Fig. 1F) and male (Fig. 1G) G6pc2 KO relative to WT mice with no differences in FPI in either female (Fig. 1H) or male (Fig. 1I) mice relative to WT.
Analysis of the effect of high-fat feeding on glucose tolerance in 129SvEv WT and G6pc2 KO mice

We have previously shown that deletion of G6pc2 does not affect glucose tolerance in chow-fed C57BL/6J (Pound et al. 2013) and 129SvEv (Boortz et al. 2016) mice, consistent with human GWAS data showing no association between G6PC2 SNPs and variations in glucose tolerance (Li et al. 2009, Rose et al. 2009, Heni et al. 2010, Ingelsson et al. 2010). Although high-fat feeding did not result in weight gain in male 129SvEv mice (Table 1) relative to chow-fed mice (Table 1), intraperitoneal glucose tolerance tests (IPGTTs) revealed a clear impairment in glucose tolerance in both WT (P<0.0002) and G6pc2 KO (P<0.0001) high-fat fed 129SvEv mice relative to chow-fed mice (Fig. 1J), suggesting the presence of either insulin resistance and/ or impaired GSIS in high-fat-fed 129SvEv mice. However, even in high-fat-fed mice, deletion of G6pc2 did not affect glucose tolerance (Fig. 1H).

Analysis of the effect of G6pc2 deletion on body weight, FBG and FPI in high-fat-fed mixed genetic background mice

A comparison of data derived from studies on C57BL/6J (Pound et al. 2013) and 129SvEv (Fig. 1, Tables 1 and 2) mice suggest that the effect of G6pc2 deletion on body weight varies with gender and genetic background. We therefore repeated these high-fat feeding analyses in mice with a mixed C57BL/6J X 129SvEv genetic background. We have previously shown that FBG is reduced in both female and male mixed C57BL/6J X 129SvEv genetic background G6pc2 KO mice relative to WT with no differences in body weight or FPI (Wang et al. 2007). After starting high-fat feeding at 8 weeks of age and continuing for 8 weeks, we observed no differences in body weight between female mixed genetic background WT and KO mice (Fig. 2A). In contrast, male mixed genetic background G6pc2 KO mice exhibited a striking protection against DIO (Fig. 2B).

No reduction in FBG was observed in high-fat-fed female KO mice relative to WT mice (Fig. 2C), whereas FBG was markedly reduced in high-fat-fed male KO mice relative to WT mice (Fig. 2D). Similarly, although no difference in FPI was observed between high-fat-fed female KO mice relative to WT mice (Fig. 2E), FPI was markedly reduced in high-fat-fed male KO mice relative to WT mice (Fig. 2F).

**Figure 2**

Effect of G6pc2 deletion on body weight and metabolic parameters in high-fat-fed mixed genetic background mice. Metabolic parameters were assessed in high-fat-fed mixed genetic background mice at 16 weeks of age after 8 weeks of high-fat feeding. Mice were fasted for 5h and then weighed (Panels A and B). One hour later, mice were anesthetized and blood was isolated. Blood glucose (Panels C and D) and plasma insulin (Panels E and F) were determined as described in the ‘Materials and methods’ section. Results are the mean ± s.e.m. of data with the genotype, gender and number of animals indicated. WT, wild type; KO, knockout; f, female; M, male. *P<8.32E-05 WT vs KO (Panel B); **P<3.45E-05 WT vs KO (Panel D); +P<6.84E-05 WT vs KO (Panel F).

