Deletion of Protein Kinase D1 in Pancreatic β-Cells Impairs Insulin Secretion in High-Fat Diet–Fed Mice

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β-Cell adaptation to insulin resistance is necessary to maintain glucose homeostasis in obesity. Failure of this mechanism is a hallmark of type 2 diabetes (T2D). Hence, factors controlling functional β-cell compensation are potentially important targets for the treatment of T2D. Protein kinase D1 (PKD1) integrates diverse signals in the β-cell and plays a critical role in the control of insulin secretion. However, the role of β-cell PKD1 in glucose homeostasis in vivo is essentially unknown. Using β-cell–specific, inducible PKD1 knockout mice (βPKD1KO), we examined the role of β-cell PKD1 under basal conditions and during high-fat feeding. βPKD1KO mice under a chow diet presented no significant difference in glucose tolerance or insulin secretion compared with mice expressing the Cre transgene alone; however, when compared with wild-type mice, both groups developed glucose intolerance. Under a high-fat diet, deletion of PKD1 in β-cells worsened hyperglycemia, hyperinsulinemia, and glucose intolerance. This was accompanied by impaired glucose-induced insulin secretion both in vivo in hyperglycemic clamps and ex vivo in isolated islets from high-fat diet–fed βPKD1KO mice without changes in islet mass. This study demonstrates an essential role for PKD1 in the β-cell adaptive secretory response to high-fat feeding in mice.

Type 2 diabetes (T2D) is characterized by insufficient insulin secretion from the pancreatic β-cell. Functional β-cell adaptation is a central mechanism by which the body overcomes insulin resistance to maintain glucose homeostasis in obese individuals. Over time, however, failure of this mechanism can lead to T2D (1). Insulin secretion is a tightly regulated process controlled by a number of metabolic, hormonal, and neural cues, many of which are mediated by G-protein–coupled receptors (2). G-protein–coupled receptors are validated targets for the treatment of T2D, and among these, the fatty acid receptor 1 (also known as GPR40/FFAR1) has been the subject of considerable interest in recent years (3). GPR40 is predominantly expressed in β-cells and is implicated in the second phase of insulin secretion in response to fatty acids in vivo and in vitro (4–6). GPR40 preferentially couples to the G-protein subunit Goq and, as shown by our group in isolated mouse islets (7), its downstream signaling cascade involves protein kinase D1 (PKD1), a serine/threonine protein kinase of the calcium/calmodulin-dependent kinase family. PKD1 is also involved in the potentiation of insulin secretion by M3 muscarinic receptor signaling via β-arrestins (8). Mechanistically, PKD1 promotes insulin vesicle fission at the trans-Golgi network (9,10) and controls remodeling of the actin cytoskeleton (7). Hence, inhibition of PKD1 activity in cultured β-cells reduces insulin secretion by preventing the replenishment of secretion–competent insulin granules at the plasma membrane (9,11). Together, these studies suggest that PKD1 activity regulates insulin granule formation and secretion. However, the precise role of β-cell PKD1 in glucose homeostasis in vivo remains to be elucidated.

To this aim, we generated tamoxifen-inducible, β-cell–specific PKD1 knockout mice and examined glucose homeostasis...
and β-cell function and mass under basal conditions and in response to high-fat feeding.

**RESEARCH DESIGN AND METHODS**

**Animals and Diets**

All procedures involving animals were approved by the institutional committee for the protection of animals at Centre Hospitalier de l’Université de Montréal. Mice were housed on a 12-h light/dark cycle with free access to water and standard laboratory chow (Teklad Global 18% protein rodent diet, category no. 2918; Harlan Teklad, Madison, WI). Mice carrying LoxP sites between exons 11 and 12 and exons 14 and 15 of one Prkd1 allele (Prkd1+/h) (12) and transgenic MIP-CreERT11p (MIP-CreERT) mice (13,14) were backcrossed to C57Bl/6N (Charles River, Saint-Constant, Quebec, Canada) for nine generations and genotyped as previously described (12,13). PKD1+/h females and MIP-CreERT;Prkd1+/h males were crossed to generate experimental groups. Tamoxifen (Sigma-Aldrich, Oakville, Ontario, Canada) was injected (125 mg/kg i.p.) at 48-h intervals for a total of three injections in 9-week-old males as previously described (15). All experimental animals received tamoxifen. Three weeks after tamoxifen injections (week 0), mice were either kept on standard chow or given a high-fat diet (60% fat, 15% protein, and 25% carbohydrate on a caloric basis, category no. S3282; Bioserv Diets, Frenchtown, NJ) for up to 13 weeks. Body weight composition was assessed with the EchoMRI-700 (Echo Medical System, Houston, TX).

