The FFA receptor GPR40 links hyperinsulinemia, hepatic steatosis, and impaired glucose homeostasis in mouse

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Summary

Obesity is typically associated with elevated levels of free fatty acids (FFAs) and is linked to glucose intolerance and type 2 diabetes. FFAs exert divergent effects on insulin secretion from β cells: acute exposure to FFAs stimulates insulin secretion, whereas chronic exposure impairs insulin secretion. The G protein-coupled receptor GPR40 is selectively expressed in β cells and is activated by FFAs. We show here that GPR40 mediates both acute and chronic effects of FFAs on insulin secretion and that GPR40 signaling is linked to impaired glucose homeostasis. *GPR40*-deficient β cells secrete less insulin in response to FFAs, and loss of G*PR40* protects mice from obesity-induced hyperinsulinemia, hepatic steatosis, hypertriglyceridemia, increased hepatic glucose output, hyperglycemia, and glucose intolerance. Conversely, overexpression of *GPR40* in β cells of mice leads to impaired β cell function, hypoinsulinemia, and diabetes. These results suggest that GPR40 plays an important role in the chain of events linking obesity and type 2 diabetes.

Introduction

Type 2 diabetes is strongly linked to visceral obesity and elevated levels of circulating FFAs (Kahn and Flier, 2000; Zraika et al., 2002; Yaney and Corkey, 2003; Kashyap et al., 2003; Moller and Kaufman, 2005). The PPAR class of nuclear receptors bind and respond to FFAs (Evans et al., 2004), and, recently, members of the G protein-coupled class of transmembrane receptors have been shown to be activated by FFAs (Briscoe et al., 2003; Itoh et al., 2003; Kotarsky et al., 2003; Hirasawa et al., 2005). Among these, GPR40 is preferentially expressed in pancreatic β cells (Briscoe et al., 2003; Itoh et al., 2003; Kotarsky et al., 2003), and long-chain fatty acids enhance glucose-stimulated insulin secretion (GSIS) from insulinoma cell lines in a GPR40-dependent manner (Itoh et al., 2003). The effects of FFAs on insulin secretion are, however, complex and divergent. Acutely increased levels of FFAs enhance GSIS (Haber et al., 2002; Zraika et al., 2002; Yaney and Corkey, 2003). In contrast, prolonged exposure of β cells to high concentrations of FFAs impairs several aspects of β cell function including GSIS (Haber et al., 2002; Zraika et al., 2002; Yaney and Corkey, 2003). Typically, the development of type 2 diabetes is characterized by an early state of hyperinsulinemia and glucose intolerance that progresses to hypoinsulinemia and overt diabetes (Kahn, 1998; Taylor, 1999; Linghor et al., 2002). The dual and opposing effects of FFAs on insulin secretion raise the possibility that FFAs contribute to both hyper- and hypoinsulinemia during the development of type 2 diabetes.

The exact mechanism underlying the negative effects of FFAs on β cell function and GSIS is not fully understood: the majority of studies have been performed on clonal β cells or isolated islets, and results have often been conflicting. Excess FFAs have been proposed to affect GSIS via the Randle or glucose/fatty acid oxidation cycle (Zhou and Grill, 1994; Zhou and Grill, 1995; Zhou et al., 1996): other studies claim that increased fatty acid oxidation does not impair glucose metabolism in FFA-exposed β cells (Segall et al., 1999; Liu et al., 1999; Boucher et al., 2004) and instead propose that FFAs perturb the glucose-mediated increase in pyruvate cycling in β cells (Iizuka et al., 2002; Boucher et al., 2004). In leptin signaling-deficient animals with impaired fatty acid oxidation in β cells, excess levels of circulating FFAs have been proposed to lead to toxic accumulation of triglycerides in β cells (Shimabukuro et al., 1998; Unger, 2002). FFAs stimulate *PPARα* activity and expression in isolated islets and insulinoma cells, and adenoviral-mediated expression of *PPARα* expression in isolated islets or insulinoma cells stimulates fatty acid oxidation and impairs GSIS (Zhou et al., 1998; Tordjman et al., 2002). Whether GPR40 contributes to the negative effects of FFA signaling on insulin secretion from β cells, however, remains to be established.

