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PANDER KO Mice On High-Fat Diet Are Glucose Intolerant Yet Resistant to Fasting Hyperglycemia and Hyperinsulinemia

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Abstract

The recent creation of the PANDER knockout (PANKO) and acute mouse models have revealed a biological function in the regulation of glycemic levels via promotion of hepatic glucose production (HGP) and pancreatic β-cell insulin secretion. Therefore, we hypothesized that the absence of PANDER may afford some degree of protection from high-fat diet (HFD) induced fasting hyperglycemia. On HFD, fasting glycemic levels were significantly lower in the PANKO mice. Also, fasting insulin levels and the in-vivo insulin response following glucose injection were inhibited in PANKO mice. The lowered fasting glycemic levels are attributed to decreased HGP due to the absence of PANDER. Overall, our findings further indicate PANDER impacts glycemic levels and may represent a potential but complicated therapeutic target.

Keywords

PANDER; insulin; glucose; hyperglycemia

1. Introduction

PANcreatic-DERived factor (PANDER, FAM3B) is a 235 amino acid protein that is predominantly expressed and secreted from the α and β-cells of the endocrine pancreas [1–3]. The biological function of PANDER has been difficult to elucidate but the recent creation of the PANDER knockout (PANKO) and acute mouse models have generated robust clues into the role of PANDER in the regulation of euglycemia [4, 5]. PANKO mice displayed glucose intolerance due to inhibited glucose-stimulated pancreatic β-cell insulin secretion [4]. In addition, hyperinsulinemic-euglycemic clamp studies demonstrated hepatic glucose production (HGP) was significantly lower in PANKO as compared to WT mice.

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Concordantly, the acute adenoviral mouse model revealed overexpression of PANDER resulted in increased fasting hyperglycemia due to increased hepatic gluconeogenic expression whereas fasting PANKO mice have decreased hepatic gluconeogenic expression. In addition, treatment of isolated murine hepatocytes with PANDER elevated gluconeogenic gene expression and glucose output. The recently created PANDER transgenic model which overexpresses PANDER specifically in pancreatic β-cells has demonstrated glucose intolerance due to increased HGP, but only while on a high-fat diet [6]. Taken together, this suggests the potential that the absence of PANDER may protect mice on a HFD from developing gross fasting hyperglycemia and that PANDER action within the liver may represent a potential target for limiting HFD-induced fasting hyperglycemia. Therefore, to better understand the biological role of PANDER, we have evaluated the newly characterized PANKO mouse while on a HFD with regard to impact on fasting hyperglycemia and glucose tolerance.

2. Materials and Methods

2.1 PANDER Knockout (PANKO) Mice

PANKO mice were initially developed and phenotyped at the Children's Hospital of Philadelphia Research Institute as previously described [4]. Successful disruption of the PANDER gene was confirmed via PCR for all mice employed in this study as detailed previously (Supplementary Figure 1). Ten male PANKO and WT mice aged 8–9 months were maintained on a 12-h light/dark cycle with access to a HFD (45% fat by calories, 4.76 kcal/g, Research diets Inc. #D12451) and water. Body weight, percentage weight gain, and food consumption were measured weekly for the first 5 weeks. Male mice were solely utilized for this study as our previous findings have indicted a sex-specific phenotype for the PANKO mice. All animals were handled according to the guidelines established by the Institutional Animal Care and Use Committee at the Children's Hospital of Philadelphia.

2.2 Glucose Tolerance Tests

Glucose tolerance tests (GTTs) were performed as previously described [4]. In brief, mice at 5 and 10 weeks post-HFD were fasted overnight for approximately 16 hours and subsequently injected intraperitoneally with 2 grams of glucose (Fisher Scientific) per kilogram of body weight. Glucose levels were measured at 0, 15, 30, 60, 90, and 120 min post-injection from tail vein blood collections.

2.3 Glycemic Blood Measurements

Blood was typically collected from the tail vein (approximately 5 μl) at various timepoints and conditions and measured with a glucometer (Freestyle).

2.4 Insulin Tolerance Tests

Insulin tolerance tests (ITTs) were performed as previously described [4]. In brief, mice at 5 and 10 weeks post-HFD were fasted for 4 hours and then injected intraperitoneally with 0.75 units of insulin (NovoLog) per kilogram of body weight. Glucose levels were measured by the same method and chronological timepoints as indicated for the GTT above. Results are shown as percentage of glucose levels at the time of injection.

2.5 Quantitative RT-PCR

Livers were collected from PANKO and WT mice and immediately snap frozen in RNA later (Qiagen). RNA was then isolated using the RNAeasy kit (Qiagen) following manufacturer's instructions. Purity and quantity of the isolated RNA was determined by the A_{260}/A_{280}. Primer and probe sets for glucose-6-phosphatase (G6P), phosphoenolpyruvate carboxykinase (PEPCK), and 18S rRNA were commercially received (Applied Biosystems). Relative levels of target gene mRNA expression were normalized to β-actin, and calculated using the $2^{\Delta\Delta CT}$ method.