**Comparison of pancreatic G6pc2 expression in 129SvEv and C57BL/6J mice**

The data derived from studies on C57BL/6J, 129SvEv and mixed genetic background mice reveal that the effect of G6pc2 deletion on body weight varies with gender and genetic background. In addition, FBG is lower in both male...
chow-fed (Boortz et al. 2016) and high-fat-fed (Fig. 1F) 129SvEv mice than that in C57BL/6J mice (Goren et al. 2004, Mazzuccara et al. 2008, Poud et al. 2013). Although there are likely multiple factors that account for these differences, one potential contributing factor could be variations in G6pc2 gene expression between C57BL/6J and 129SvEv mice. To address this possibility, we compared pancreatic G6pc2 and Ins2 gene expression in both mouse strains. There was no difference in the ratio of G6pc2 to Ins2 gene expression between female and male chow-fed 129SvEv mice (Fig. 3A) or between female and male chow-fed C57BL/6J mice (Fig. 3B). There was also no difference in the ratio of G6pc2 to Ins2 gene expression between chow-fed female 129SvEv and C57BL/6J mice (Fig. 3C) or between chow-fed male 129SvEv and C57BL/6J mice (Fig. 3D). In contrast, although there was no difference in the ratio of G6pc2 to Ins2 gene expression between female and male high-fat-fed 129SvEv mice (Fig. 3E), there was a difference between female and male high-fat-fed C57BL/6J mice (Fig. 3F). Similarly, although there was no difference in the ratio of G6pc2 to Ins2 gene expression between high-fat-fed female 129SvEv and C57BL/6J mice (Fig. 3G), there was a difference between high-fat-fed male 129SvEv and C57BL/6J mice (Fig. 3H). These data suggest that G6pc2 expression is induced by high-fat feeding relative to Ins2 expression in male C57BL/6J mice, which may contribute to differences in the effect of high fat feeding between male and female C57BL/6J mice and between male C57BL/6J and 129SvEv mice.

Analysis of the effect of G6pc2 deletion on plasma cholesterol in 129SvEv, C57BL/6J and mixed genetic background mice

As multiple plasma lipids change in response to high-fat feeding (Eisinger et al. 2014), we also compared plasma cholesterol levels in G6pc2 KO mice on different genetic backgrounds. We previously observed no change in cholesterol levels in male or female mixed genetic background G6pc2 KO mice relative to WT (Wang et al. 2007). However, when we repeated these analyses in chow-fed 129SvEv and C57BL/6J mice along with high-fat fed 129SvEv, C57BL/6J and mixed genetic background mice, we observed that plasma cholesterol levels were reduced in chow-fed male C57BL/6J KO mice (Fig. 4D), high-fat-fed female (Fig. 4G) and male (Fig. 4H) C57BL/6J KO mice and high-fat-fed mixed genetic background male KO mice (Fig. 4J).

Analysis of the relationship between G6PC2 SNPs and metabolic parameters in humans using BioVU

Our results in mice demonstrate that the effect of G6pc2 deletion on cholesterol levels varies with gender and genetic background. We next used Vanderbilt’s BioVU DNA databank to determine whether G6PC2 affects these parameters in humans. BioVU individuals with extant genotyping at the intronic G6PC2 SNP rs560887 were screened to identify associations with cholesterol.
and triglyceride measurements. The rs560887-G allele, which enhances G6PC2 pre-mRNA splicing (Baerenwald et al. 2013), was associated with increased cholesterol (total cholesterol: $\beta = 1.0$, $P = 0.039$; LDL-C: $\beta = 1.1$, $P = 0.006$), but not triglyceride levels ($\beta = 0.90$, $P = 0.46$) or HDL-C ($\beta = -0.07$, $P = 0.75$) (Table 3). We further analyzed the population by sex and found that rs560887-G significantly associated with increased LDL-C in males ($P = 0.009$) but not in females ($P = 0.15$), although SNP and sex interaction is not significant ($P = 0.30$) (Table 3). Rs560887 did not associate with diabetes status ($P = 0.37$). Thus, as in mice, the impact in humans of modulating G6PC2 expression on plasma lipids is dependent on gender.

### Analysis of the effect of G6pc2 deletion on food intake

A key question that arises from these studies is how G6PC2, which is thought to be expressed exclusively in pancreatic islet beta cells (Arden et al. 1999, Martin et al. 2001), could be affecting body weight. One possible explanation for the link between G6PC2 and body weight is that G6PC2 affects satiety. Thus, the leftward shift in the dose–response curve for GSIS observed in G6pc2 KO mice (Pound et al. 2013) might result in a faster rise in plasma insulin levels after eating or glucose injection in an IPGTT. As insulin is a satiety factor (Woods et al. 2006), this faster rise in insulin could promote a quicker cessation of feeding and ultimately reduced food intake. To address this hypothesis, we measured the intake of high-fat food...
Table 3  Association between G6PC2 SNP rs560887 and plasma lipid measurements using electronic health record (EHR)-derived phenotype analyses.