Elevated levels of FFAs promote lipid accumulation and insulin resistance in target tissues (Kahn and Flier, 2000; Haber et al., 2002; Zraika et al., 2002; Yaney and Corkey, 2003; Kashyap et al., 2003; Moller and Kaufman, 2005). Hyperinsulinemia and elevated hepatic glucose output are hallmarks of insulin resistance, and hyperinsulinemia per se has been proposed to contribute to the development of insulin resistance, fatty liver/hepatic steatosis, and increased hepatic glucose output (Kahn and Flier, 2000; Wolfrum et al., 2004; Moller and Kaufman, 2005). Thus, under conditions of visceral obesity, FFA-stimulated insulin secretion may promote hyperinsulinemia that contributes to hepatic steatosis, increased hepatic glucose output, and impaired glucose homeostasis. The role, if any, of GPR40 in the development of obesity-induced hyperinsulinemia and hepatic dysfunction remains, however, to be elucidated.

We have used genetic approaches in mice to address the role of GPR40 in insulin secretion and glucose homeostasis. Our results provide evidence that GPR40 mediates both acute and chronic effects of FFAs on insulin secretion in β cells. Loss of *GPR40* protects mice from obesity-induced hyperinsulinemia, hepatic steatosis, hypertriglyceridemia, increased hepatic glucose output, hyperglycemia, and glucose intolerance, whereas transgenic overexpression of *GPR40* in β cells leads to impaired β cell function, hypoinsulinemia, and diabetes, mimicking the overt diabetic state.

Results

Impaired insulin secretory response to FFA in *GPR40*$^{-/-}$ mice

GPR40 is expressed in adult β cells of rodents with little or no expression reported in peripheral tissues like liver, skeletal muscle, or adipose tissue, and, although expression has been reported in human brain, expression is not seen in brain of mouse or rat (Briscoe et al., 2003; Itoh et al., 2003; Kotarsky et al., 2003; see Figure S1 in the Supplemental Data available with this article online). *GPR40* expression was also observed in insulin-positive cells of mice at embryonic day (E) 15 and 17 and in neonates (Figure 1A). Insulin-expressing cells are readily detectable already at E13, but these cells do not express GPR40 (data not shown), suggesting that *GPR40* is expressed preferentially in differentiated β cells. To investigate the role(s) of *GPR40* in vivo, we generated *GPR40*-deficient mice through homologous recombination (Figures 1B–1D). The targeted *GPR40* allele contained lacZ-cDNA encoding the marker protein β-galactosidase (β-gal), thus allowing identification of tissues in which the GPR40 promoter is active in heterozygous and homozygous mutant mice. β-gal staining of tissues isolated from *GPR40*$^{+/-}$ mice confirmed the preferential expression of GPR40 in pancreatic β cells, whereas no expression could be detected in liver, fat, skeletal muscle, or brain (Figure S2). *GPR40*$^{-/-}$ mice were viable, apparently healthy, lean, and fertile. They showed no signs of diabetes, and fasting blood glucose levels and glucose tolerance were normal (Figure 1E). *GPR40*$^{-/-}$ mice displayed lower fasted serum insulin levels than *GPR40*$^{+/+}$ mice but maintained a biphasic insulin secretory response upon glucose challenge (Figure 1F). Together, these data provide evidence that, under normal nutritional conditions, GPR40-mediated FFA signaling is not essential for insulin secretion or maintenance of glucose homeostasis.

To test whether loss of *GPR40* affects the β cell response to FFAs, we examined whether islets from GPR40 null mice had a perturbed insulin secretory response to FFAs. Palmitic acid (PA) robustly stimulated insulin secretion from control islets but not from islets isolated from *GPR40*$^{-/-}$ mice (Figures 2A and 2B), although these islets responded to increased glucose levels (Figures 2A and 2B). These results confirm the proposed role for GPR40 in mediating FFA stimulation of insulin secretion (Itoh et al., 2003). To elucidate whether GPR40 also mediated the long-term negative effect of FFAs on GSIS, we exposed islets isolated from *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice to PA for 48 hr. Long-term (48 hr) incubation of islets in the absence of FFAs revealed a difference in insulin secretion from *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ islets that was not observed when performing short-term (14 hr) incubation (Figures 2B and 2C). However, in vitro culture of islets isolated from *GPR40*$^{+/+}$ mice in the presence of PA for 48 hr resulted, as expected, in attenuation of GSIS compared to nonexposed islets (Figure 2C). In contrast, insulin secretion from islets isolated from *GPR40*$^{-/-}$ mice was not impaired by 48 hr exposure to PA (Figure 2C), demonstrating that *GPR40*$^{-/-}$ islets cultured in vitro are protected from the long-term negative effect of FFAs on GSIS. Taken together, the combined data derived from the short- and long-term exposure of *GPR40*$^{-/-}$ and control islets to PA in vitro provide evidence that GPR40 mediates both the short- and long-term effects of FFA on GSIS.