2.5 Insulin Measurement

Measurement of serum insulin concentration during the GTT was performed by tail vein blood collection in a Microvette CB 300 (Sarstedt), followed by centrifugation for serum separation and analysis. Sera samples were stored frozen at −80°C prior to measurement for insulin levels. Insulin content was determined by using the Ultra Sensitive Mouse Insulin ELISA kit (Crystal Chem).

2.6 Statistical analysis

Data are typically presented as mean ± SEM or for certain assays such as the GTT results are also analyzed by total area under the curve (AUC). Statistical significance between groups was determined by Student *t* test (Graphpad Prism version 5.01). *P* value less than 0.05 was considered significant.

3. RESULTS

3.1 PANKO mice exhibit similar body weight, % weight gain, and food consumption

To determine if PANDER could impact HFD induced fasting and postprandial hyperglycemia, PANKO and WT littermate control male mice aged 8 to 9 months were placed on a 10 week HFD (45% fat). Initial testing was performed to evaluate potential regulation of body weight and food consumption. Mice were weighed weekly and initial mean body weights indicated PANKO mice weighed less than WT (36.7 ± 1.6 g. vs. 40.5 ± 2.1 g.) but the difference was not statistically significant and after 5 weeks weights were almost identical (Fig. 1A). Similar results were obtained when evaluating % weight gain and food consumption whereby no significant differences were observed between PANKO and WT mice (Figs. 1B and 1C). These findings indicate that absence of PANDER does not inhibit or alter HFD induced weight gain or food consumption.

3.2 Metabolic evaluation of PANKO mice

Our previous report has indicated that the PANKO mouse displays post-prandial glucose intolerance [4]. Therefore, we measured if this result is exaggerated in the PANKO mouse during HFD conditions. The GTT revealed that following injection, glucose levels were higher in PANKO mice as compared to WT controls at both 5 and 10 weeks post-HFD (Figs. 2A and 2C) with a significant difference observed when determining total AUC for all glucose measurements during the course of the GTT (Figs. 2B and 2D). To measure insulin sensitivity, ITTs were also performed at both 5 and 10 weeks post-HFD and revealed similar

insulin tolerance between PANKO and WT mice (Figs. 2E and 2F). In addition, the observed decreased insulin sensitivity observed at 10 weeks was of similar magnitude for both PANKO and WT mice (compare Figs 2E to 2F). Furthermore, we confirmed that the high fat diet had the expected effects on fasting blood glucose and plasma insulin when compared to mice fed a normal chow diet (11% fat by calories) (Supplementary Figure 2).

3.3 Fasting glycemic levels of PANKO mice

We have previously demonstrated that PANDER can increase fasting hyperglycemic levels due to induced HGP during adenoviral expression or recombinant protein application [5]. The liver has also been demonstrated as a potential target for PANDER via binding studies [7]. Therefore, we examined if the absence of PANDER could provide a protective effect in HFD induced fasting hyperglycemia. PANKO and WT mice were fasted for either 24 and/or 48 hours and evaluated at both 5 and 10 weeks post-HFD. Fasting glucose levels were not significantly different between both groups prior to initiation of HFD (data not shown) and at 5 weeks HFD (Fig 3A, left grouping). However, following 10 weeks HFD, fasting glycemic levels were higher in the WT as compared to PANKO mice at 24 hours (213.7 ± 20.1 vs. 142.8 ± 16.2 mg/dl, respectively; $P < 0.05$) and 48 hours (133.1 ± 6.2 vs. 107.7 ± 8.7 mg/dl, respectively; $P < 0.05$) fasting (Fig 3A, middle and right grouping). To investigate the potential mechanism of decreased fasting glycemia, the relative expression of the critical gluconeogenic enzymes of G6pase and PEPCK were examined. RT-PCR analysis of livers from mice fasted for 24 hours indicated significantly decreased gluconeogenic expression from the PANKO as compared to WT mice (Figure 3B), indicating that the absence of PANDER has a protective effect on HFD induced fasting hyperglycemia via the decreased impact on hepatic gluconeogenic expression.

