G6PC2: A Negative Regulator of Basal Glucose-Stimulated Insulin Secretion

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Elevated fasting blood glucose (FBG) is associated with increased risk for the development of type 2 diabetes and cardiovascular-associated mortality. Genome-wide association studies (GWAS) have linked polymorphisms in *G6PC2* with variations in FBG and body fat, although not insulin sensitivity or glucose tolerance. *G6PC2* encodes an islet-specific, endoplasmic reticulum–resident glucose-6-phosphatase catalytic subunit. A combination of in situ perfused pancreas, in vitro isolated islet, and in vivo analyses were used to explore the function of G6pc2 in mice. *G6pc2* deletion had little effect on insulin sensitivity and glucose tolerance, whereas body fat was reduced in female *G6pc2* knockout (KO) mice on both a chow and high-fat diet, observations that are all consistent with human GWAS data. *G6pc2* deletion resulted in a leftward shift in the dose-response curve for glucose-stimulated insulin secretion (GSIS). As a consequence, under fasting conditions in which plasma insulin levels were identical, blood glucose levels were reduced in *G6pc2* KO mice, again consistent with human GWAS data. Glucose-6-phosphatase activity was reduced, whereas basal cytoplasmic calcium levels were elevated in islets isolated from *G6pc2* KO mice. These data suggest that G6pc2 represents a novel, negative regulator of basal GSIS that acts by hydrolyzing glucose-6-phosphate, thereby reducing glycolytic flux. *Diabetes* 62:1547–1556, 2013

Numerous studies have investigated the biological impact of variations in fasting blood glucose (FBG) levels in humans. Elevated FBG has been shown to be associated with increased risk for the development of type 2 diabetes (1). In addition, a number of studies have found that even mild variations in FBG can have significant consequences on the risk of cardiovascular-associated mortality (CAM). For example, a study performed on a European male population demonstrated that a small increase in FBG levels from just 90 mg/dL to between 99 and 108 mg/dL was associated with a 30% increase in the risk of CAM (2). Conversely, a study performed in an Asian population found that a reduction in FBG levels from 99 to 90 mg/dL was associated with a 25% reduction in CAM (3). The risk of CAM increases still further in individuals with the very high FBG levels characteristic of diabetes (2–5). Because of the apparent biological importance of tightly regulating FBG levels, there has been tremendous interest in understanding how this parameter is controlled.

Recent genome-wide association studies (GWAS) have shed light on this question by demonstrating that single nucleotide polymorphisms (SNPs) within the human *G6PC2* gene are associated with variations in FBG (6,7). These GWAS data are consistent with the observation that global deletion of *G6pc2* in mice on a mixed 129SvEv × C57BL/6J genetic background results in a ~15% reduction in FBG levels compared with wild-type (WT) littermates (8). These observations raise the key question as to how G6pc2 modulates FBG. *G6PC2*, formerly known as *IGRP*, is primarily expressed in islet β-cells and encodes a glucose-6-phosphatase catalytic subunit isoform that catalyzes the hydrolysis of glucose-6-phosphate (G6P) to produce glucose and inorganic phosphate within the endoplasmic reticulum lumen (9,10).

Historically, the biological purpose and even existence of glucose-6-phosphatase activity in islets has been highly controversial (9,11,12). Although there is now general agreement that glucose-6-phosphatase activity is present in pancreatic islets, the issue as to whether the level of activity is enough to result in significant glucose cycling and hence affect glycolytic flux and glucose-stimulated insulin secretion (GSIS) and, therefore, be of physiological significance has remained unclear (13,14). To address this controversy over the importance of glucose-6-phosphatase activity in pancreatic islets, we have analyzed GSIS in *G6pc2* knockout (KO) mice on a pure C57BL/6J genetic background. We provide evidence that G6pc2 acts as a novel, negative regulator of basal GSIS by hydrolyzing G6P and thereby opposing the action of the glucose sensor, glucokinase (15,16). This glucokinase/G6pc2 futile substrate cycle is predicted to reduce glycolytic flux and hence insulin secretion. Consistent with this model and human GWAS data, we show that a reduction in *G6pc2* expression results in a leftward shift in the dose-response curve for GSIS such that under fasting conditions, blood glucose levels are reduced.

RESEARCH DESIGN AND METHODS

Animal care. The animal housing and surgical facilities used for this study meet the standards set by the American Association for the Accreditation of Laboratory Animal Care standards. 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 12 weeks. Food and water were provided ad libitum.

Generation of *G6pc2* KO mice. *G6pc2* KO mice that had been generated on a mixed 129/SvEv[BRD] × C57BL/6J genetic background (8) were backcrossed
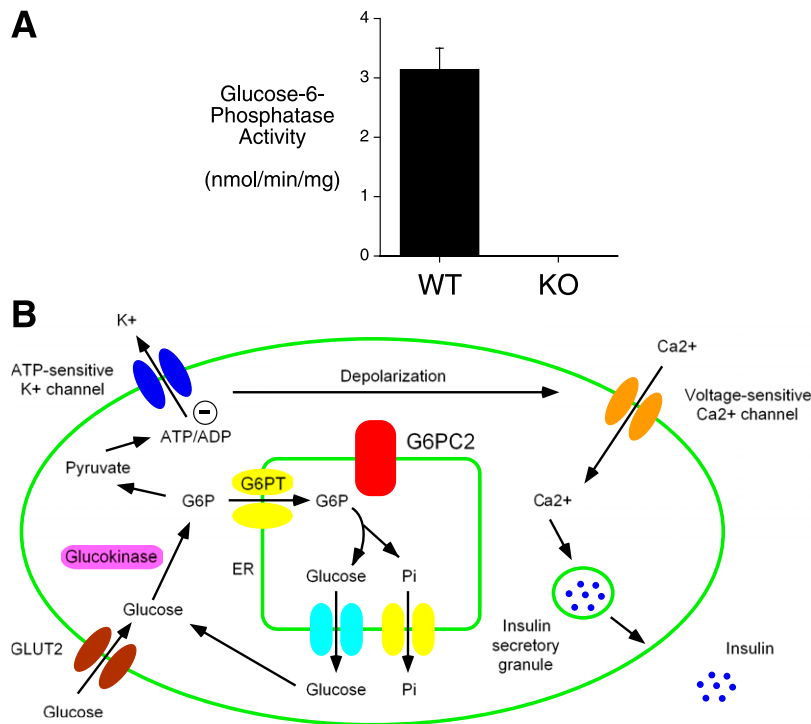
FIG. 1. G6PC2: a negative regulator of basal GSIS. *A*: Glucose-6-phosphatase activity was compared in two independent islet preparations isolated from *G6pc2* WT and KO mice as described in RESEARCH DESIGN AND METHODS. The results show mean glucose-6-phosphatase activity ± SD. *B*: A simplified model for GSIS proposing the existence of a glucokinase/G6PC2 futile cycle. The best-characterized pathway for GSIS is shown, although other pathways clearly contribute (40). Overexpression of *Gck* increases glycolytic flux and results in a leftward shift in the $S_{0.5}$ for GSIS (31,32). We hypothesize that G6PC2 is a negative regulator of basal GSIS such that a reduction in *G6PC2* expression augments glycolytic flux and causes a leftward shift in the dose-response curve for GSIS. The G6P transporter (G6PT) is a G6P–Pi antiporter (41). ER, endoplasmic reticulum.

onto a pure C57BL/6J genetic background using a speed congenic breeding strategy (17).