GPR40$^{-/-}$ mice do not develop hyperinsulinemia and glucose intolerance on HFD

To investigate how lack of GPR40 function affects insulin secretion and glucose homeostasis in vivo under conditions of obesity/increased FFA levels, we exposed *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice to a high-fat diet (HFD) for 8 weeks. HFD leads to obesity and a variety of metabolic disturbances including elevated FFA plasma levels, insulin resistance, hyperinsulinemia, and glucose intolerance (Gregoire et al., 2002; Rossmeisl et al., 2003; Wolfrum et al., 2004). *GPR40*$^{-/-}$ and *GPR40*$^{+/+}$ littermates gained weight similarly on the HFD and became obese (Figures 3A and 3B). As expected, *GPR40*$^{+/+}$ mice kept on HFD became hyperinsulinemic (Table 1 and Figures 3C and 3D); in contrast, fasted serum insulin levels and glucose-stimulated insulin secretion were significantly lower in *GPR40*$^{-/-}$ mice (Table 1; Figures 3C and 3D). *GPR40*$^{+/+}$ mice showed elevated fasted blood glucose levels and were glucose intolerant (Table 1; Figures 3E and 3F), whereas *GPR40*$^{-/-}$ mice did not develop significant glucose intolerance, and both nonfasted and fasted blood glucose levels were lower than in control littermates (Table 1; Figures 3E and 3F and data not shown). To begin to unravel the mechanism underlying the protection from diet-induced glucose intolerance in *GPR40*$^{-/-}$ mice, we compared insulin sensitivity in these mice. No difference in insulin sensitivity was observed between *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice on control diet (CD) (Figure 3G). On HFD, both *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice developed insulin resistance but *GPR40*$^{-/-}$ mice to a lesser extent (Figure 3H). Taken together, these data show that, compared to *GPR40*$^{+/+}$ littermates, *GPR40*$^{-/-}$ mice kept on HFD secrete less insulin, do not develop severe glucose intolerance, and become less insulin resistant.

GPR40$^{-/-}$ mice are protected from hepatic steatosis and hypertriglyceridemia

Mice kept on HFD develop fatty liver/hepatic steatosis and hypertriglyceridemia and show elevated hepatic glucose output (Gregoire et al., 2002; Rossmeisl et al., 2003; Wolfrum et al., 2004). Hyperinsulinemia per se has been proposed to trigger hepatic steatosis and hepatic insulin resistance (Kahn and Flier, 2000; Wolfrum et al., 2004; Moller and Kaufman, 2005). We therefore examined whether HFD-fed *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice developed hepatic steatosis and hypertriglyceridemia. HFD treatment of *GPR40*$^{+/+}$ mice led to a dramatic increase in stored lipids in livers (Figure 4A). Serum triglyceride levels were also increased in the HFD-fed *GPR40*$^{+/+}$ mice (Table 1). No similar increases were observed in HFD-treated *GPR40*$^{-/-}$ mice (Figure 4A; Table 1). In several obese and diabetic animal models, hepatic steatosis is correlated with increased hepatic glucose output that results from hepatic insu-