3.4 Glucose stimulated insulin levels

The observed impaired glucose intolerance in PANKO mice did not appear to be the result of impaired insulin sensitivity and suggested inhibited insulin secretion. Our previous characterization of the PANKO mice indicated impaired glucose-stimulated insulin secretion (GSIS) and altered calcium response in pancreatic islets [4]. To evaluate GSIS of PANKO mice under HFD conditions, serum insulin levels were measured at various time points following glucose injection after 10 weeks of HFD. Serum insulin levels were significantly higher in the WT as compared to PANKO mice following glucose injection at 15 (3.5 ± 0.6 vs. 1.5 ± 0.2 ng/ml, respectively; $P < 0.05$) and 30 minutes (2.9 ± 0.5 vs. 1.5 ± 0.4 ng/ml, respectively; $P < 0.05$) post-IP glucose injection (Fig 4). Also, fasting levels of insulin were also significantly decreased in the WT as compared to PANKO mice (1.9 ± 0.4 vs. 0.9 ± 0.1 ng/ml, respectively; $P < 0.05$) with levels approximately two-fold higher in the WT mice. Taken together, these results indicate that PANKO mice have impaired GSIS and decreased basal insulinemia under HFD.

4. Discussion

In summary, our results are highly concordant with our previous characterization of the PANKO and acute mouse models revealing that PANDER may have a pleiotropic role in regulating glycemia via both the liver and the pancreatic islet. Nonetheless, this HFD diet

study is not just merely confirmatory but has allowed a further exaggeration of the phenotype and additional elucidation of the biological function of PANDER. For example, the previous characterization of PANKO mice while on a normal chow diet (Purina Diet #5008, 16.7% fat) demonstrated decreased hepatic glucose production, yet fasting levels of PANKO mice were not statistically different from WT. Under HFD conditions, WT mice have significantly higher glycemic fasting levels at both 24 and 48 hours post-fast. HGP due to gluconeogenesis is critical during extensive fasting (24 hours) and responsible for up to 90% of endogenous glucose production after 40 hours of fasting [8]. The significantly decreased glycemic levels of PANKO mice after 48 hours certainly suggests that this is the result of decreased HGP and this is further supported by the reduced gluconeogenic expression of G6pase and PEPCK.

Rodent models of type 2 diabetes mellitus (T2DM) typically have fasting hyperglycemia with increased expression of these critical genes [9–12]. Also, elevated fasting hyperglycemia in T2DM is a typical metabolic abnormality and is attributed to increased HGP due to decreased insulin sensitivity and hyperglucagonemia [13, 14]. In addition, high-fat feeding in animal models has been demonstrated to significantly contribute to insulin resistance, which is one of the major features of metabolic syndrome [15]. Our data indicates that the absence of PANDER provides some degree of protection against HFD induced fasting hyperglycemia and potentially provides an attractive therapeutic target for T2DM intervention. Interestingly, we have recently demonstrated that circulating PANDER levels are chronically elevated in fat, aged wild-type C57Bl/6 mice (Supplementary Figure 3) [16]. In lean (3 months) and fat (8 months) wild type C57Bl/6 mice, PANDER levels were evaluated during feeding and fasting conditions. Two primary differences in PANDER levels were observed in these mice: (1) PANDER levels were significantly higher in the fat mice compared to lean mice during feeding and fasting and (2) serum PANDER levels in fat mice did not decrease with fasting as seen with lean mice. These data indicate a positive correlation between increased body weight and elevated PANDER levels. Therefore, increased PANDER levels in heavier mice may be an important contributor to fasting hyperglycemia due to induced increased hepatic glucose production.

However, our results demonstrate that PANKO mice on HFD still display post-prandial glucose intolerance despite decreased fasting glycemia. It appears that in the presence of high insulin as found following post-glucose injection, the absence of PANDER is not additive with regard to the suppressive impact of insulin on HGP. However, when insulin is low as found during fasting conditions, the absence of PANDER is reflected by decreased HGP and this result is supported by the acute model which demonstrates that overexpression of PANDER increases fasting hyperglycemia. In the knockout, this impact on fasting hyperglycemia is only evident during HFD and this may be attributed to increased insulin resistance. Following the 10 week HFD, both PANKO and WT mice were clearly becoming increasingly insulin resistant (Figure 2E) and this may have exaggerated the phenotypic impact on fasting glycemic levels in the PANKO mouse.

Interestingly, the only physiological condition under which circulating PANDER levels are chronically elevated is in fat, aged mice (Supplementary Figure 3) [16]. In lean (3 months) and fat (8 months) wild type C57Bl/6 mice, ad libitum fed glucose levels were not

significantly different between the two groups, but after a 24-hr fast, older mice were hyperglycemic compared to younger mice. Two primary differences in PANDER levels were observed in these mice: (1) PANDER levels were significantly higher in the fat mice compared to lean mice both during feeding and fasting and (2) serum levels in fat mice did not decrease with fasting as with lean mice, but remained high. These data suggest that there is a positive correlation between increased body weight and elevated PANDER levels and the increased PANDER levels in heavier mice may be an important contributor to fasting hyperglycemia due to PANDER-induced hepatic glucose production.