**Oral Glucose Tolerance Test and Hyperglycemic Clamps**

Oral glucose tolerance tests (OGTTs) were performed in 4-h–fasted mice by measuring tail blood glucose and plasma insulin after oral glucose administration (2 g/kg) by gavage as previously described (16).

One-step hyperglycemic clamps were performed in conscious, ad libitum–fed animals as previously described (6). Briefly, a 20% dextrose solution (Baxter, Mississauga, ON) was infused via a jugular catheter. Mice initially received a 90-s bolus (140 mg/kg/min) and then the glucose infusion rate (GIR) was adjusted to maintain blood glucose between 18 and 21 mmol/L for 80 min. The insulin sensitivity index (M/I) was calculated as the glucose infusion rate (M) divided by the average insulinemia during the last 30 min of the clamp (I). Insulin clearance (insulin/C-peptide) was estimated as the average insulinemia divided by the average C-peptide during the last 30 min of the clamp. Blood samples were collected from the tail to measure glucose using the handheld glucometer Accu-Chek (Roche, Indianapolis, IN) and plasma insulin, C-peptide, and proinsulin were measured with ELISA (Alpco Diagnostics, Salem, NH) at the time points indicated in the figure legends.

**Islet Mass Measurements**

Immediately after the hyperglycemic clamp, the pancreas was removed and islet mass was measured on paraffin sections using an anti–chromogranin A antibody (Abcam, Toronto, Ontario, Canada) to label endocrine cells as previously described (17).

**Static Incubations**

Islets were isolated by collagenase (Sigma-Aldrich) digestion as previously described (4) and recovered in RPMI-1640 (Gibco Life Technologies, Burlington, Ontario, Canada) supplemented with 10% (wt/vol) FBS (Gibco Life Technologies), 100 units/mL penicillin/streptomycin, and 11 mmol/L glucose. Triplicate batches of 10 islets each were incubated in Krebs-Ringer bicarbonate HEPES buffer with 0.1% BSA and 2.8 mmol/L glucose twice for 20 min followed by a 1-h static incubation in Krebs-Ringer bicarbonate HEPES buffer in the presence of glucose, oleate (0.5 mmol/L; Sigma-Aldrich), carbachol (500 μmol/L; Millipore, Billerica, MA), or glucagon-like peptide 1 (GLP-1) (0.1 μmol/L; Bachem, Torrance, CA), as indicated in the figure legends. Oleate was complexed for 1 h at 37°C with fatty acid–free BSA (BAH66; Equet Bio, Kerrville, TX) to a final molar ratio of 1:5 prior to use as previously described (18). Control conditions contained the same amount of BSA and vehicle (50% [vol/vol] ethanol). Secreted insulin was measured in the supernatant and intracellular insulin content was measured after acid-alcohol extraction by radioimmunoassay using a rat insulin RIA kit (Millipore).

**Western Blots**

Islet protein extracts were subjected to 10% SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with primary antibodies against PKD1 (1:500; Cell Signaling, New England Biolabs, Whitby, Ontario, Canada) or tubulin (1:5,000; Abcam, Toronto, Ontario, Canada) and then horseradish peroxidase–labeled anti-rabbit IgG secondary antibodies in 5% (wt/vol) milk and visualized using Western Lighting Plus-ECL (Perkin Elmer, Woodbridge, Ontario, Canada). Band intensity was quantified using ImageJ software (National Institutes of Health).