Radioisotopic glucose-6-phosphatase assay in permeabilized islets. Isolated islets (~100) were precipitated by centrifugation, resuspended in 20 µL 10% (weight for volume) sucrose in 10 mmol MES pH 6.5 buffer, and permeabilized by freeze/thawing. Glucose-6-phosphatase assays were performed for 2 h at 30°C in a final volume of 120 µL containing 4 mmol/L G6P, 150 mmol/L 2-glycerophosphate, 100 mmol/L MES pH 6.5, and 1 µCi [U-C14] G6P (262 mCi/mmol; Moravek Biochem, Brea, CA). A total of 200 µL 10% (weight for volume) sucrose in 10 mmol/L MES pH 6.5 was then added to each tube, the contents sonicated for 1 min (sonication water bath), and then mixed with 245 µL 1 mol/L $ZnSO_4$ followed by 285 µL $Ba(OH)_2$ and centrifugation at 20,000 g at 4°C for 5 min. A 300-µL sample of the supernatant was mixed with 3 mL Microscint scintillation fluid (PerkinElmer) and radioactivity determined by liquid scintillation counting. Enzyme activity was expressed as namomoles G6P hydrolyzed per minute per milligram cellular protein. Protein was determined by Bio-Rad dye binding assay (Bio-Rad) using BSA as standard.

Pancreas perfusions. In situ pancreas perfusions were performed on ~14-week-old mice following a 3-h fast according to the method of Bonnevie-Neilsen et al. (18), modified as previously described (19).

Intraperitoneal and oral glucose tolerance tests, insulin tolerance tests, islet isolations, and GSIS assays. Intraperitoneal glucose tolerance tests (IPGTTs), oral glucose tolerance tests (OGTTs), insulin tolerance tests, and islet isolations for GSIS assays were performed on ~13-week-old male mice as previously described (20).

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 isofluorane, and blood samples were isolated from the retro-orbital venous plexus. Glucose concentrations were measured in whole blood using a glucose monitor (Accu-Chek Advantage; Roche, Indianapolis, IN). EDTA (5 µL; 0.5 mol/L) was then added to blood samples prior to isolation of plasma by centrifugation. Insulin samples were assayed using radioimmunoassay (21) by the Vanderbilt Diabetes Research and Training Center Hormone Assay & Analytical Services Core.

Measurement of cytoplasmic calcium. Islet cytoplasmic calcium concentration was measured with the Ca^{2+}-sensitive dye fura-2 acetoxymethyl ester (Molecular Probes, Eugene, OR) as previously described (22). Briefly, dispersed mouse islet cells were dye-loaded by incubation for 20 min at 37°C in

a pH 7.3 solution containing 2 µmol/L fura-2, 119 mmol/L NaCl, 2.5 mmol/L $CaCl_2 \cdot [(H_2O)_6]$, 4.7 mmol/L KCl, 10 mmol/L HEPES, 1.2 mmol/L $MgSO_4$, 1.2 mmol/L KH_2PO_4, and 5.6 mmol/L glucose. Fluorescence imaging was performed using a Nikon Eclipse TE2000-U microscope equipped with an epi-fluorescent illuminator (Sutter Instruments, Novato, CA), a CoolSNAP HQ2 camera (Photometrics, Tucson, AZ), and Nikon Elements software (Nikon, Tokyo, Japan). The cytoplasmic calcium concentration ratios of emitted fluorescence intensities at excitation wavelengths of 340 and 380 nm ($F340/F380$) were determined every 5 s with background subtraction. Cells were perifused at 37°C at a flow of 2 mL/min; the solutions used during the experiments were the same as the loading solution.
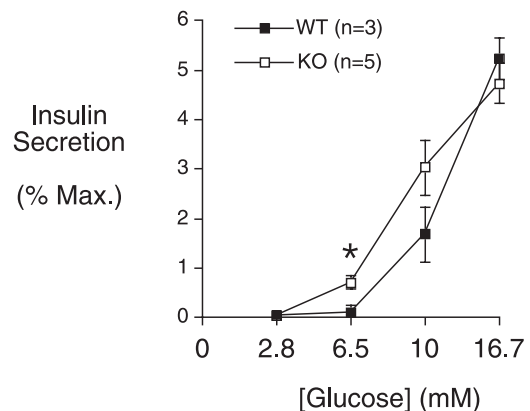

FIG. 2. In situ perfused pancreas experiments demonstrate that *G6pc2* deletion results in a leftward shift in the dose-response curve for GSIS. GSIS from perfused ~14-week-old male WT and *G6pc2* KO mouse pancreata was assayed in situ as described in RESEARCH DESIGN AND METHODS. The results show the mean insulin concentrations ± SEM determined using three WT and five KO animals. *P < 0.05 vs. WT. Max., maximum.

RESULTS

Analysis of glucose-6-phosphatase activity in *G6pc2* KO mouse islets in vitro. Petrolonis et al. (10) have previously shown that G6PC2 can hydrolyze G6P. Consistent with this observation, Fig. 1A shows that glucose-6-phosphatase activity is reduced in islets isolated from *G6pc2* KO mice. The level of glucose-6-phosphatase activity in islets (Fig. 1A) is much lower than in liver, again consistent with published data (9,11,12). Based on its ability to hydrolyze G6P, we hypothesized that *G6pc2* opposes the action of the glucose

sensor glucokinase, thereby creating a futile substrate cycle and reducing glycolytic flux, the ATP/ADP ratio, and hence insulin secretion (Fig. 1B). If correct, deletion of *G6pc2* should result in a leftward shift in the dose-response curve for GSIS.