This study has also indicated that PANDER impacts pancreatic β-cell function and post-prandial glucose tolerance. Initial characterization of the knockout was highly confounded by the fact that the GTT in PANKO mice on normal chow displayed higher levels of insulin secretion despite *in-vivo* glucose intolerance and *in-vitro* islet perifusion and calcium imaging studies demonstrating abnormal responses of the PANKO islets to glucose stimulation. This conflicting report was attributed to compensatory and protective mechanisms that exist *in vivo* to preserve and maintain pancreatic β-cell function whereas defects in GSIS are exaggerated and pronounced in isolated islets as has been observed in other knockout models [17, 18]. However, as shown by this study, for the PANKO mice under HFD conditions GSIS was inhibited and accompanied by post-glucose injection glucose intolerance thus further supporting a role for PANDER in pancreatic β-cell function. Under insulin resistant conditions as induced by the HFD, it is possible that *in-vivo* compensation was inadequate due to additional pressure on the pancreatic β-cell to secrete more insulin in the scenario of increasing resistance. Nonetheless, this result has demonstrated that the pleiotropic role of PANDER will certainly complicate its utility as a potential T2DM drug target in that overall PANDER inhibition will result in contradictory outcomes with regard to reducing fasting glycemic levels but having impaired post-prandial glucose tolerance.

In a physiological context, PANDER appears to serve varying roles with regard to either interaction with the liver or pancreatic islets, depending on the fed or fasted state. In the fed state, as demonstrated by our previously published knockout mouse, PANDER strongly impacts pancreatic β-cell function in a glucose-stimulated manner by either regulating or facilitating insulin secretion. PANKO pancreatic islets have both inhibited insulin secretion and abnormal calcium handling. This phenotype is even further exaggerated in our presented high-fat model, whereas the PANKO mice displayed glucose intolerance to a more severe extent as compared to the WT mice with a concordant decrease in GSIS. However, in the fasted state, as demonstrated by our acute mouse model, PANDER stimulates cAMP and CREB dependent pathways that increase gluconeogenic gene expression and overall HGP. This finding is supported by our PANKO mouse during normal diet conditions that demonstrated decreased HGP during hyperinsulinemic-euglycemic clamp studies. Our findings in this high fat diet study strongly confirm this fasting role, whereas PANKO mice display decreased fasting glycemic levels.

Overall, our study strongly indicates that PANDER is a novel hormone regulating glycemic levels via pancreatic β-cell insulin secretion and hepatic gluconeogenic expression. In addition, the absence of PANDER provides some degree of protection against HFD induced

fasting hyperglycemia and may provide a potential therapeutic T2DM drug target but only with regard to the specific inhibition of distal action on the liver.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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ABBREVIATIONS

G6pase	glucose-6-phosphatase
GSIS	glucose-stimulated insulin secretion
GTT	glucose tolerance tests
HFD	high-fat diet
HGP	hepatic glucose production
ITT	insulin tolerance tests
PANDER	pancreatic-derived factor
PEPCK	phosphoenolpyruvate carboxykinase
T2DM	type 2 diabetes mellitus

REFERENCES

1. Zhu Y, Xu G, Patel A, McLaughlin MM, Silverman C, Knecht K, Sweitzer S, Li X, McDonnell P, Mirabile R, et al. Cloning, expression, and initial characterization of a novel cytokine-like gene family. Genomics. 2002; 80:144–150. [PubMed: 12160727]
2. Cao X, Gao Z, Robert CE, Greene S, Xu G, Xu W, Bell E, Campbell D, Zhu Y, Young R, et al. Pancreatic-derived factor (FAM3B), a novel islet cytokine, induces apoptosis of insulin-secreting beta-cells. Diabetes. 2002; 52:2296–2303.
3. Carnegie JR, Robert-Cooperman CE, Wu J, Young RA, Wolf BA, Burkhardt BR. Characterization of the expression, localization, and secretion of PANDER in alpha-cells. Mol Cell Endocrinol. 2010; 325:36–45. [PubMed: 20638985]
4. Robert-Cooperman CE, Carnegie JR, Wilson CG, Yang J, Cook JR, Wu J, Young RA, Wolf BA, Burkhardt BR. Targeted disruption of pancreatic-derived factor (PANDER, FAM3B) impairs pancreatic beta-cell function. Diabetes. 2010; 59:2209–2218. [PubMed: 20566664]
5. Wilson CG, Schupp M, Burkhardt BR, Wu J, Young RA, Wolf BA. Liver-specific overexpression of pancreatic-derived factor (PANDER) induces fasting hyperglycemia in mice. Endocrinology. 2010; 151:5174–5184. [PubMed: 20844005]
6. Robert CE, Wu J, Burkhardt Br, Wolf B. Transgenic mice overexpressing the novel islet specific cytokine, PANDER, exhibit glucose intolerance. Diabetes. 2005; 54:A400.
7. Yang J, Wang C, Li J, Burkhardt BR, Robert-Cooperman CE, Wilson C, Gao Z, Wolf BA. PANDER binds to the liver cell membrane and inhibits insulin signaling in HepG2 cells. FEBS Lett. 2009; 583:3009–3015. [PubMed: 19683528]

