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.

Key Words

- ▶ glucose-6-phosphatase
- ▶ fasting blood glucose
- ▶ cholesterol
- ▶ body weight

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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, Iynedjian 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 5 h 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'-CCCTGATGGTGGTGGCTCTA-3'
G6pc2 reverse 5'-GTCTGTGGGTGGAGCAGGAC-3'
Ins2 forward 5'-CACCCAGGCTTTGTCAAGC-3'
Ins2 reverse 5'-CCAGTGCCAAGGTCTGAAGG-3'
 Mouse *Ppia* forward 5'-GGCCGATGACGAGCCC-3'
 Mouse *Ppia* reverse 5'-TGTCTTTGGAACCTTGTCTGCAA-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

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

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

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.

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

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

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.

Figure 1

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 $\pm$ 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 $\pm$ S.E.M. of data with the genotype, gender and number of animals indicated. WT, wild type; F, female; M, male. $*P<4.61E-10$ high-fat-fed vs chow-fed females; $*P<3.94E-05$ 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 $\pm$ 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 $\pm$ 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 $\pm$ 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 2) 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 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 5 h 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 $\pm$ S.E.M. of data with the genotype, gender and number of animals indicated. WT, wild type; KO, knockout; F, female; M, male. * $P < 8.32 \times 10^{-5}$ WT vs KO (Panel B); * $P < 3.45 \times 10^{-5}$ WT vs KO (Panel D); * $P < 6.84 \times 10^{-5}$ 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

Figure 3

Comparison of pancreatic *G6pc2* expression in 129SvEv and C57BL/6J mice. Pancreatic RNA was isolated after a 6-h fast from chow-fed (Panels A, B, C and D) 129SvEv (129) or C57BL/6J (C57) mice or mice fed a high-fat diet for 2 weeks (Panels E, F, G and H). *G6pc2* and *Ins2* expression were quantitated by real-time PCR. Results show the ratio of *G6pc2* to *Ins2* expression $\pm$ S.E.M. in 3–5 pancreata. * $P < 0.05$ vs control.

chow-fed (Boortz *et al.* 2016) and high-fat-fed (Fig. 1F) 129SvEv mice than that in C57BL/6J mice (Goren *et al.* 2004, Mazzaccara *et al.* 2008, Pound *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

Figure 4

Effect of *G6pc2* deletion on plasma cholesterol in 129SvEv, C57BL/6J and mixed genetic background mice. Panels A, B, C and D: At 17 weeks of age, chow-fed 129SvEv or C57BL/6J mice were fasted for 5 h and then weighed. One hour later, mice were anesthetized and blood was isolated. Panels E, F, G and H: At 21 weeks of age, after 13 weeks of high-fat feeding, 129SvEv and C57BL/6J mice were fasted for 5 h and then weighed. One hour later, mice were anesthetized and blood was isolated. Panels I and J: At 16 weeks of age, following 8 weeks of high-fat feeding, mixed genetic background mice were fasted for 5 h and then weighed. One hour later, mice were anesthetized and blood was isolated. Plasma cholesterol was determined as described in the 'Materials and methods' section. Results are the mean $\pm$ S.E.M. of data with the genotype, gender and number of animals indicated. WT, wild type; KO, knockout; F, female; M, male. * $P < 0.0069$ WT vs KO (Panel D); * $P < 0.02$ WT vs KO (Panel G); * $P < 7.01 \times 10^{-6}$ WT vs KO (Panel H); * $P < 0.001$ WT vs KO (Panel J).

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.

Lab	Population	N	Beta (G)	95% CI	P-value	Allele		
						GG	GA	AA
LDL-C	All	13,087	1.15	0.33–1.96	0.006	102.13±31.43	101.58±31.33	99.08±31.25
LDL-C	Male	5863	1.60	0.4–2.8	0.009	97.5±31.23	95.77±29.41	94.29±32.15
LDL-C	Female	7224	0.82	–0.29–1.93	0.148	105.9±31.09	106.33±32.05	102.73±30.07
Cholesterol	All	14,349	1.00	0.05–1.95	0.039	183.62±38.55	183.25±38.96	181.18±40.03
Cholesterol	Male	6412	1.49	0.09–2.87	0.037	173.36±37.46	171.53±36.79	170.63±38.45
Cholesterol	Female	7937	0.68	–0.59–1.95	0.29	191.97±37.39	192.81±38.06	189.05±39.39
Triglycerides	All	14,213	0.90	–1.48–3.28	0.459	149.92±98.13	149.41±95.16	148.37±99.99
Triglycerides	Male	6398	–0.08	–4.07–3.9	0.967	157.72±109.51	157.17±105.84	157.87±107.95
Triglycerides	Female	7815	1.80	–1.02–4.62	0.211	143.49±87.14	143.00±84.82	141.09±92.86
HDL-C	All	13,457	–0.07	–0.46–0.33	0.746	51.42±17.11	51.36±17.47	51.76±17.56
HDL-C	Male	6006	0.28	–0.22–0.78	0.269	44.00±13.49	43.54±12.93	43.72±13.33
HDL-C	Female	7451	–0.35	–0.94–0.24	0.245	57.43±17.38	57.70±18.09	57.85±17.93