Analysis of GSIS from perfused *G6pc2* KO mouse pancreata in situ. To begin to assess our hypothesis, we measured insulin secretion in response to varying glucose concentrations from perfused pancreata in situ. When challenged with a 6.5 mmol/L submaximal concentration of glucose, the pancreata of *G6pc2* KO mice secreted ~2.5-fold more insulin than WT mouse pancreata (Fig. 2). A trend toward increased insulin secretion was also observed at 10 mmol/L glucose (Fig. 2). These data suggest that there is
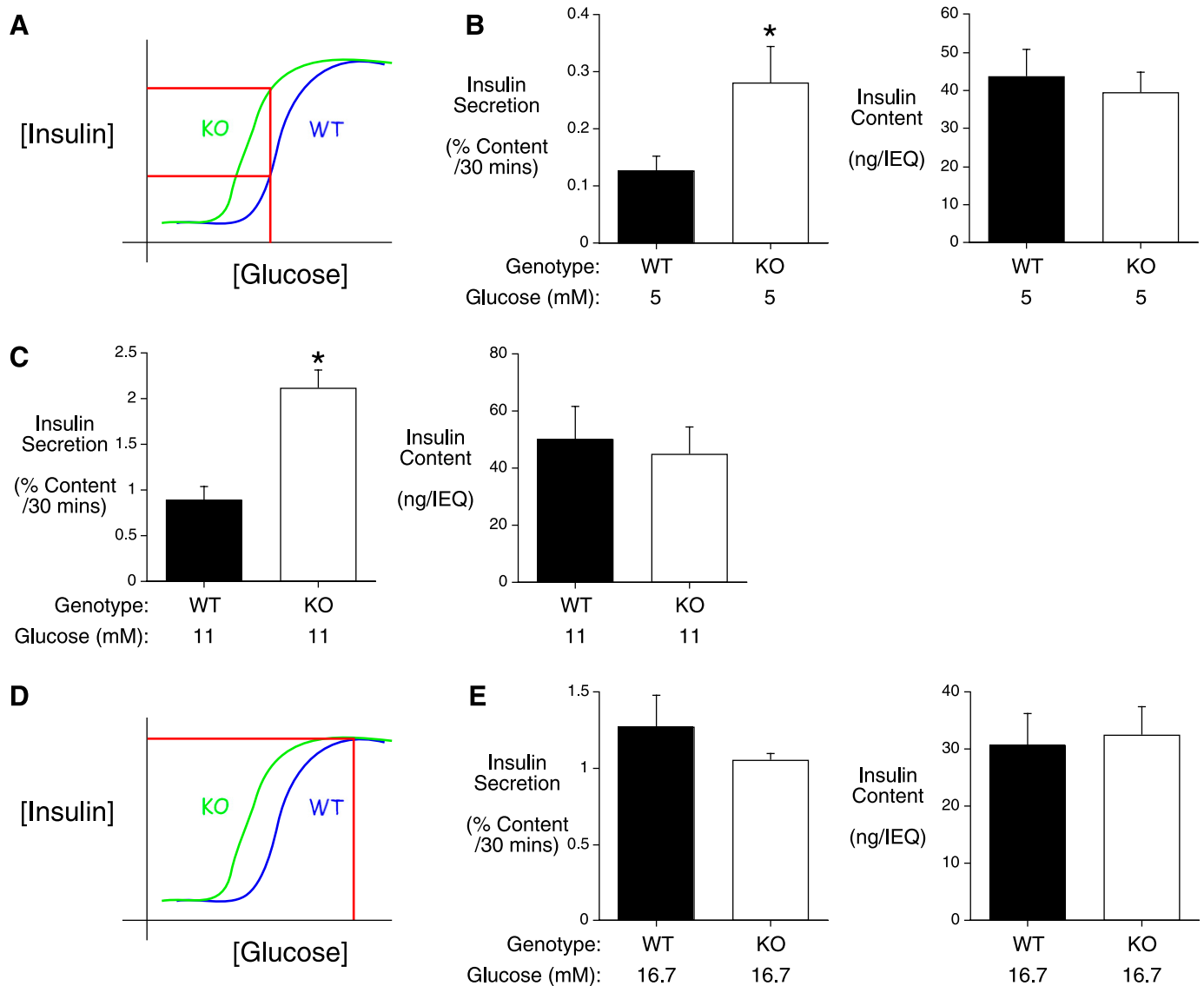