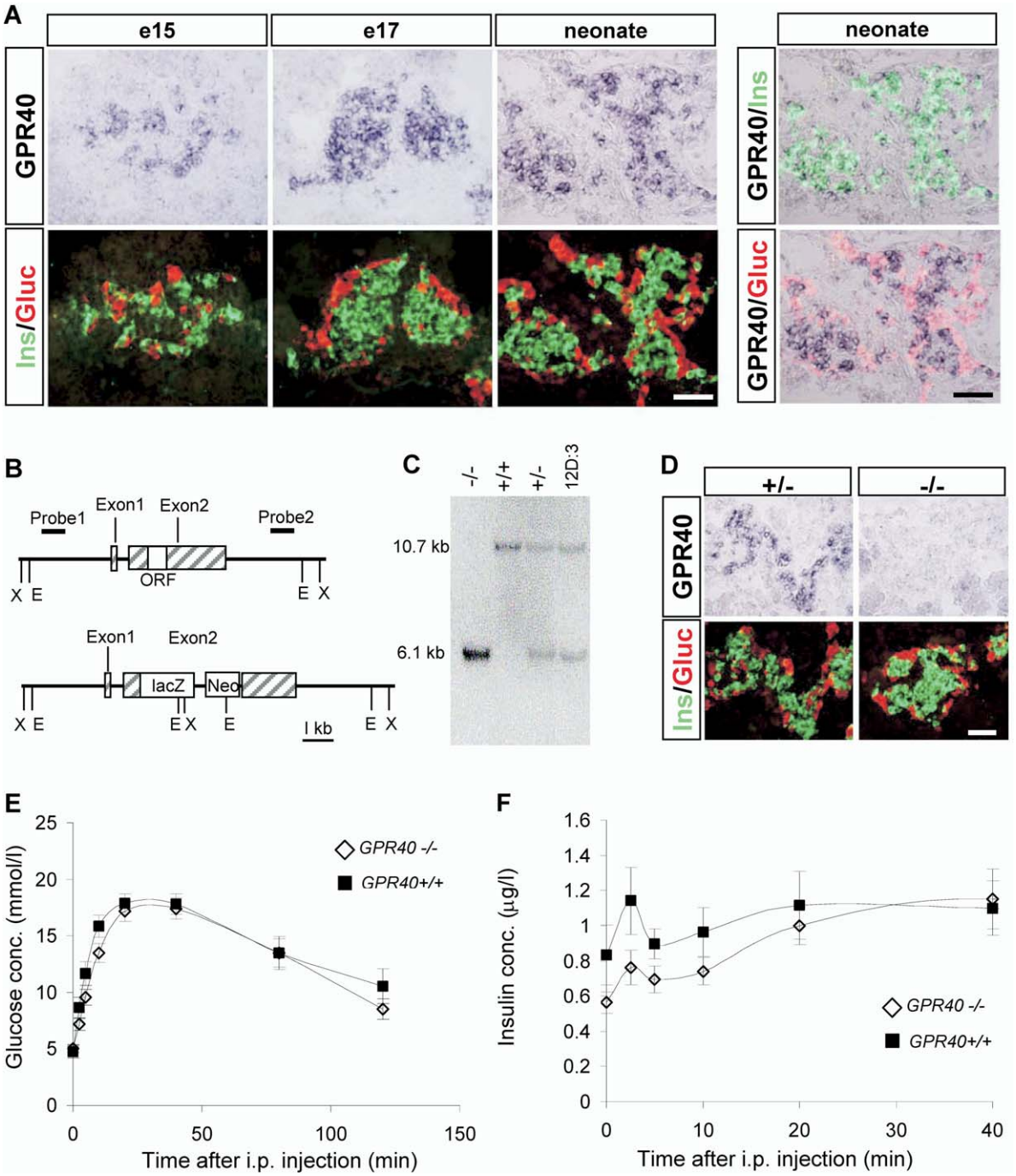

Figure 1. *GPR40* expression and generation of *GPR40*$^{-/-}$ mice

A) *GPR40* is expressed in fetal β cells. In situ hybridisation of E15, E17, and neonatal mouse pancreas using a DIG-labeled *GPR40* cDNA probe, counterstained with antibodies against insulin and/or glucagon as indicated.

B) Targeted disruption of the *GPR40* gene. Structure of the wild-type and recombinant *GPR40* locus; the localization of the probes (probe 1 and 2) used for genotyping is indicated. X, XbaI; E, EcoRI.

C) Southern blot analysis of XbaI-digested genomic DNA from +/+, +/–, and –/– mice and the targeted ES cells clone (12D:3).

D) In situ hybridization of E16.5 wild-type and *GPR40*$^{-/-}$ pancreas for *GPR40* mRNA. Scale bars, 20 μm.