**Statistical Analyses**

Data are expressed as mean ± SEM. Significance was tested using one-way ANOVA with Tukey or Dunnett post hoc test or two-way ANOVA with post hoc adjustment for multiple comparisons, as appropriate, using GraphPad InStat (GraphPad Software, San Diego, CA). P < 0.05 was considered significant.

**RESULTS**

**Efficient β-Cell–Specific Deletion of PKD1**

MIP-CreERT;Prkd1+/h (βPKD1KO) mice were born at the expected Mendelian ratio. At 9 weeks of age, male mice from all four experimental groups were injected with tamoxifen. Three weeks later, PKD1 protein levels were assessed by Western blotting in isolated islets. PKD1 protein levels were significantly reduced in βPKD1KO mice compared with MIP-CreERT, Prkd1+/h (FL), or wild-type (WT) littermates (Supplementary Fig. 1A and B).
**MIP-CreERT and bPKD1KO Mice Develop Glucose Intolerance**

We first performed sequential OGTTs in a first cohort of chow-fed animals from all four genotypes starting 3 weeks after tamoxifen injections (week 0). As shown in Fig. 1, glucose tolerance was reduced, though not significantly, in MIP-CreERT and bPKD1KO mice compared with WT mice at week 0 (Fig. 1A). At week 8, glucose tolerance of MIP-CreERT and bPKD1KO mice became significantly different from WT mice (Fig. 1B), and it remained so for bPKD1KO mice at week 12 (Fig. 1C). Insulin levels were unaffected (Fig. 1D–F). The development of glucose intolerance in both MIP-CreERT and bPKD1KO mice suggests that this phenotype is likely due to the presence of the Cre transgene rather than PKD1 deletion.

Second, for further examination of the impact of the Cre transgene on insulin secretion in vivo, animals from the four genotypes were subjected to hyperglycemic clamps at week 13 (Fig. 2). Mice were catheterized at the beginning of week 13 and allowed to recover for 3–5 days. During the clamp, target blood glucose levels were achieved in all four groups and were stable between 50 and 80 min (Fig. 2A). Although insulin levels during the clamp were not significantly different (Fig. 2B and C), plasma C-peptide levels were significantly reduced in MIP-CreERT and bPKD1KO mice compared with WT mice (Fig. 2D). Both MIP-CreERT and bPKD1KO mice had reduced GIR compared with WT mice (Fig. 2E), although the difference was statistically significant only for bPKD1KO mice. However, the M/I (Fig. 2F) and the insulin–to–C-peptide ratio (Supplementary Fig. 2A) were not significantly different between all four groups.

Third, to examine whether the lower C-peptide levels in MIP-CreERT and bPKD1KO mice during the clamp reflected an intrinsic insulin secretory defect, we performed 1-h static incubations in islets isolated at week 0 (Fig. 3). As shown in Fig. 3A, glucose-stimulated insulin secretion (GSIS) was similar in all four groups. Whereas the potentiation of GSIS by oleate was similar between MIP-CreERT, FL, and WT islets, it was slightly but significantly diminished clamp, target blood glucose levels were achieved in all four groups and were stable between 50 and 80 min (Fig. 2A). Although insulin levels during the clamp were not significantly different (Fig. 2B and C), plasma C-peptide levels were significantly reduced in MIP-CreERT and bPKD1KO mice compared with WT mice (Fig. 2D). Both MIP-CreERT and bPKD1KO mice had reduced GIR compared with WT mice (Fig. 2E), although the difference was statistically significant only for bPKD1KO mice. However, the M/I (Fig. 2F) and the insulin–to–C-peptide ratio (Supplementary Fig. 2A) were not significantly different between all four groups.

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in βPKD1KO versus WT islets (Fig. 3A). In contrast, the potentiation of GSIS by carbachol was diminished in MIP-CreERT, FL, and βPKD1KO versus WT islets (Fig. 3A). Neither the potentiation of GSIS by GLP-1 nor insulin content was significantly different between the four genotypes (Fig. 3A and B). Overall, these data suggest that 1) expression of the MIP-CreERT transgene results in glucose intolerance and lower glucose-induced C-peptide secretion in vivo but no obvious defect in GSIS ex vivo and 2) β-cell PKD1 is dispensable for normal glucose homeostasis in mice under basal conditions. Given the phenotype of the MIP-CreERT mice, this group was used as a control against which the βPKD1KO mice were compared in all subsequent experiments.