FIG. 3. In vitro isolated islet experiments demonstrate that *G6pc2* deletion results in a leftward shift in the dose-response curve for GSIS. *A* and *D*: Schematics to explain the effect of *G6pc2* deletion on the dose-response curve for GSIS. The model in *A* proposes that at submaximal glucose concentrations, insulin secretion from *G6pc2* KO mouse islets will be enhanced relative to WT islets due to a leftward shift in the dose-response curve for GSIS. The model in *D* proposes that at high glucose concentrations, flux through glucokinase is much greater than flux through G6pc2 such that deletion of *G6pc2* does not affect the V_{max} of GSIS. *B*, *C*, and *E*: GSIS from and insulin content in isolated ~13-week-old male WT and *G6pc2* KO mouse islets were assayed in vitro as described in RESEARCH DESIGN AND METHODS following stimulation with 5 (*B*), 11 (*C*), or 16.7 mmol/L (*E*) glucose. The results in *B*, *C*, and *E* show the mean insulin concentrations ± SEM determined using six (at 5 mmol/L) or three (at 11 and 16.7 mmol/L) independent islet preparations. The experiments shown in *B*, *C*, and *E* were done at separate times and because the actual level of insulin secretion can vary significantly between different islet preparations the level of secretion between the graphs is not directly comparable. *$P < 0.05$ vs. WT. IEQ, islet equivalents.
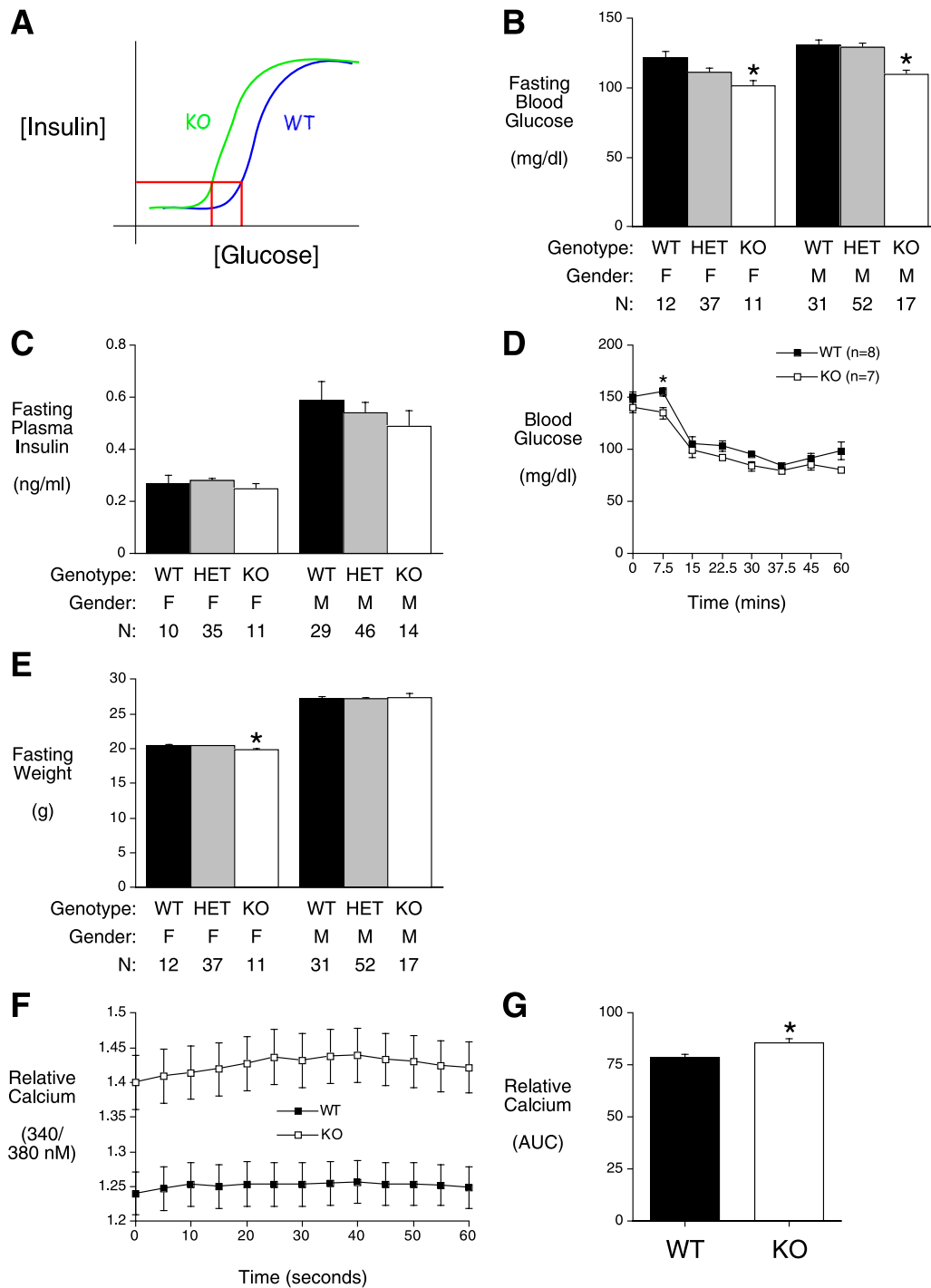
FIG. 4. FBG is reduced in chow-fed *G6pc2* KO mice. *A*: A schematic to explain the effect of *G6pc2* deletion on FBG levels. The model proposes that in 6-h–fasted mice, in which insulin levels are the same in WT and *G6pc2* KO mice (*C*), FBG is reduced in *G6pc2* KO mice due to a leftward shift in the dose-response curve for GSIS relative to WT mice. *B*, *C*, and *E*: At 17 weeks of age, mice were fasted for 5 h and then weighed (*E*). Weight results are the mean ± SEM of data from the following number of animals: male WT, 31; male heterozygous (HET), 52: male KO, 17; female WT, 12; female HET, 37; and female KO, 11. One hour later, mice were anesthetized and blood isolated. Blood glucose (*B*) and plasma insulin (*C*) were determined as described in RESEARCH DESIGN AND METHODS. Glucose results are the mean ± SEM of data from the following number of animals: male WT, 31; male HET, 52; male KO, 17; female WT, 12; female HET, 37; and female KO, 11. Insulin results are the mean ± SEM of data from the following number of animals: male WT, 29; male HET, 46; male KO, 14; female WT, 10; female HET, 35; and female KO, 11. *D*: Insulin tolerance tests using 0.75 units/kg insulin were performed on 5-h–fasted conscious ~14-week-old WT and *G6pc2* KO male mice as described in RESEARCH DESIGN AND METHODS. The results show the mean glucose concentrations ± SEM determined using eight WT and seven KO animals. *F* and *G*: Cytoplasmic calcium levels at 5.6 mmol/L glucose were compared in dispersed cells generated from three independent WT and *G6pc2* KO mouse islet preparations as described in RESEARCH DESIGN AND METHODS. Results are the mean ± SEM of 288 measurements from WT cells and 236 measurements from KO cells. *$P < 0.05$ vs.WT. AUC, area under the curve; F, female; M, male.

a leftward shift in the dose-response curve for GSIS in *G6pc2* KO mice.

Analysis of GSIS in isolated *G6pc2* KO mouse islets in vitro. To directly assess the role of G6pc2 in islet function, GSIS from isolated male WT and *G6pc2* KO mouse islets was compared in static 30-min incubations. Islets were stimulated with submaximal glucose concentrations (5 and 11 mmol/L) to address the hypothesis that deletion of *G6pc2* affects the dose-response curve for GSIS (Fig. 3*A*). GSIS from *G6pc2* KO mouse islets was enhanced ~2.2-fold at 5 mmol/L glucose (Fig. 3*B*) and ~2.2-fold at 11 mmol/L glucose (Fig. 3*C*) relative to GSIS from WT mouse islets. No differences in insulin content were detected between WT and *G6pc2* KO mouse islets (Fig. 3*B* and *C*). These observations are consistent with the in situ pancreas perfusion data (Fig. 2) and support the presence of a leftward shift in the dose-response curve for GSIS following *G6pc2* deletion (Fig. 3*A*).

Because the extent of organ perfusion is highly variable between individual pancreata, the data in Fig. 2 have to be expressed as a percentage of the insulin secretion observed in the presence of 16.7 mmol/L glucose and 20 mmol/L arginine. As such, the pancreas perfusion assay cannot provide information as to whether the absence of G6pc2 also affects the maximum rate (V_{max}) of GSIS. We hypothesized that at high glucose concentrations, flux through glucokinase would be much greater than flux through G6pc2 such that deletion of *G6pc2* would not affect the V_{max} of GSIS (Fig. 3*D*). To address this hypothesis, islets were stimulated with a high glucose concentration (16.7 mmol/L). Fig. 3*E* shows that no difference in insulin secretion or insulin content was observed between WT and *G6pc2* KO mouse islets at 16.7 mmol/L glucose. This observation suggests that the absence of G6pc2 does not affect the V_{max} of GSIS.

Analysis of FBG in *G6pc2* KO mice in vivo and basal cytoplasmic calcium in *G6pc2* KO mouse islets in vitro. If G6pc2 only affects the dose-response curve for GSIS, it would be predicted that, under fasting conditions, insulin levels should be identical regardless of the level of *G6pc2* expression, and a leftward shift in the dose-response curve for GSIS should result in a reduction in FBG in *G6pc2* KO mice in vivo (Fig. 4*A*). Fig. 4*B* shows that, following a 6-h fast, blood glucose levels were indeed reduced in both male and female *G6pc2* KO mice relative to their WT littermates (16.4 and 14.4%, respectively), whereas fasting insulin levels were unchanged (Fig. 4*C*). This reduction in FBG is consistent with our previous data obtained with mixed genetic background mice (8). The absence of a difference in fasting insulin is consistent with the results of insulin tolerance tests showing little difference in insulin sensitivity between male WT and *G6pc2* KO

mice (Fig. 4*D*) and no differences in body weights between male mice (Fig. 4*E*). Similarly, human GWAS data show no association between *G6PC2* and insulin sensitivity (23–26).