<table>
<thead>
<tr>
<th>Lab</th>
<th>Population</th>
<th>N</th>
<th>Beta (G)</th>
<th>95% CI</th>
<th>P-value</th>
<th>Allele</th>
</tr>
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<tbody>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>GG</td>
</tr>
<tr>
<td>LDL-C</td>
<td>All</td>
<td>13,087</td>
<td>1.15</td>
<td>0.33–1.96</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>LDL-C</td>
<td>Male</td>
<td>5863</td>
<td>1.60</td>
<td>0.4–2.8</td>
<td>0.009</td>
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<tr>
<td>LDL-C</td>
<td>Female</td>
<td>7224</td>
<td>0.82</td>
<td>−0.29–1.93</td>
<td>0.148</td>
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<tr>
<td>Cholesterol</td>
<td>All</td>
<td>14,349</td>
<td>1.00</td>
<td>0.05–1.95</td>
<td>0.039</td>
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<tr>
<td>Cholesterol</td>
<td>Male</td>
<td>6412</td>
<td>1.49</td>
<td>0.09–2.87</td>
<td>0.037</td>
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<tr>
<td>Cholesterol</td>
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<td>7937</td>
<td>0.68</td>
<td>−0.59–1.95</td>
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<tr>
<td>Triglycerides</td>
<td>All</td>
<td>14,213</td>
<td>0.90</td>
<td>−1.48–3.28</td>
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<tr>
<td>Triglycerides</td>
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<td>6398</td>
<td>−0.08</td>
<td>−4.07–3.9</td>
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<td>Triglycerides</td>
<td>Female</td>
<td>7815</td>
<td>1.80</td>
<td>−1.02–4.62</td>
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<tr>
<td>HDL-C</td>
<td>All</td>
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<td>−0.07</td>
<td>−0.46–0.33</td>
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<td>Male</td>
<td>6006</td>
<td>0.28</td>
<td>−0.22–0.78</td>
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<tr>
<td>HDL-C</td>
<td>Female</td>
<td>7451</td>
<td>−0.35</td>
<td>−0.94–0.24</td>
<td>0.245</td>
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Plasma lipid measurements were obtained from routine lipid panels in Vanderbilt University Medical Center’s EHR repository. For each laboratory of each individual, the associations are tested against the median of all laboratory results for that test. All associations were adjusted for age, sex and body mass index using linear regression. The unit for all measurements is mg/dL. Cholesterol, total cholesterol; HDL-C, calculated high-density lipoprotein; LDL-C, calculated low-density lipoprotein.

in female C57BL/6J WT and G6pc2 KO mice. Although female C57BL/6J G6pc2 KO mice are lighter than wild-type (WT) littermates on both a chow-fed and high-fat-fed diet (Pound et al. 2013), no difference in food intake was detected (Fig. 5).

Discussion

Our results demonstrate that the effect of G6pc2 deletion in mice on FBG closely parallels human GWAS data in that the effect of G6pc2 deletion on FBG is largely independent of gender and genetic background. We previously showed that, relative to WT mice, FBG is reduced in both female and male chow-fed and high-fat-fed G6pc2 KO on a pure C57BL/6J genetic background (Pound et al. 2013), female and male chow-fed G6pc2 KO mice on a mixed genetic background (Wang et al. 2007) and male mice on a 129SvEv genetic background (Boortz et al. 2016).

We show here that FBG is also reduced in 129SvEv chow-fed female mice (Fig. 1D) and high-fat-fed female (Fig. 1F) and male (Fig. 1G) mice. Similarly, FBG is reduced in male high-fat-fed mixed genetic background G6pc2 KO relative to WT mice (Fig. 2D). FBG was not reduced in female high-fat-fed mixed genetic background G6pc2 KO mice (Fig. 2C), though the n value in this study was relatively low. These observations are largely consistent with human GWAS data showing an association between G6PC2 and FBG in multiple different populations (Prokopenko et al. 2008, Bouatia-Naji et al. 2009, Hu et al. 2009, 2010, Reiling et al. 2009, Dupuis et al. 2010, Tam et al. 2010, Wang et al. 2013).