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 $\pm$ 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 FBI 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, Mahajan *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 body 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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References

- Almind K & Kahn CR 2004 Genetic determinants of energy expenditure and insulin resistance in diet-induced obesity in mice. *Diabetes* **53** 3274–3285. (doi:10.2337/diabetes.53.12.3274)
- Arden SD, Zahn T, Steegers S, Webb S, Bergman B, O'Brien RM & Hutton JC 1999 Molecular cloning of a pancreatic islet-specific glucose-6-phosphatase catalytic subunit-related protein. *Diabetes* **48** 531–542. (doi:10.2337/diabetes.48.3.531)
- Aulchenko YS, Ripatti S, Lindqvist I, Boomsma D, Heid IM, Pramstaller PP, Penninx BW, Janssens AC, Wilson JF, Spector T, *et al.* 2009 Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. *Nature Genetics* **41** 47–55. (doi:10.1038/ng.269)
- Baerenwald DA, Bonnefond A, Bouatia-Naji N, Flemming BP, Umunakwe OC, Oeser JK, Pound LD, Conley NL, Cauchi S, Lobbens S, *et al.* 2013 Multiple functional polymorphisms in the *G6PC2* gene contribute to the association with higher fasting plasma glucose levels. *Diabetologia* **56** 1306–1316. (doi:10.1007/s00125-013-2875-3)
- Boortz KA, Syring KE, Dai C, Pound LD, Oeser JK, Jacobson DA, Wang JC, McGuinness OP, Powers AC & O'Brien RM 2016 *G6PC2* modulates fasting blood glucose in male mice in response to stress. *Endocrinology* **157** 3002–3008. (doi:10.1210/en.2016-1245)
- Bouatia-Naji N, Rocheleau G, Van Lommel L, Lemaire K, Schuit F, Cavalcanti-Proenca C, Marchand M, Hartikainen AL, Sovio U, De Graeve F, *et al.* 2008 A polymorphism within the *G6PC2* gene is associated with fasting plasma glucose levels. *Science* **320** 1085–1088. (doi:10.1126/science.1156849)
- Bouatia-Naji N, Bonnefond A, Cavalcanti-Proenca C, Sparso T, Holmkvist J, Marchand M, Delplanque J, Lobbens S, Rocheleau G, Durand E, *et al.* 2009 A variant near *MTNR1B* is associated with increased fasting plasma glucose levels and type 2 diabetes risk. *Nature Genetics* **41** 89–94. (doi:10.1038/ng.277)
- Bouatia-Naji N, Bonnefond A, Baerenwald DA, Marchand M, Bugliani M, Marchetti P, Pattou F, Printz RL, Flemming BP, Umunakwe OC, *et al.* 2010 Genetic and functional assessment of the role of the rs13431652-A and rs573225-A alleles in the *G6PC2* promoter that strongly associate with elevated fasting glucose levels. *Diabetes* **59** 2662–2671. (doi:10.2337/db10-0389)
- Burcelin R, Crivelli V, Dacosta A, Roy-Tirelli A & Thorens B 2002 Heterogeneous metabolic adaptation of C57BL/6J mice to high-fat diet. *American Journal of Physiology: Endocrinology and Metabolism* **282** E834–E842. (doi.org/10.1152/ajpendo.00332.2001)
- Chen WM, Erdos MR, Jackson AU, Saxena R, Sanna S, Silver KD, Timpson NJ, Hansen T, Orru M, Grazia Piras M, *et al.* 2008 Variations in the *G6PC2/ABCB11* genomic region are associated with fasting glucose levels. *Journal of Clinical Investigation* **118** 2620–2628. (doi:10.1172/JCI32723)
- Dupuis J, Langenberg C, Prokopenko I, Saxena R, Soranzo N, Jackson AU, Wheeler E, Glazer NL, Bouatia-Naji N, Gloyn AL, *et al.* 2010 New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. *Nature Genetics* **42** 105–116. (doi:10.1038/ng.520)
- Ebert DH, Bischof LJ, Streeter RS, Chapman SC, Svitek CA, Goldman JK, Mathews CE, Leiter EH, Hutton JC & O'Brien RM 1999 Structure and promoter activity of an islet-specific glucose-6-phosphatase catalytic subunit-related gene. *Diabetes* **48** 543–551. (doi:10.2337/diabetes.48.3.543)
- Eisinger K, Liebisch G, Schmitz G, Aslanidis C, Krautbauer S & Buechler C 2014 Lipidomic analysis of serum from high fat diet