Our model (Fig. 1*B*) predicts that this reduction in FBG resulting from the absence of G6pc2 should be associated with enhanced glycolytic flux and hence cytoplasmic calcium levels. Fig. 4*F* and *G* demonstrate that basal cytoplasmic calcium levels are enhanced in islets isolated from *G6pc2* KO mice.

Overall, these observations are consistent with the in situ pancreas perfusion data (Fig. 2) and in vitro–isolated islet data (Fig. 3*B* and *C*) and further support the presence of a leftward shift in the dose-response curve for GSIS following *G6pc2* deletion.

Analysis of the effect of *G6pc2* deletion on body weight and composition. Human GWAS data show that the SNP within the *G6PC2* gene (specifically the A allele of rs560887) that is associated with reduced fasting glycemia is also associated with reduced BMI and body fat (23). No differences in weight (Fig. 4*E*) or body fat (Table 1) were observed between chow-fed male *G6pc2* KO mice and WT littermates (Fig. 4*E*) following a 6-h fast. In contrast, chow-fed female *G6pc2* KO mice were slightly lighter than WT littermates (Fig. 4*E*) and had reduced body fat (Table 1).

To further explore this difference in body fat, we investigated the effect of high-fat feeding, a standard nutritional challenge in the field of obesity and diabetes research that induces insulin resistance and is considered to model human disease (27). High-fat feeding was started at 8 weeks of age and continued for 12 weeks. Weekly measurements of body weight in nonfasted mice showed that female (Fig. 5*A*) but not male (Fig. 5*B*) *G6pc2* KO mice were protected from diet-induced obesity, relative to WT and heterozygous mice. An analysis of body weights and fat composition following a 6-h fast confirmed that high-fat–fed female *G6pc2* KO mice were lighter than WT littermates (Fig. 5*C*), and a difference in body fat was again apparent (Table 2). These results are consistent with the human GWAS data (23), at least with respect to female mice.

FBG (Fig. 5*D*) and plasma insulin (Fig. 5*E*) were both markedly elevated in high-fat–fed mice compared with chow-fed animals (Fig. 4*B* and *C*), consistent with the presence of insulin resistance. In high-fat–fed mice there was no difference in fasting plasma insulin between WT and *G6pc2* KO mice (Fig. 5*E*), whereas FBG was higher in WT mice (Fig. 5*D*), again consistent with the chow-fed mouse data (Fig. 4*B* and *C*) and a leftward shift in the dose-response curve for GSIS.

Analysis of GSIS in *G6pc2* KO mice in vivo during glucose tolerance tests. Glucose tolerance tests were performed to determine the effect of *G6pc2* deletion on

TABLE 1
Nuclear magnetic resonance analysis of chow-fed *G6pc2* KO mouse body composition

Gender and genotype	Fat (g)	Muscle (g)	Free fluid (g)	Fat (%)	Muscle (%)	Free fluid (%)
Female WT	1.68 ± 0.09 (11)	15.7 ± 0.3 (11)	0.50 ± 0.04 (11)	8.3 ± 0.5 (11)	77.3 ± 1.0 (11)	2.47 ± 0.18 (11)
Female $^{-/+}$	1.52 ± 0.06 (33)	15.9 ± 0.2 (33)	0.52 ± 0.03 (33)	7.5 ± 0.3 (33)	78.6 ± 0.3 (33)	2.56 ± 0.12 (33)
Female KO	1.28 ± 0.07 (10)***	15.4 ± 0.2 (10)	0.47 ± 0.03 (10)	6.6 ± 0.4 (10)***	79.2 ± 0.6 (10)	2.46 ± 0.15 (10)
Male WT	1.98 ± 0.11 (18)	20.4 ± 0.3 (18)	0.34 ± 0.03 (18)	7.6 ± 0.4 (18)	77.7 ± 0.4 (18)	1.29 ± 0.11 (18)
Male $^{-/+}$	2.20 ± 0.09 (36)	20.4 ± 0.2 (36)	0.32 ± 0.02 (36)	8.3 ± 0.3 (36)	77.1 ± 0.3 (36)	1.20 ± 0.06 (36)
Male KO	1.96 ± 0.18 (9)	19.9 ± 0.5 (9)	0.25 ± 0.03 (9)	7.6 ± 0.7 (9)	77.3 ± 0.5 (9)	0.96 ± 0.13 (9)