E) Glucose tolerance test in *GPR40*$^{-/-}$ (◇; n = 12) and *GPR40*$^{+/+}$ (■; n = 12) littermates. Blood glucose was measured at the indicated time points following intraperitoneal (i.p.) injection of glucose.

F) Insulin secretion from pancreas of *GPR40*$^{-/-}$ (n = 7) and *GPR40*$^{+/+}$ (n = 10) littermates during glucose tolerance test. Data represent the mean ± SEM.

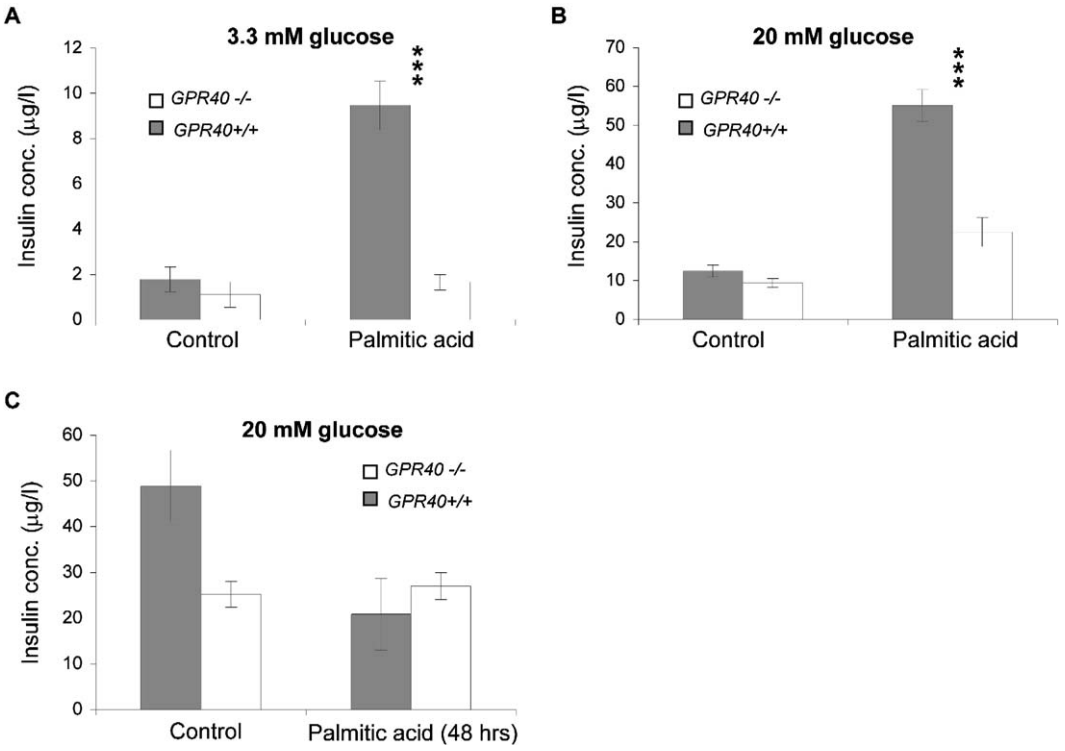

Figure 2. FFA stimulation of insulin secretion in isolated islets

A–C) The effect on GSIS after short-term (2 hr; [**A**] and [**B**]) and long-term (48 hr; [**C**]) exposure to palmitic acid (0.5 mM) was measured using islets derived from *GPR40*$^{-/-}$ (white bars; n = 4 in [**A**] and [**B**]; n = 3 in [**C**]) and wild-type (gray bars; n = 3 in [**A**] and [**B**]; n = 3 in [**C**]) littermates. Data represent the average values ± SEM. ***p < 0.001 for *GPR40*$^{-/-}$ versus *GPR40*$^{+/+}$.

lin resistance, and reversal of hepatic steatosis increases hepatic insulin sensitivity (Matsusue et al., 2003; Wolfrum et al., 2004). Thus, we next compared gluconeogenesis in HFD-fed mice. In HFD-fed *GPR40*$^{+/+}$ mice, administration of the gluconeogenic substrate pyruvate (Miyake et al., 2002) led to a significant increase in blood glucose levels that persisted at 120 min (Figure 4C). However, pyruvate injection into HFD-fed *GPR40*$^{-/-}$ mice resulted in a more modest increase in blood glucose concentration that returned almost to basal levels by 120 min, similar to results obtained with CD-fed *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice (Figure 4B). Collectively, these data provide evidence that *GPR40*$^{-/-}$ mice are protected from HFD-induced hepatic steatosis, hypertriglyceridemia, and increased hepatic glucose output.