**Deletion of PKD1 in β-Cells Exacerbates Hyperglycemia, Hyperinsulinemia, and Glucose Intolerance Under High-Fat Diet**

We administered a high-fat diet to a second cohort of MIP-CreERT and βPKD1KO mice for 12 weeks beginning at week 0 (3 weeks after tamoxifen injections). PKD1 protein levels assessed by Western blot of isolated islets confirmed that PKD1 remained significantly reduced in βPKD1KO islets compared with MIP-CreERT islets after 12 weeks of diet (Supplementary Fig. 1C and D). During the 12-week diet, caloric intake (Fig. 4A) and weight gain (Fig. 4B) were not different between both groups, except for a transient increase in weight gain in βPKD1KO mice between weeks 5 and 7 (Fig. 4B). Fed and fasted glucose (Fig. 4C and D) and insulin (Fig. 4E and F) levels were similar between normal-chow fed βPKD1KO and MIP-CreERT mice. In contrast, high-fat diet–fed βPKD1KO mice became more severely hyperglycemic than high-fat diet–fed MIP-CreERT mice after 8 weeks, especially in the fed state (Fig. 4C and D). This was accompanied by elevated levels of circulating insulin (Fig. 4E and F). Lean (Fig. 4G) and fat (Fig. 4H) mass, respectively, decreased and increased in response to high-fat diet–feeding but were not different between both genotypes after the 12-week diet. Proinsulin levels were not significantly different in βPKD1KO compared with MIP-CreERT after 8 and 12 weeks of high-fat diet (Fig. 4I).

OGTTs were performed after a 4-h fast in chow-fed and high-fat diet–fed groups at weeks 8 and 12 (Fig. 5). As expected, glucose intolerance (Fig. 5A and B) and hyperinsulinemia (Fig. 5C and F) were observed in both high-fat diet–fed groups during the OGTT. βPKD1KO mice were more severely glucose intolerant than MIP-CreERT mice at week 8 (Fig. 5A), although this difference was no longer significant at week 12 (Fig. 5B). Insulin levels during the OGTT were not different between both genotypes after the 12-week diet. Proinsulin levels were not significantly different in βPKD1KO compared with MIP-CreERT after 8 and 12 weeks of high-fat diet (Fig. 4I).
deletion exacerbates high-fat diet–induced hyperglycemia, hyperinsulinemia, and glucose intolerance.

**Deletion of PKD1 in β-Cells Is Associated With Defective GSIS in High-Fat Diet–Fed Mice**

A cohort of MIP-CreERT and βPKD1KO mice underwent catheterization under general anesthesia after 12 weeks of diet and were allowed to recover from surgery while on the same respective diet regimen, after which insulin secretion in vivo was assessed by hyperglycemic clamps (Fig. 6). Target blood glucose levels during the clamp were achieved in all four groups and were stable between 50 and 80 min (Fig. 6A). High-fat diet–fed MIP-CreERT mice displayed a robust insulin response to glucose during the clamp (Fig. 6B) both at early time points (Fig. 6C) and during the steady-state period (Fig. 6D). In contrast, the glucose-stimulated insulin response of high-fat diet–fed βPKD1KO mice was significantly reduced compared with high-fat diet–fed MIP-CreERT mice (Fig. 6B) and indistinguishable from that of chow-fed controls (Fig. 6B–D). Similar to insulin, C-peptide levels during the steady state of the clamp were increased in high-fat diet–fed MIP-CreERT but not βPKD1KO mice (Fig. 6E). The GIR (Fig. 6F) during the clamp and M/I (Fig. 6G) trended lower in both high-fat diet–fed groups, suggestive of insulin resistance, but were not different between βPKD1KO and MIP-CreERT mice, and the same was seen for the insulin–to–C-peptide ratio (Supplementary Fig. 2B). Taken together, these data indicate that deletion of PKD1 in β-cells impairs insulin secretion in vivo after high-fat diet feeding.