Body composition was assessed using an mq10 NMR analyzer (Bruker Optics) on 6-h–fasted animals at 16 weeks of age. Results are means ± SEM obtained from the number of animals indicated in parentheses. $^{-/+}$, heterozygous. *$P < 0.01$ for female WT vs. KO. **$P < 0.05$ for female heterozygous vs. KO. ***$P < 0.05$ for female WT vs. KO.
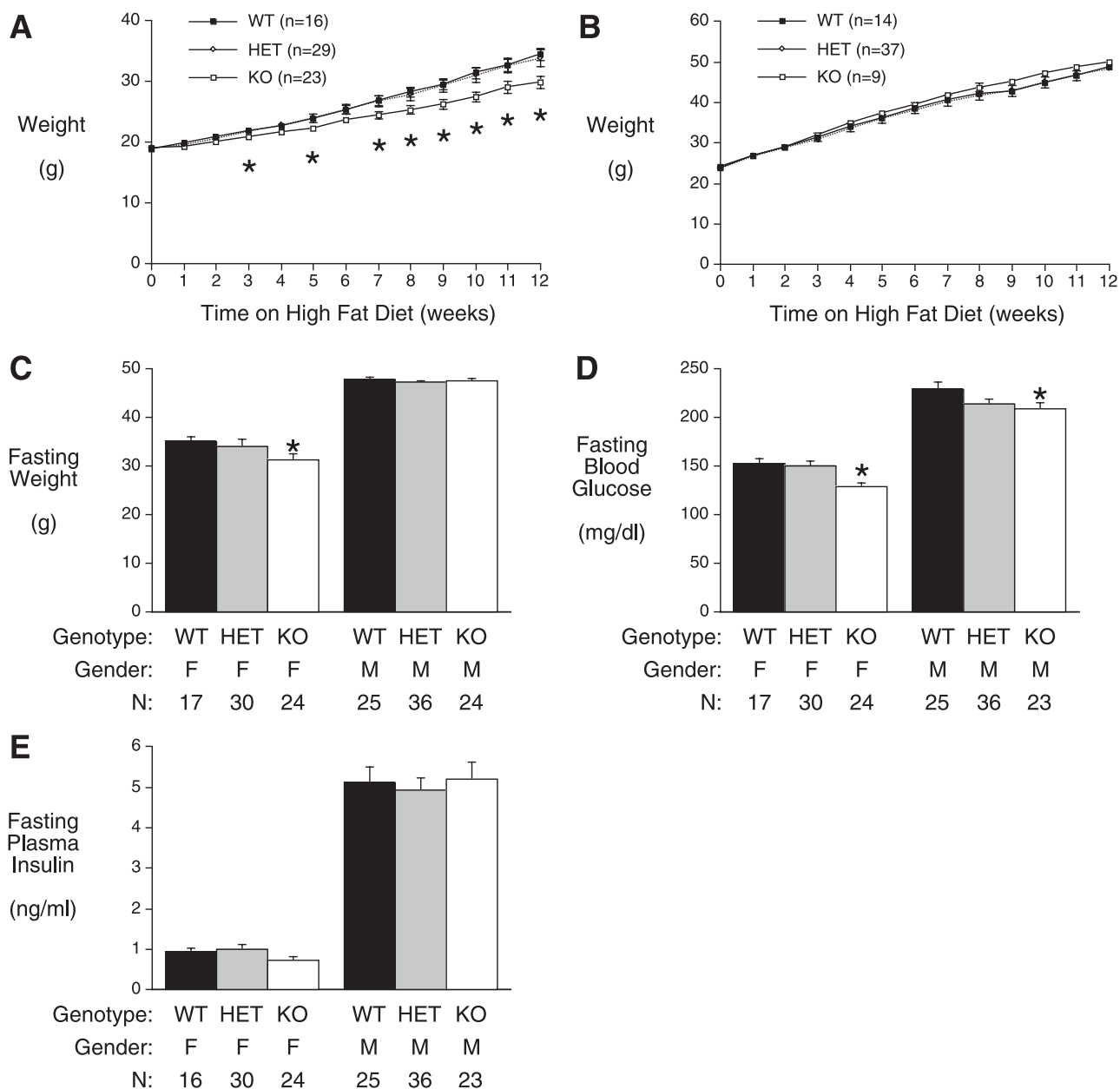
FIG. 5. FBG is reduced in high-fat–fed *G6pc2* KO mice. Starting at 8 weeks of age, female (*A*) and male (*B*) mice were fed a high-fat diet with nonfasting body weights measured weekly. Results are the mean ± SEM of data from the following number of animals: male WT, 14; male heterozygous (HET), 37; male KO, 9; female WT, 16; female HET, 29; and female KO, 23. *C–E*: At 21 weeks of age, mice were fasted for 5 h and then weighed (*C*). One hour later, mice were anesthetized and blood isolated. Weight results are the mean ± SEM of data from the following number of animals: male WT, 25; male HET, 36; male KO, 24; female WT, 17; female HET, 30; and female KO, 24. Blood glucose (*D*) and plasma insulin (*E*) were determined as described in RESEARCH DESIGN AND METHODS. Glucose results are the mean ± SEM of data from the following number of animals: male WT, 25; male HET, 36; male KO, 23; female WT, 17; female HET, 30; and female KO, 24. Insulin results are the mean ± SEM of data from the following number of animals: male WT, 25; male HET, 36; male KO, 23; female WT, 16; female HET, 30; and female KO, 24. *P < 0.05 vs. WT. F, female; M, male.

insulin secretion in mice in vivo following a glucose challenge. Because *G6pc2* deletion resulted in no change in GSIS in isolated islets at high glucose concentrations (Fig. 3*E*), we hypothesized that no difference in glucose tolerance or insulin secretion would be detected between *G6pc2* KO mice and WT mice following a challenge with a high glucose concentration. IPGTTs were performed to explore this hypothesis. Following a 6-h fast, male WT and KO mice were injected with a 2.0 g/kg body weight dose of glucose, and blood glucose was then measured over a 120-min period. Fig. 6*A* shows that surprisingly a slight impairment in glucose tolerance was observed in *G6pc2* KO

mice relative to WT mice, but only at one time point. We next directly measured insulin secretion 15 min following intraperitoneal injection of 2.0 g/kg glucose. Fig. 6*B–D* demonstrate that no difference in insulin secretion was detected between *G6pc2* KO mice relative to WT mice. This observation is consistent with isolated islet experiments that showed no difference in insulin secretion between WT and *G6pc2* KO mouse islets at high glucose concentrations (Fig. 3*E*).

Because *G6pc2* deletion resulted in improved GSIS in perfused pancreata (Fig. 2) and in isolated islets (Fig. 3) at submaximal glucose concentrations, we hypothesized that

TABLE 2
Nuclear magnetic resonance analysis of high-fat–fed *G6pc2* KO mouse body composition

Gender and genotype	Fat (g)	Muscle (g)	Free fluid (g)	Fat (%)	Muscle (%)	Water (%)
Female WT	13.0 ± 0.6 (14)	19.4 ± 0.2 (14)	0.64 ± 0.02 (14)	37.8 ± 1.1 (14)	56.9 ± 0.8 (14)	1.90 ± 0.09 (14)
Female$^{-/+}$	11.9 ± 1.1 (29)	19.0 ± 0.3 (29)	0.59 ± 0.02 (29)	34.1 ± 2.2 (29)	59.9 ± 1.6 (29)	1.84 ± 0.06 (29)
Female KO	10.2 ± 1.1 (24)	18.5 ± 0.2 (24)*	0.60 ± 0.02 (24)	31.2 ± 2.4 (24)***	61.7 ± 1.7 (24)****	1.99 ± 0.07 (24)
Male WT	20.6 ± 0.3 (11)	25.2 ± 0.4 (11)	0.70 ± 0.05 (11)	42.9 ± 0.5 (11)	52.3 ± 0.4 (11)	1.44 ± 0.09 (11)
Male$^{-/+}$	20.1 ± 0.2 (33)	24.8 ± 0.2 (33)	0.74 ± 0.02 (33)	42.6 ± 0.3 (33)	52.5 ± 0.2 (33)	1.57 ± 0.05 (33)
Male KO	20.9 ± 0.5 (9)	25.5 ± 0.3 (9)**	0.74 ± 0.04 (9)	42.8 ± 0.6 (9)	52.3 ± 0.4 (9)	1.52 ± 0.07 (9)

Body composition was assessed using an mq10 NMR analyzer (Bruker Optics) on 6-h–fasted animals at 20 weeks of age following 12 weeks of high-fat feeding. Results are means ± SEM obtained from the number of animals indicated in parentheses. $^{-/+}$, heterozygous. *$P < 0.05$ for female WT vs. KO. **$P < 0.05$ for male heterozygous vs. KO. ***$P < 0.05$ for female WT vs. KO. ****$P < 0.05$ for female WT vs. KO.