- induced obese mice. *International Journal of Molecular Sciences* **15** 2991–3002. (doi:10.3390/ijms15022991)
- Ellacott KL, Morton GJ, Woods SC, Tso P & Schwartz MW 2010 Assessment of feeding behavior in laboratory mice. *Cell Metabolism* **12** 10–17. (doi:10.1016/j.cmet.2010.06.001)
- Frigeri C, Martin CC, Svitek CA, Oeser JK, Hutton JC, Gannon M & O'Brien RM 2004 The proximal islet-specific glucose-6-phosphatase catalytic subunit related protein (IGRP) autoantigen promoter is sufficient to initiate but not maintain transgene expression in mouse islets in vivo. *Diabetes* **53** 1754–1764. (doi:10.2337/diabetes.53.7.1754)
- Goh BH, Khan A, Efendic S & Portwood N 2006 Expression of glucose-6-phosphatase system genes in murine cortex and hypothalamus. *Hormone and Metabolic Research* **38** 1–7. (doi:10.1055/s-2006-924964)
- Goren HJ, Kulkarni RN & Kahn CR 2004 Glucose homeostasis and tissue transcript content of insulin signaling intermediates in four inbred strains of mice: C57BL/6, C57BLKS/6, DBA/2, and 129X1. *Endocrinology* **145** 3307–3323. (doi:10.1210/en.2003-1400)
- Heni M, Ketterer C, Hart LM, Ranta F, van Haeften TW, Eekhoff EM, Dekker JM, Boomsma DI, Nijpels G, Kramer MH, et al. 2010 The impact of genetic variation in the *G6PC2* gene on insulin secretion depends on glycemia. *Journal of Clinical Endocrinology and Metabolism* **95** E479–E484. (doi:10.1210/jc.2010-0860)
- Hu C, Zhang R, Wang C, Ma X, Wang C, Fang Q, Bao Y, Xiang K & Jia W 2009 A genetic variant of *G6PC2* is associated with type 2 diabetes and fasting plasma glucose level in the Chinese population. *Diabetologia* **52** 451–456. (doi:10.1007/s00125-008-1241-3)
- Hu C, Zhang R, Wang C, Yu W, Lu J, Ma X, Wang J, Jiang F, Tang S, Bao Y, et al. 2010 Effects of *GCK*, *GCKR*, *G6PC2* and *MTNR1B* variants on glucose metabolism and insulin secretion. *PLoS ONE* **5** e11761. (doi:10.1371/journal.pone.0011761)
- Hutton JC & O'Brien RM 2009 The glucose-6-phosphatase catalytic subunit gene family. *Journal of Biological Chemistry* **284** 29241–29245. (doi:10.1074/jbc.R109.025544)
- Ingelsson E, Langenberg C, Hivert MF, Prokopenko I, Lysenko V, Dupuis J, Magi R, Sharp S, Jackson AU, Assimes TL, et al. 2010 Detailed physiologic characterization reveals diverse mechanisms for novel genetic loci regulating glucose and insulin metabolism in humans. *Diabetes* **59** 1266–1275. (doi:10.2337/db09-1568)
- Iynedjian PB 2009 Molecular physiology of mammalian glucokinase. *Cellular and Molecular Life Sciences* **66** 27–42. (doi:10.1007/s00018-008-8322-9)
- Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, et al. 2009 Common variants at 30 loci contribute to polygenic dyslipidemia. *Nature Genetics* **41** 56–65. (doi:10.1038/ng.291)
- Kozak RA, Nikonova L, Hogan J, Rim JS, Mendoza T, Faulk C, Skaf J & Kozak LP 2006 Changes in gene expression foreshadow diet-induced obesity in genetically identical mice. *PLoS Genetics* **2** e81. (doi:10.1371/journal.pgen.0020081)
- Li X, Shu YH, Xiang AH, Trigo E, Kuusisto J, Hartiala J, Swift AJ, Kawakubo M, Stringham HM, Bonnycastle LL, et al. 2009 Additive effects of genetic variation in *Gck* and *G6pc2* on insulin secretion and fasting glucose. *Diabetes* **58** 2946–2953. (doi:10.2337/db09-0228)
- Lu Y, Day FR, Gustafsson S, Buchkovich ML, Na J, Bataille V, Cousminer DL, Dastani Z, Drong AW, Esko T, et al. 2016 New loci for body fat percentage reveal link between adiposity and cardiometabolic disease risk. *Nature Communications* **7** 10495. (doi:10.1038/ncomms10495)
- Mahajan A, Sim X, Ng HJ, Manning A, Rivas MA, Highland HM, Locke AE, Grarup N, Im HK, Cingolani P, et al. 2015 Identification and functional characterization of *G6PC2* coding variants influencing glycemic traits define an effector transcript at the *G6PC2-ABC11* locus. *PLoS Genetics* **11** e1004876. (doi:10.1371/journal.pgen.1004876)