To examine whether this phenotype was associated with defective GSIS ex vivo, we performed 1-h static incubations of islets isolated from high-fat diet–fed mice. While insulin secretion in response to 2.8 and 8.3 mmol/L glucose was similar between βPKD1KO and MIP-CreERT mice (Fig. 7A), the stimulation index, calculated as insulin secretion at 16.7 mmol/L glucose/insulin secretion at 2.8 mmol/L glucose, was significantly reduced in βPKD1KO islets (Fig. 7B) without changes in insulin content (Fig. 7C). This was not associated with significant changes in islet area, islet mass, or total islet number (Supplementary Fig. 3A–C), although high-fat diet–fed βPKD1KO mice tended to have a greater proportion of larger islets (Supplementary Fig. 3D).

**DISCUSSION**

The objective of this study was to delineate the contribution of PKD1 in β-cells to glucose homeostasis. To this aim, we analyzed the metabolic consequence of β-cell–specific PKD1 deletion in mice under basal conditions and in response to high-fat feeding. We showed that βPKD1KO mice became...
more hyperglycemic, hyperinsulinemic, and glucose intolerant than control MIP-CreERT mice under a high-fat diet. High-fat diet–fed βPKD1KO mice had defective GSIS in hyperglycemic clamps in vivo and in isolated islets ex vivo without significant changes in islet mass. Our findings demonstrate a key contribution of PKD1 to the β-cell functional adaptation to high-fat feeding.

Under a normal diet, both MIP-CreERT and βPKD1KO mice became glucose intolerant with age compared with FL or WT mice (Fig. 1), suggesting that this phenotype was due to the MIP-CreERT transgene rather than deletion of PKD1. Although insulin levels were not significantly lower during the OGTT (Fig. 1), C-peptide levels during the hyperglycemic clamps were significantly reduced (Fig. 2), which likely contributes to the glucose intolerance. As all mice in our study were on the same C57BL/6N background, the difference between Cre-expressing (MIP-CreERT and βPKD1KO) and non-Cre-expressing (FL and WT) mice is likely due to the human growth hormone minigene included in the MIP-CreERT transgene (14). In a previous study (14), we observed normal glucose tolerance in MIP-CreERT mice of similar age (equivalent to week 0 in this study) on the C57BL/6J background—an observation subsequently confirmed by Carboneau et al. (19). We attribute this apparent discrepancy (at weeks 8 and 12 in this study) to the fact that both of these previous studies used MIP-CreERT mice on a C57BL/6J background, while ours were on a C57BL/6N background. Such a possibility is supported by the known phenotypic differences between the 6J and 6N substrains (16,20–23). These findings further highlight the importance of including Cre-expressing mice as controls for β-cell–specific knockout mice generated with the MIP-CreERT construct (13,14). βPKD1KO mice were very similar to MIP-CreERT mice under a normal diet (Figs. 1 and 2), suggesting that β-cell PKD1 is dispensable for glucose tolerance and insulin secretion under basal conditions, although we cannot entirely rule out the possibility that the glucose intolerance due to expression of the Cre transgene might have masked a subtle phenotype induced by PKD1 deletion.

As expected, oleate potentiation of GSIS was reduced in islets from βPKD1KO mice (Fig. 3), although this reduction was less pronounced than previously observed in islets from FL mice transduced with an adenovirus encoding Cre (7). In contrast to previous studies using PKD1-targeting short hairpin RNA (8), M3 muscarinic potentiation of GSIS in βPKD1KO islets was not significantly reduced compared with MIP-CreERT islets (Fig. 3). These results suggest that sustained deletion of PKD1 for several weeks in the current study might have triggered compensatory mechanisms not observed upon acute PKD1 knockdown in isolated islets.