To elucidate the mechanism underlying this differential susceptibility to HFD-induced hepatic steatosis, we compared the expression of genes that regulate lipid content, i.e., genes involved in fatty acid synthesis, fatty acid oxidation, and fatty acid uptake in *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice kept on CD and HFD. In agreement with previous observations (Kim et al., 2004), the expression of genes involved in fatty acid synthesis was either decreased (fatty acid synthase [FAS] and stearoyl-CoA desaturase [SCD]) or unchanged (malic enzyme [MAL]) for both groups of HFD-fed mice compared to mice on CD (Figure 4D). Accordingly, the expression of the transcription factor sterol-regulatory element binding protein-1c (SREBP-1c) that transcriptionally activates expression of lipogenic genes was reduced in the HFD-fed mice compared to CD-fed mice (Figure 4D). The expression of CPT-1 was also unchanged in livers of HFD-fed *GPR40*$^{+/+}$ mice (Figure 4D), providing evidence that β oxidation is not decreased in the HFD-fed *GPR40*$^{+/+}$ mice. *PPARγ* expression is increased in livers of obese and diabetic animal models (Chao et al., 2000; Memon et al., 2000; Bedoucha et al., 2001; Rahimian et al., 2001) and has been directly linked to the development of fatty liver and hepatic insulin resistance. Adenovirus-mediated overexpression of PPARγ in livers of mice leads to hepatic steatosis, and selective inactivation of PPARγ in livers of *ob/ob* and AZIP-F-1 mice reduces hepatic steatosis (Gavrilova et al., 2003; Matsusue et al., 2003; Yu et al., 2003). The expression of PPARγ was upregulated in livers of HFD-fed *GPR40*$^{+/+}$ but not HFD-fed *GPR40*$^{-/-}$ mice (Figure 4D). Consistent with this, expression of the PPARγ target gene fatty acid translocase (FAT/CD36) (Memon et al., 2000; Yu et al., 2003) that stimulates fatty acid uptake by cells (Schaffer, 2002) was also upregulated in livers of HFD-fed *GPR40*$^{+/+}$ mice (Figure 4D). Foxa2 activity in the liver has been suggested to be regulated by insulin and to be inactive and constitutively located to the cytoplasm in insulin-resistant and/or hyperinsulinemic mice (Wolfrum et al., 2004). We did not observe this phenomenon in our animals: we find Foxa2 protein in the nucleus of insulin-resistant, hyperinsulinemic, nonfasted, HFD-fed mice (Figure S3). Collectively, these data suggest that the hepatic steatosis observed in the HFD-fed control mice is the consequence of increased fatty acid uptake rather