- Martin CC, Bischof LJ, Bergman B, Hornbuckle LA, Hilliker C, Frigeri C, Wahl D, Svitek CA, Wong R, Goldman JK, et al. 2001 Cloning and characterization of the human and rat islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP) genes. *Journal of Biological Chemistry* **276** 25197–25207. (doi:10.1074/jbc.M101549200)
- Matschinsky FM 2005 Glucokinase, glucose homeostasis, and diabetes mellitus. *Current Diabetes Reports* **5** 171–176. (doi:10.1007/s11892-005-0005-4)
- Mazzaccara C, Labruna G, Cito G, Scarfo M, De Felice M, Pastore L & Sacchetti L 2008 Age-related reference intervals of the main biochemical and hematological parameters in C57BL/6J, 129SV/EV and C3H/HeJ mouse strains. *PLoS ONE* **3** e3772. (doi:10.1371/journal.pone.0003772)
- Morgan CR & Lazarow AL 1963 Immunoassay of insulin: two antibody system: plasma insulin of normal, subdiabetic, and diabetic rats. *American Journal of the Medical Sciences* **257** 415–419.
- Morton GJ, Cummings DE, Baskin DG, Barsh GS & Schwartz MW 2006 Central nervous system control of food intake and body weight. *Nature* **443** 289–295. (doi:10.1038/nature05026)
- O'Brien RM 2013 Moving on from GWAS: functional studies on the *G6PC2* gene implicated in the regulation of fasting blood glucose. *Current Diabetes Reports* **13** 768–777. (doi:10.1007/s11892-013-0422-8)
- Oey H, Isbel L, Hickey P, Ebaid B & Whitelaw E 2015 Genetic and epigenetic variation among inbred mouse littermates: identification of inter-individual differentially methylated regions. *Epigenetics and Chromatin* **8** 54. (doi:10.1186/s13072-015-0047-z)
- Petrolonis AJ, Yang Q, Tummino PJ, Fish SM, Prack AE, Jain S, Parsons TF, Li P, Dales NA, Ge L, et al. 2004 Enzymatic characterization of the pancreatic islet-specific glucose-6-phosphatase-related protein (IGRP). *Journal of Biological Chemistry* **279** 13976–13983. (doi:10.1074/jbc.M307756200)
- Pound LD, Sarkar SA, Ustione A, Dadi PK, Shadoan MK, Lee CE, Walters JA, Shiota M, McGuinness OP, Jacobson DA, et al. 2012 The physiological effects of deleting the mouse *slc30a8* gene encoding zinc transporter-8 are influenced by gender and genetic background. *PLoS ONE* **7** e40972. (doi:10.1371/journal.pone.0040972)
- Pound LD, Oeser JK, O'Brien TP, Wang Y, Faulman CJ, Dadi PK, Jacobson DA, Hutton JC, McGuinness OP, Shiota M, et al. 2013 *G6PC2*: a negative regulator of basal glucose-stimulated insulin secretion. *Diabetes* **62** 1547–1556. (doi:10.2337/db12-1067)
- Prokopenko I, Langenberg C, Florez JC, Saxena R, Soranzo N, Thorleifsson G, Loos RJ, Manning AK, Jackson AU, Aulchenko Y, et al. 2008 Variants in *MTNR1B* influence fasting glucose levels. *Nature Genetics* **41** 77–81. (doi:10.1038/ng.290)
- Pulley J, Clayton E, Bernard GR, Roden DM & Masys DR 2010 Principles of human subjects protections applied in an opt-out, de-identified biobank. *Clinical and Translational Science* **3** 42–48. (doi:10.1111/j.1752-8062.2010.00175.x)
- Rasmussen-Torvik LJ, Li M, Kao WH, Couper D, Boerwinkle E, Bielinski SJ, Folsom AR & Pankow JS 2011 Association of a fasting glucose genetic risk score with subclinical atherosclerosis: The Atherosclerosis Risk in Communities (ARIC) study. *Diabetes* **60** 331–335. (doi:10.2337/db10-0839)
- Reiling E, van 't Riet E, Groenewoud MJ, Welschen LM, van Hove EC, Nijpels G, Maassen JA, Dekker JM & Hart LM 2009 Combined effects of single-nucleotide polymorphisms in *GCK*, *GCKR*, *G6PC2* and *MTNR1B* on fasting plasma glucose and type 2 diabetes risk. *Diabetologia* **52** 1866–1870. (doi:10.1007/s00125-009-1413-9)
- Roden DM, Pulley JM, Basford MA, Bernard GR, Clayton EW, Balser JR & Masys DR 2008 Development of a large-scale de-identified DNA biobank to enable personalized medicine. *Clinical Pharmacology and Therapeutics* **84** 362–369. (doi:10.1038/clpt.2008.89)
- Rose CS, Grarup N, Krarup NT, Poulsen P, Wegner L, Nielsen T, Banasik K, Faerch K, Andersen G, Albrechtsen A, et al. 2009 A