8. Chandramouli V, Ekberg K, Schumann WC, Kalhan SC, Wahren J, Landau BR. Quantifying gluconeogenesis during fasting. Am J Physiol. 1997; 273:E1209–E1215. [PubMed: 9435538]
9. Robinson SW, Dinulescu DM, Cone RD. Genetic models of obesity and energy balance in the mouse. Annu Rev Genet. 2000; 34:687–745. [PubMed: 11092843]
10. Veneziale CM, Donofrio JC, Nishimura H. The concentration of P-enolpyruvate carboxykinase protein in murine tissues in diabetes of chemical and genetic origin. J Biol Chem. 1983; 258:14257–14262. [PubMed: 6643479]
11. Haber BA, Chin S, Chuang E, Buikhuisen W, Naji A, Taub R. High levels of glucose-6-phosphatase gene and protein expression reflect an adaptive response in proliferating liver and diabetes. J Clin Invest. 1995; 95:832–841. [PubMed: 7860767]
12. Mosseri R, Waner T, Shefi M, Shafrir E, Meyerovitch J. Gluconeogenesis in non-obese diabetic (NOD) mice: in vivo effects of vandadate treatment on hepatic glucose-6-phoshatase and phosphoenolpyruvate carboxykinase. Metabolism. 2000; 49:321–325. [PubMed: 10726908]
13. Leahy JL. Pathogenesis of type 2 diabetes mellitus. Arch Med Res. 2005; 36:197–209. [PubMed: 15925010]
14. Fisher SJ, Kahn CR. Insulin signaling is required for insulin's direct and indirect action on hepatic glucose production. J Clin Invest. 2003; 111:463–468. [PubMed: 12588884]
15. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest. 2006; 116:1793–1801. [PubMed: 16823477]
16. Wilson, C. Characterization of the effect of Pancreatic Derived Factor (PANDER) on hepatic regulation of glucose homeostasis. Ph.D. dissertation. Philadelphia: University of Pennsylvania; 2010.
17. Gupta RK, Vatamaniuk MZ, Lee CS, Flaschen RC, Fulmer JT, Matschinsky FM, Duncan SA, Kaestner KH. The MODY1 gene HNF-4alpha regulates selected genes involved in insulin secretion. J Clin Invest. 2005; 115:1006–1015. [PubMed: 15761495]
18. Sorensen H, Winzell MS, Brand CL, Fosgerau K, Gelling RW, Nishimura E, Ahren B. Glucagon receptor knockout mice display increased insulin sensitivity and impaired beta-cell function. Diabetes. 2006; 55:3463–3469. [PubMed: 17130493]