With respect to FPI, we previously showed that, relative to WT mice, FPI is unchanged in both female and male chow-fed and high-fat-fed G6pc2 KO mice on a pure C57BL/6J genetic background (Pound et al. 2013), female and male chow-fed G6pc2 KO mice on a mixed

Figure 5  Effect of G6pc2 deletion on high-fat food intake in female C57BL/6J WT and G6pc2 KO mice. Female C57BL/6J WT and G6pc2 KO mice were switched from chow food to high-fat food at 8 weeks of age, and food intake was measured daily for 21 days. Results show the mean food intake ± s.e.m. in 6 WT and 6 G6pc2 KO mice.
genetic background (Wang et al. 2007) and male mice on a 129SvEv genetic background (Boortz et al. 2016). We show here that FPI is also unchanged in 129SvEv chow-fed female mice (Fig. 1E) and high-fat-fed female (Fig. 1H) and male (Fig. 1I) mice. Similarly, FPI is unchanged in female high-fat-fed mixed genetic background G6pc2 KO mice (Fig. 2E). A reduction in FPI was observed in male high-fat-fed mixed genetic background G6pc2 KO mice (Fig. 2F), but this is presumably secondary to the marked effect of G6pc2 deletion on body weight in males (fig. 2B). These observations are consistent with human GWAS data showing no association between G6PC2 and FPI in multiple different populations (Bouatia-Naji et al. 2008, Chen et al. 2008, Mahajani et al. 2015, Wessel et al. 2015).

In contrast to these data showing largely consistent effects of G6pc2 deletion on FBG and FPI regardless of gender, diet and genetic background, the effect of G6pc2 deletion on weight and body composition is highly dependent on these variables. We previously showed that female, but not male, G6pc2 KO mice on a pure C57BL/6J genetic background had reduced body weight and body fat on both a chow- and high-fat diet relative to WT mice (Pound et al. 2013). In contrast, we show here that deletion of G6pc2 in female mice on the 129SvEv genetic background has no effect on body weight or body fat on either a chow (Table 1) or high-fat (Table 2) diet relative to WT mice. Similarly, deletion of G6pc2 in female mice on a mixed 129SvEv X C57BL/6J genetic background has no effect on body weight on either a chow (Wang et al. 2007) or high-fat (Fig. 2A) diet relative to WT mice. In males, deletion of G6pc2 on the 129SvEv genetic background was associated with reduced body weight on a chow diet (Table 1) but increased body weight on a high-fat diet (Table 2). In contrast, deletion of G6pc2 in male mice on a mixed 129SvEv X C57BL/6J genetic background had no effect on body weight on a chow diet (Wang et al. 2007), whereas this conferred a marked protection against DIO on a high-fat diet (Fig. 2B). Overall, our results suggest that FBG is a much more tightly regulated variable than body weight. Thus, although FBG levels are relatively similar in chow-fed C57BL/6J (Pound et al. 2013), 129SvEv (Boortz et al. 2016) and mixed (Wang et al. 2007) genetic background mice, the increase in body weight and body fat in response to high-fat feeding is markedly different in C57BL/6J (Surwit et al. 1988, Winzell & Ahren 2004, Pound et al. 2013) and 129SvEv mice (Fig. 1) (Almind & Kahn 2004). Interestingly, the response to DIO varies remarkably even within inbred mice through poorly understood epigenetic mechanisms (Burcelin et al. 2002, Koza et al. 2006, Oey et al. 2015) though whether such mechanisms and/or environmental factors or modifier genes contribute to the variable effects of G6pc2 deletion on body weight and composition is unknown.