- 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)
- Shieh JJ, Pan CJ, Mansfield BC & Chou JY 2003 A glucose-6-phosphate hydrolase, widely expressed outside the liver, can explain age-dependent resolution of hypoglycemia in glycogen storage disease type Ia. *Journal of Biological Chemistry* **278** 47098–47103. (doi:10.1074/jbc.M309472200)
- Surwit RS, Kuhn CM, Cochrane C, McCubbin JA & Feinglos MN 1988 Diet-induced type II diabetes in C57BL/6J mice. *Diabetes* **37** 1163–1167. (doi:10.2337/diab.37.9.1163)
- Tam CH, Ho JS, Wang Y, Lee HM, Lam VK, Germer S, Martin M, So WY, Ma RC, Chan JC, *et al.* 2010 Common polymorphisms in *MTNR1B*, *G6PC2* and *GCK* are associated with increased fasting plasma glucose and impaired beta-cell function in Chinese subjects. *PLoS ONE* **5** e11428. (doi:10.1371/journal.pone.0011428)
- Wall ML, Pound LD, Trenary I, O'Brien RM & Young JD 2015 Novel stable isotope analyses demonstrate significant rates of glucose cycling in mouse pancreatic islets. *Diabetes* **64** 2129–2137. (doi:10.2337/db14-0745)
- Wang Y, Martin CC, Oeser JK, Sarkar S, McGuinness OP, Hutton JC & O'Brien RM 2007 Deletion of the gene encoding the islet-specific glucose-6-phosphatase catalytic subunit-related protein autoantigen results in a mild metabolic phenotype. *Diabetologia* **50** 774–778. (doi:10.1007/s00125-006-0564-1)
- Wang Y, Flemming BP, Martin CC, Allen SR, Walters J, Oeser JK, Hutton JC & O'Brien RM 2008 Long-range enhancers are required to maintain expression of the autoantigen islet-specific glucose-6-phosphatase catalytic subunit-related protein in adult mouse islets in vivo. *Diabetes* **57** 133–141. (doi:10.2337/db07-0092)
- Wang H, Liu L, Zhao J, Cui G, Chen C, Ding H & Wang DW 2013 Large scale meta-analyses of fasting plasma glucose raising variants in *GCK*, *GCKR*, *MTNR1B* and *G6PC2* and their impacts on type 2 diabetes mellitus risk. *PLoS ONE* **8** e67665. (doi:10.1371/journal.pone.0067665)
- Wessel J, Chu AY, Willems SM, Wang S, Yaghootkar H, Brody JA, Dauriz M, Hivert MF, Raghavan S, Lipovich L, *et al.* 2015 Low-frequency and rare exome chip variants associate with fasting glucose and type 2 diabetes susceptibility. *Nature Communications* **6** 5897. (doi:10.1038/ncomms6897)
- Winzell MS & Ahren B 2004 The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. *Diabetes* **53** (Supplement 3) S215–S219. (doi:10.2337/diabetes.53.suppl_3.S215)
- Woods SC, Lutz TA, Geary N & Langhans W 2006 Pancreatic signals controlling food intake; insulin, glucagon and amylin. *Philosophical Transactions of the Royal Society B: Biological Sciences* **361** 1219–1235. (doi:10.1098/rstb.2006.1858)
- Young GS & Kirkland JB 2007 Rat models of caloric intake and activity: relationships to animal physiology and human health. *Applied Physiology, Nutrition, and Metabolism* **32** 161–176. (doi:10.1139/h06-082)

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