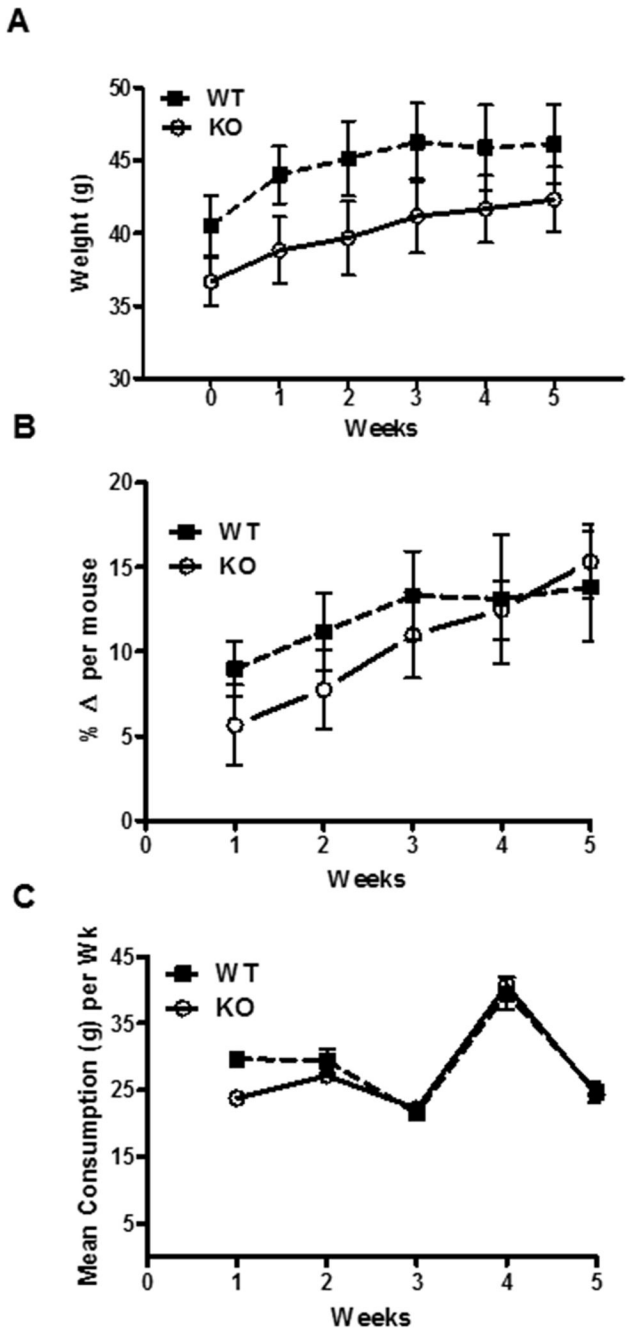

Figure 1. Body mass, weight gain, and food consumption of PANKO mice on HFD

Average body weight, (B) Percentage change weight gain and, (C) Food consumption per mouse per week of PANKO and WT mice monitored weekly. Male mice aged 8–9 months were evaluated ($n = 8$). Values are means ± SE. KO, knockout; WT, wild-type.

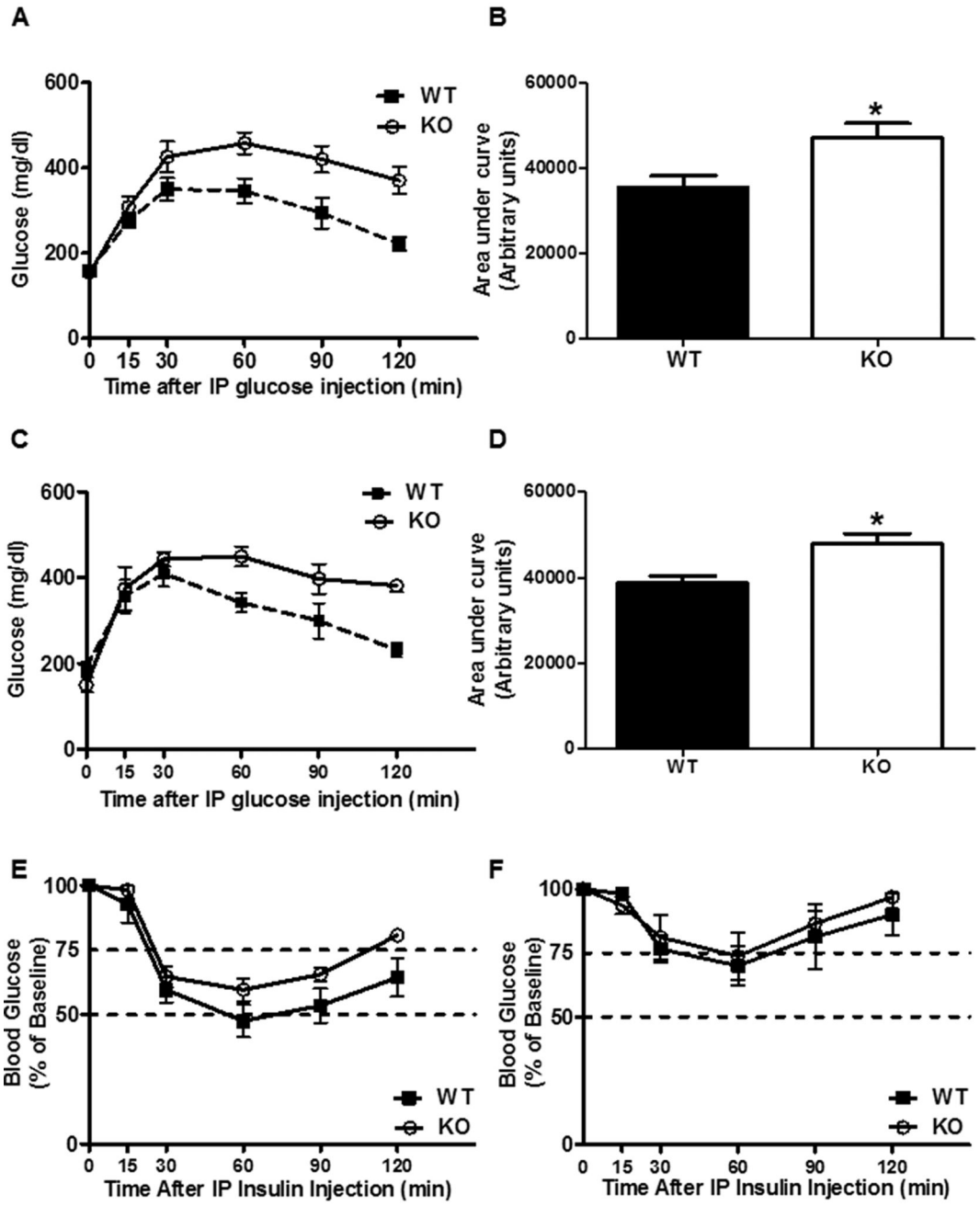

Figure 2. Glucose intolerance in PANKO mice

(A) Intraperitoneal GTT performed by injecting mice with 2 g/kg of glucose and measuring serum glucose concentration at indicated time points following 5 weeks of HFD. (B) AUC calculated from measured glucose levels during entire course of GTT. (C) GTT as described above following 10 weeks of HFD. (D) AUC of GTT at 10 weeks of HFD. (E) Intraperitoneal ITT performed by injecting mice with insulin at 0.75 units/kg and measuring glucose concentration at indicated time points following 5 weeks of HFD. Results are