glucose tolerance would be improved in the *G6pc2* KO mice relative to WT mice following a challenge with a moderate glucose concentration due to enhanced insulin secretion. IPGTTs were performed to explore this hypothesis. Following a 6-h fast, male WT and KO mice were injected with a 0.4 g/kg body weight dose of glucose, and blood glucose was then measured over a 90-min period. Fig. 6*E* shows that a slight improvement in glucose tolerance was observed in the *G6pc2* KO mice relative to WT mice. We next directly measured insulin secretion 15 min following intraperitoneal injection of 0.4 g/kg glucose. Fig. 6*F–H* show that no difference in insulin secretion was detected between *G6pc2* KO mice and WT mice. When IPGTTs were repeated using a higher 0.75 g/kg body weight dose of glucose, no differences in glucose tolerance or insulin secretion were detected between *G6pc2* KO and WT mice (Figs. 6*I–L*).

OGTTs were performed to further explore the effect of *G6pc2* deletion on insulin secretion in vivo. Following a 6-h fast, male WT and *G6pc2* KO mice were challenged with a 2.0 g/kg body weight dose of glucose administered by oral gavage, and blood glucose was then measured over a 120-min period. Fig. 6*M* shows that a small improvement in glucose tolerance was observed in the *G6pc2* KO mice relative to WT mice. We next directly measured insulin secretion 15 min following oral gavage with 2.0 g/kg glucose. Fig. 6*N–P* show that no difference in insulin secretion was detected between *G6pc2* KO mice and WT mice. Overall, these data show that deletion of *G6pc2* has surprisingly little effect on glucose tolerance or insulin secretion in vivo. Potential explanations for the absence of a change in insulin secretion are discussed below; however, the absence of marked changes in glucose tolerance is consistent with human GWAS data showing no association between *G6PC2* and glucose tolerance (23–26), and it suggests that G6pc2 primarily regulates FBG in vivo.

DISCUSSION

In the current study, we have investigated the effect of a global deletion of *G6pc2* in vivo, a mouse model that is directly relevant to the control of FBG in humans (6,7). Data from in situ pancreas perfusion (Fig. 2), in vitro isolated islet (Fig. 3), and in vivo fasting studies (Fig. 4) demonstrate that deletion of *G6pc2* in C57BL/6J mice results in a leftward shift in the dose-response curve for GSIS. This results in increased insulin secretion at submaximal glucose concentrations (Figs. 2 and 3) and reduced blood glucose levels during fasting (Fig. 4). Importantly, these observations are consistent with human GWAS data in which the rs560887-A and rs13431652-G

alleles, which are predicted to reduce *G6PC2* expression, were found to associate with reduced FBG levels (6,7,28). These data are also consistent with a model in which G6PC2 opposes the action of glucokinase, the β-cell glucose sensor (15,16), by hydrolyzing G6P (Fig. 1). This would create a futile cycle, thereby reducing glycolytic flux, the ATP/ADP ratio, and hence GSIS. To support this conclusion, future studies will directly compare glucose cycling and glycolytic flux in WT and *G6pc2* KO mouse islets. The latter will be important because the consumption of ATP by glucokinase in β-cells is negligible when compared with ATP consumption by Na^+/K^+ ATPase and other ATPase pumps (29). As such, we hypothesize that the absence of G6pc2 affects ATP levels in β-cells by enhancing glycolytic flux (Fig. 1) rather than ATP consumption by glucokinase. This model is consistent with the concept that glucose phosphorylation rather than transport is the major control point in GSIS (15). This conclusion is supported by studies demonstrating that a reduction in glucose transporter 2 by >90% is required to affect GSIS (30). In contrast, overexpression of glucokinase increases GSIS (31,32), while reducing glucokinase expression impairs GSIS (33). Although overexpression of G6pc2 leads to diabetes, this is likely due to the induction of endoplasmic reticulum stress rather than a direct effect on the kinetics of GSIS (34).

Human GWAS data show that *G6PC2* is also associated with altered body fat and BMI (23). Both chow-fed (Table 1 and Fig. 4*E*) and high-fat–fed (Table 2 and Fig. 5*C*) female *G6pc2* KO mice show reduced body fat and body weight, consistent with the human GWAS data (23). The reason why similar differences are not observed in male mice is unclear. Of greater interest will be future studies exploring how deletion of *G6pc2*, which is selectively expressed in islets (9), could impact body fat and body weight.