During the course of high-fat feeding, βPKD1KO mice exhibited more severe hyperglycemia, hyperinsulinemia, and glucose intolerance than their MIP-CreERT littermates (Figs. 4 and 5). The possibility that this was due to more severe insulin resistance in high-fat diet–fed βPKD1KO mice appears unlikely, since the M/I and the insulin–to–C-peptide ratio calculated from the hyperglycemic clamps at the end of the diet period were not different from those of MIP-CreERT mice (Fig. 6 and Supplementary Fig. 2). The difference in glucose tolerance between high-fat diet–fed βPKD1KO and MIP-CreERT mice was no longer significant after 12 weeks (Fig. 5). This could be due to the fact that glucose tolerance in mice is largely independent of insulin secretion dynamics, especially under elevated insulin concentrations (24). Regardless of any differences in insulin sensitivity or glucose effectiveness between βPKD1KO and MIP-CreERT mice, fasting and fed hyperglycemia (Fig. 4) and oral glucose intolerance (Fig. 5) in high-fat diet–fed βPKD1KO mice are indicative of a defect in β-cell compensation. This conclusion is supported by the results of the hyperglycemic clamps (Fig. 6) and static incubations of isolated islets (Fig. 7), both demonstrating a defect in GSIS after 13 weeks of high-fat diet.

The observed defect in GSIS in βPKD1KO mice is in agreement with previous findings indicating a positive correlation between PKD1 activity and insulin secretion. An increase in PKD1 activity in β-cells resulting from the loss of mitogen-activated protein kinase p38 δ (11) or peroxisome proliferator–activated receptor β/δ (25) enhances GSIS and improves glucose tolerance. Conversely, downregulation of PKD1 in insulin-secreting cell lines reduces GSIS (9,11) and defective PKD1 activation in phosphorylation-deficient M(3)-muscarinic receptor mutant mice decreases GSIS and glucose tolerance (8). In contrast, our data indicating that islet mass was not altered in βPKD1KO mice (Supplementary Fig. 3) diverge from previous studies supporting a role of PKD1 in β-cell proliferation and survival (11,25). However, the current study was conducted using adult β-cell–specific knockout mice, whereas evidence supporting a role of PKD1 in β-cell proliferation and survival is based on indirect PKD1 gain-of-function studies performed during neonatal development (25) or in a nonphysiological setting of streptozocin-induced apoptosis (11), respectively. Hence, the contribution of PKD1 to β-cell mass regulation may be context dependent.

Previously we demonstrated that PKD1 is necessary for GPR40-mediated potentiation of GSIS in response to long-chain

Figure 7—Insulin secretion from high-fat diet–fed βPKD1KO and MIP-CreERT mouse islets ex vivo. Insulin secretion presented as a percentage of islet insulin content was assessed in 1-h static incubations in response to glucose (A). Stimulation index calculated by the ratio of insulin secretion at 16.7 mmol/L to insulin secretion at 2.8 mmol/L glucose (B) and total islet insulin content (C). Data are mean ± SEM of 6–7 replicate experiments. *P < 0.05 compared with MIP-CreERT following one-tailed Student t test.
fatty acids (7). High-fat diet–fed mice deficient for GPR40 secrete significantly less insulin in response to glucose (5), a profile that resembles the phenotype of the high-fat diet–fed βPKD1KO mice described in this study. This similarity is consistent with an important role of PKD1 in the GPR40–signaling cascade. PKD1 is necessary for the replenishment of insulin granules (7,9,10); hence, it is tempting to speculate that altered vesicle formation and recruitment in βPKD1KO mice may yield a more profound phenotype under metabolic stress of high-fat feeding, when insulin demand is higher, than under basal conditions. However, the absence of a significant change in proinsulin levels in high-fat diet–fed βPKD1KO mice (Fig. 4) suggests that insulin granule maturation is intact. Further studies will be required to elucidate the precise molecular events downstream of PKD1 controlling functional β-cell compensation.

In conclusion, we provide the first in vivo and ex vivo analysis of a β-cell–specific PKD1 knockout mouse and show that PKD1 in β-cells is dispensable under basal conditions but necessary for the compensatory response in GSIS in response to high-fat feeding, furthering our understanding of the implication of this kinase in the control of β-cell function.

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