expressed as percentage of baseline glucose reading. (F) ITT as described above following 10 weeks of HFD. Values are means ± SE (n = 6–8). *P < 0.05 by Student t test.

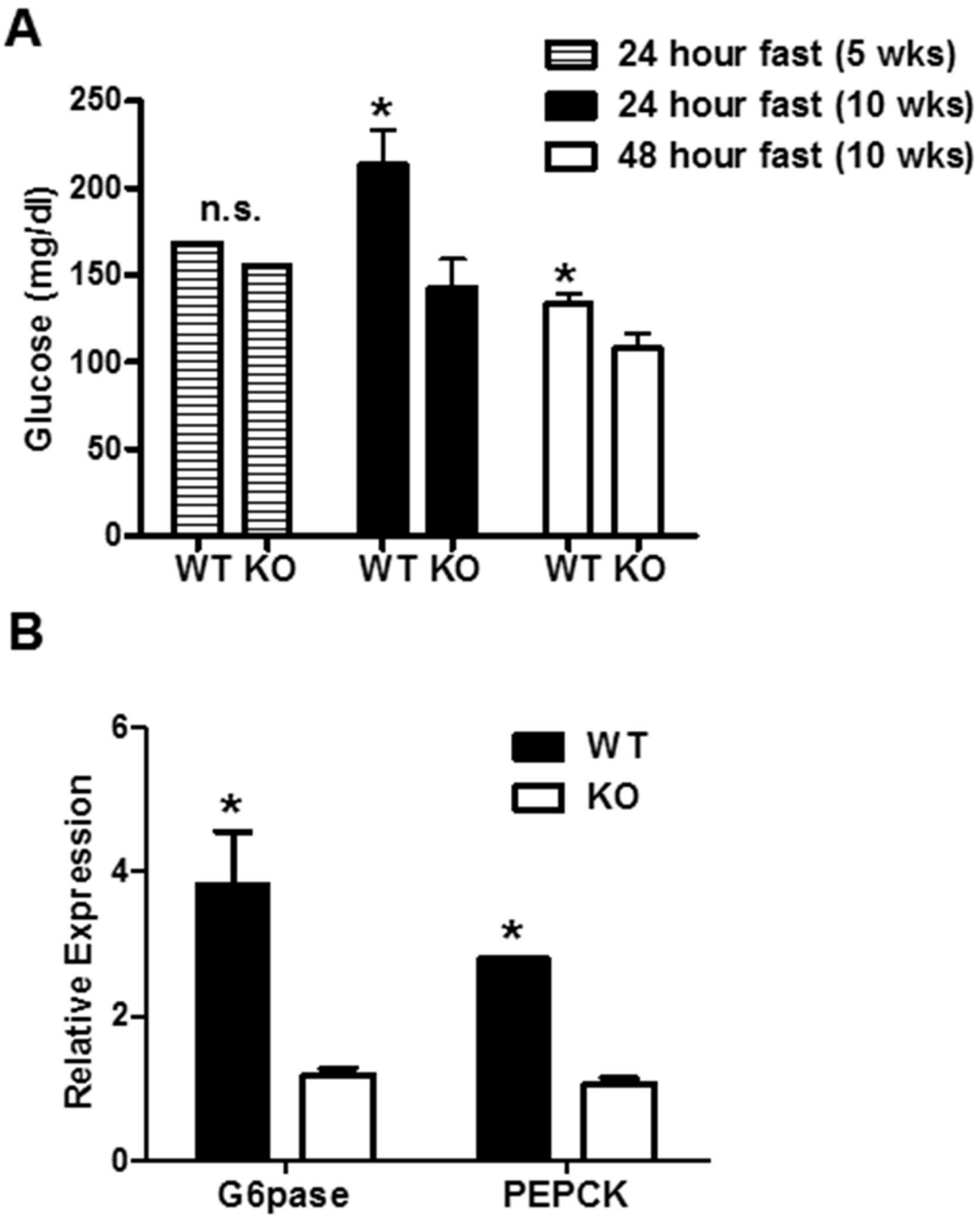

Figure 3. Decreased fasting glycemia and gluconeogenic expression in PANKO mice

(A) Fasting glucose levels of PANKO and WT mice evaluated from tail vein blood collection and measured with glucometer (Freestyle) at 5 weeks HFD/ 24 hour fast (*left grouping*), 10 weeks HFD/ 24 hour fast (*middle grouping*), and 10 weeks HFD/ 48 hour fast (*right grouping*) ($n = 6–8$). (B) Quantitative RT-PCR analysis of G6pase and PEPCK gene expression in fasted livers of PANKO and WT mice ($n = 3$). Results are expressed as fold above PANKO gene expression. Values are means ± SE. *$P < 0.05$ by Student t test.