Neither IPGTTs nor OGTTs show major differences in glucose tolerance or insulin secretion between WT and *G6pc2* KO mice over a range of glucose concentrations (Fig. 6). These observations were surprising given the expectation from the perfused pancreas (Fig. 2) and isolated islet (Fig. 3) data that increased insulin secretion at submaximal glucose concentrations would result in improved glucose tolerance in *G6pc2* KO mice. However, these observations are consistent with human GWAS data that show no association between *G6PC2* and glucose tolerance (23–26). These data are not explained by a difference in insulin sensitivity in *G6pc2* KO mice (Fig. 4*D*). Similarly, *G6PC2* is not associated with variations in insulin sensitivity in humans (23–26). Although these data suggest that *G6pc2* primarily regulates FBG in vivo, the
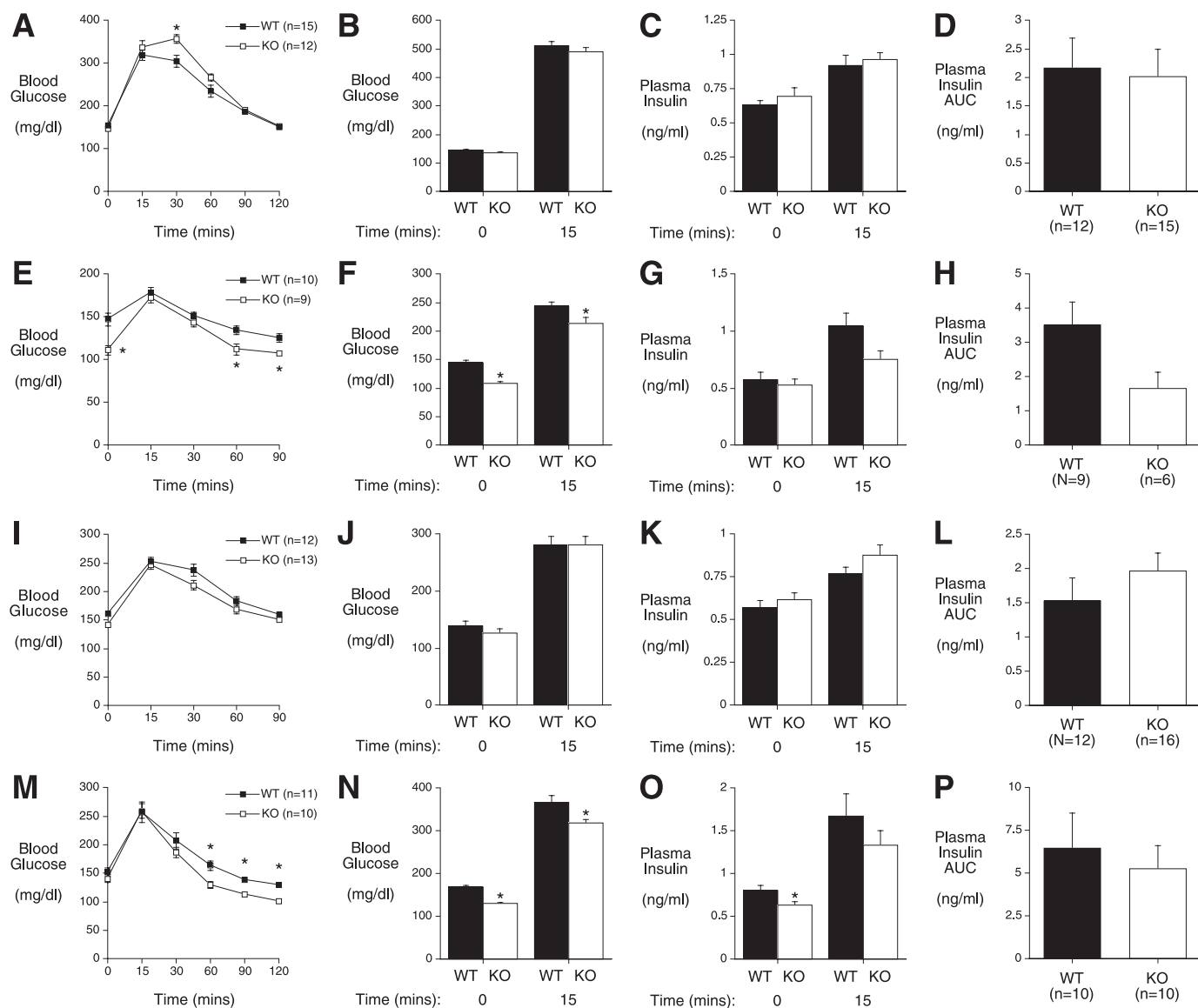
FIG. 6. *G6pc2* deletion has complex effects on glucose tolerance and insulin secretion in vivo. IPGTTs using 2.0 g/kg glucose (*A–D*), 0.4 g/kg glucose (*E–H*), or 0.75 g/kg glucose (*I–L*) and OGTTs using 2.0 g/kg glucose (*M–P*) were performed on 6-h–fasted conscious WT and *G6pc2* KO male mice as described in RESEARCH DESIGN AND METHODS. The results show the mean glucose or insulin concentrations ± SEM. The number of animals used in experiments in which only glucose was measured were as follows: *A*: 15 WT, 12 KO; *E*: 10 WT, 9 KO; *I*: 12 WT, 13 KO; and *M*: 11 WT, 10 KO. The number of animals used in experiments in which both glucose and insulin were measured were as follows: *B–D*: 12 WT, 15 KO; *F–H*: 9 WT, 6 KO; *J–L*: 12 WT, 16 KO; and *N–P*: 10 WT, 10 KO. *$P < 0.05$ vs. WT. AUC, area under the curve.

key issue is why insulin secretion is not enhanced in *G6pc2* KO mice during submaximal glucose tolerance tests. Insight into this question is provided by human GWAS data showing that the SNP within the *G6PC2* gene (specifically the A allele of rs560887) that is associated with reduced fasting glycemia is also associated with a paradoxical reduction in insulin secretion during glucose tolerance tests (23–26). This observation is paradoxical for two reasons. First, based on its association with reduced fasting glycemia it was expected that the A allele of rs560887 would be associated with increased rather than reduced insulin secretion in vivo. Second, as mentioned above, despite the association with reduced insulin secretion in vivo, this SNP is not associated with a counterbalancing change in glucose tolerance or insulin sensitivity (23–26). These data suggest that *G6pc2* not only opposes the action of glucokinase but also influences other aspects

of β-cell function and GSIS. The observed reduction in insulin secretion during glucose tolerance tests in humans has been hypothesized to indicate that either G6PC2 affects the pulsatility of insulin secretion (23) or that it affects hepatic glucose production rather than β-cell function (26). The latter explanation appears less likely since human *G6PC2* (6,35) and mouse *G6pc2* (9,36) are only expressed in islets and not in liver. A similar reduction in insulin secretion was not observed in *G6pc2* KO mice during glucose tolerance tests (Fig. 6), but this may simply be due to the relatively low number of animals analyzed ($n = 6$–16) relative to the vast number of humans analyzed in GWAS studies ($n > 5,000$) (23) and the fact that significant variations in insulin sensitivity, and hence insulin secretion, are observed even within inbred C57BL/6J mice (37). Based on these observations in humans and mice, future studies will be designed to assess whether the

absence of G6pc2 influences other aspects of β-cell function such as pulsatile insulin secretion.

The severe diabetic phenotype observed in mice with a β-cell–specific deletion of the *Gck* gene (38) contrasts with the mild phenotype of *G6pc2* KO mice (Table 1), suggesting that glucokinase is the major determinant of glycolytic flux with G6pc2 playing a modulatory role. Whether the same applies in humans is unclear since mutations resulting in the activation or inactivation of G6PC2 have not been reported. In contrast, multiple glucokinase mutations have been identified in humans, and the data clearly indicate a major role for glucokinase in glucose sensing (39). Thus, heterozygous inactivating mutations in glucokinase are one cause of maturity-onset diabetes of the young, which is characterized by mild fasting hyperglycemia, whereas homozygous inactivating glucokinase mutations result in permanent neonatal diabetes mellitus, which is characterized by severe hyperglycemia (39). In contrast, glucokinase-activating mutations result in hyperinsulinemia, leading to hypoglycemia (39).

In conclusion, the data presented in this study show that G6pc2 acts as a negative regulator of basal GSIS. However, the utility of regulating the set point for basal GSIS remains unresolved. The function of G6pc2 is clearly not to block insulin secretion during fasting (Fig. 4), and although the regulation of FBG levels is important for disease risk, the diseases involved are those of old age and therefore not subject to evolutionary pressure. This suggests that perhaps controlling FBG is more important for other aspects of metabolism than appreciated. We have also considered the possibility that, although G6pc2 is not required for blocking insulin secretion under basal conditions, activation of G6pc2 might be important for blocking insulin secretion under specific physiological conditions (for example, during exercise or in the fetal state). Future studies will examine these ideas.

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