In humans, a GWAS performed in a cohort of Mexican Americans linked the G6PC2 rs560887-A allele with a small decrease in BMI and adiposity in this population (Li et al. 2009); however, other GWAS have not associated G6PC2 with variations in body mass index, fat mass or fat distribution (Lu et al. 2016). Similarly, a strong association between G6PC2 and cholesterol was not detected using GWAS (Aulchenko et al. 2009, Kathiresan et al. 2009), though a weak association can be detected using BioVU (Table 3). These observations suggest that either the effects of G6pc2 on body mass and cholesterol are quantitatively only easily detected in mice on specific genetic backgrounds or that there is a threshold effect such that G6PC2 will markedly affect these parameters in some human populations but only after a substantial change in expression rather than the subtle changes associated with common SNPs (Bouatia-Naji et al. 2010, Baerenwald et al. 2013). If correct, the association observed by GWAS between common SNPs in G6PC2 and subclinical atherosclerosis (Rasmussen-Torvik et al. 2011) is likely secondary to the effect of G6PC2 on FBG rather than a direct effect of G6PC2 on cholesterol metabolism.

A key question that remains to be addressed is how G6PC2, which is thought to be expressed exclusively in pancreatic islet beta cells (Arden et al. 1999, Martin et al. 2001), could be affecting body weight. One possibility, as proposed by Li and coworkers (Li et al. 2009), is that the differences in body weight they observed in humans are secondary effects due to altered insulin signaling efficacy that arise due to an effect of G6PC2 on the pulsatility of insulin secretion. Another possibility is that G6PC2 expression in other tissues that affect body weight has been overlooked. Indeed although RNA blotting showed no evidence for G6PC2 expression in brain (Martin et al. 2001) and transgenic mouse studies gave inconsistent results (Frigeri et al. 2004, Wang et al. 2008), one group has reported G6pc2 expression in the mouse hypothalamus (Goh et al. 2006), a region critical for the control of body weight (Morton et al. 2006). However, this expression was only detected using very high template concentrations and PCR cycles (Goh et al. 2006). Moreover, although low levels of expression were detected, it is unlikely to be biologically consequential as expression of the G6pc3 isoform was detected at much higher levels (Goh et al. 2006) and G6pc3 is
enzymatically more active than G6pc2 (Shieh et al. 2003, Petrolonis et al. 2004). One other potential explanation for the link between G6PC2 and body weight is that G6PC2 affects satiety. Thus, the leftward shift in the dose–response curve for GSIS observed in G6pc2 KO mice (Pound et al. 2013) might result in a faster rise in plasma insulin levels after eating. As insulin is a satiety factor (Woods et al. 2006), this faster rise in insulin could promote a quicker cessation of feeding and ultimately reduced food intake. However, an analysis of food intake did not detect a difference between female C57BL/6J WT and G6pc2 KO mice (Fig. 5). Future studies will examine the alternate possibility, specifically whether deletion of G6pc2 affects energy expenditure (Ellacott et al. 2010). Although food intake did not differ between C57BL/6J WT and G6pc2 KO mice, an effect of G6PC2 on the timing of GSIS during glucose tolerance tests could explain the counterintuitive observation that the rs560887-G allele, which confers elevated G6PC2 expression (Baerenwald et al. 2013), is associated with elevated FBG but also higher insulin levels at the 30-min time point in a glucose tolerance test (Li et al. 2009). We hypothesize that insulin levels may peak earlier in individuals with the rs560887-A allele, which confers lower G6PC2 expression (Baerenwald et al. 2013).

Declaration of interest
R O’B is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of data and the accuracy of data analysis. None of the authors have a conflict of interest relating to this study.

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Author contribution statement
K A B performed most of the mouse phenotyping studies and manuscript writing. K E S performed some of the mouse studies. L D P performed some of the mouse studies. H M performed association studies with EHR data and manuscript editing. L B performed association studies with EHR data. J K O performed some of the mouse studies. O P M contributed to the design of experiments and manuscript editing. J C D contributed to design of EHR studies and manuscript editing. R M O performed some of the mouse studies and manuscript writing.

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variant in the G6PC2/ABCB11 locus is associated with increased fasting plasma glucose, increased basal hepatic glucose production and increased insulin release after oral and intravenous glucose loads. *Diabetologia* **52** 2122–2129. (doi:10.1007/s00125-009-1463-z)


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