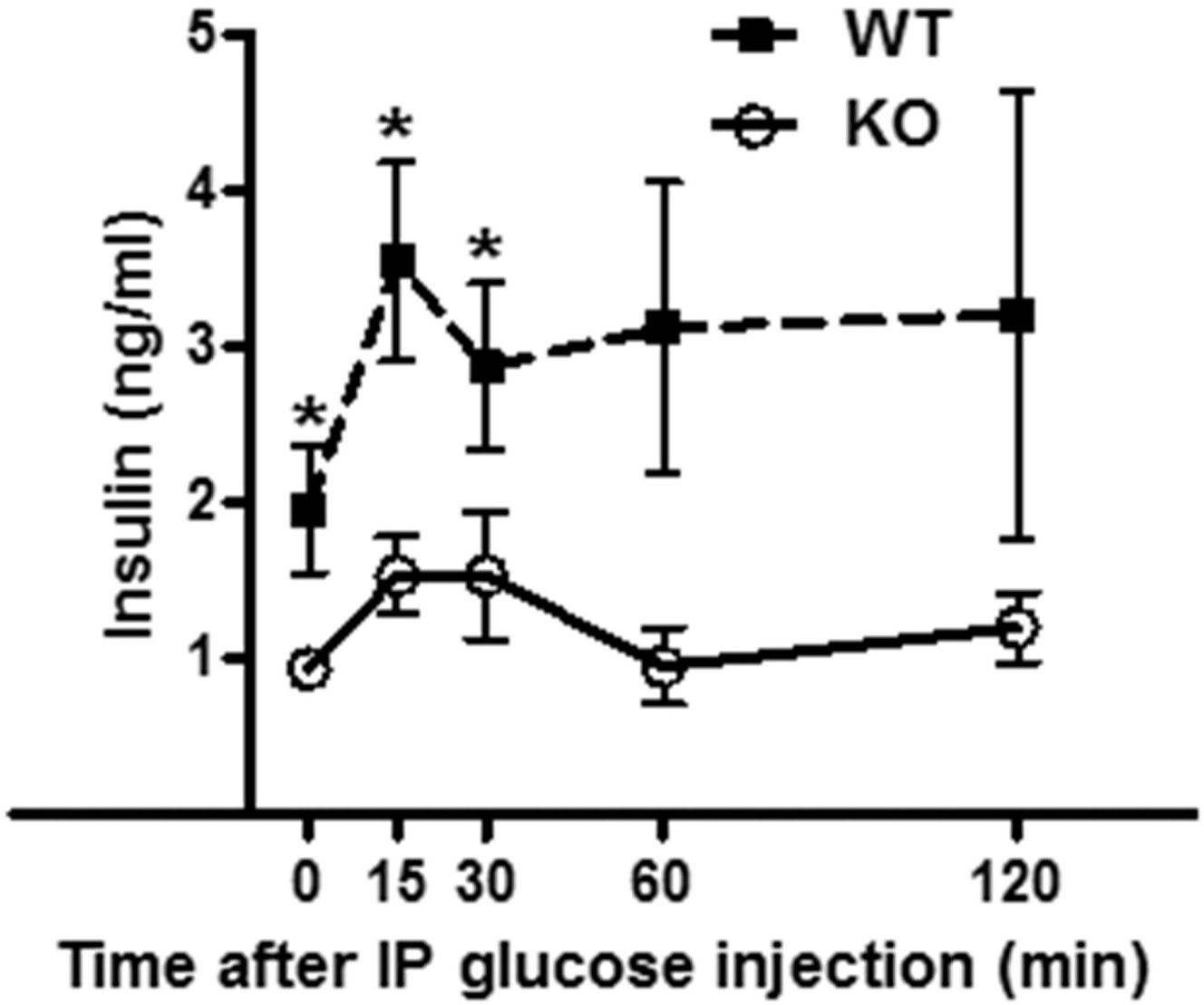

Figure 4. Inhibited glucose-stimulated insulin secretion in PANKO mice on HFD

Insulin levels were measured during the course of the GTT in PANKO and WT mice following 10 week HFD ($n = 5$). Sera samples were collected from tail vein and insulin measurements were evaluated by ELISA (Crystal Chem). Values are means ± SE. *$P < 0.05$ by Student *t* test.