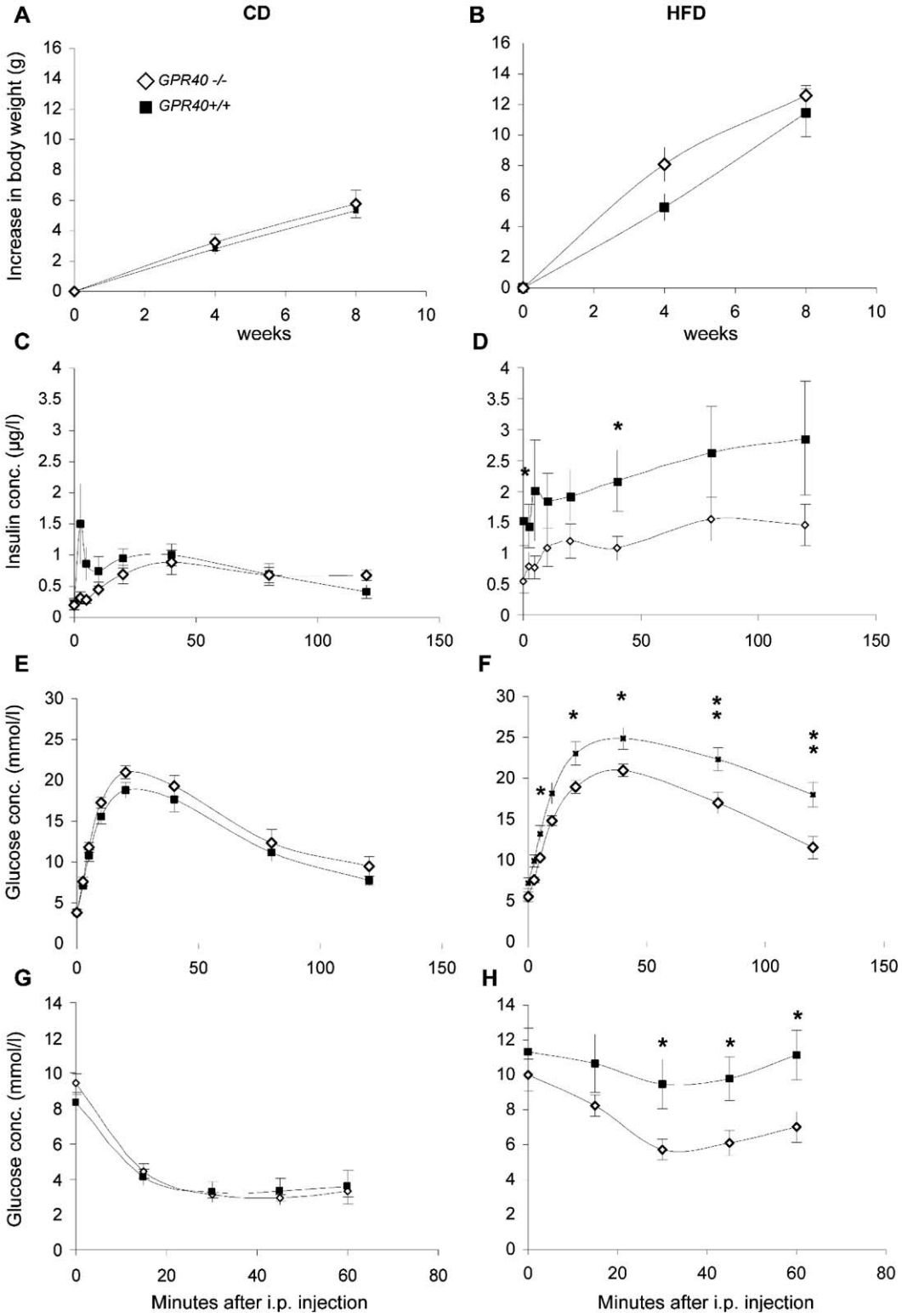

Figure 3. Diet-induced obesity, hyperinsulinemia, and glucose intolerance

Weight increase (**A** and **B**), insulin secretion during glucose tolerance test (**C** and **D**), glucose tolerance (**E** and **F**), and insulin tolerance (**G** and **H**) were determined in *GPR40*$^{-/-}$ (◇) and *GPR40*$^{+/+}$ (■) mice fed on control (CD) (**A**, **C**, **E**, and **G**) and high-fat diet (HFD) (**B**, **D**, **F**, and **H**) for 8 weeks.

A and B) Weight increase of *GPR40*$^{-/-}$ (CD, n = 6; HFD, n = 8) and *GPR40*$^{+/+}$ (CD, n = 8; HFD, n = 13) mice was determined at 4 and 8 weeks of diet.

C and D) Serum insulin levels after i.p. injection of glucose in *GPR40*$^{-/-}$ (CD, n = 9; HFD, n = 9) and *GPR40*$^{+/+}$ (CD, n = 6; HFD, n = 6) mice.

E and F) Blood glucose concentrations at the indicated time points following i.p. injection of glucose in *GPR40*$^{-/-}$ (CD, n = 6; HFD, n = 8) and *GPR40*$^{+/+}$ (CD, n = 7; HFD, n = 14) mice.

G and H) Blood glucose concentrations at the indicated time points following after i.p. injection of human insulin (0.75 units/kg body weight), in *GPR40*$^{-/-}$ (CD, n = 4; HFD, n = 9), and *GPR40*$^{+/+}$ (CD, n = 7; HFD, n = 9) mice. *$p < 0.05$ and **$p < 0.01$ for *GPR40*$^{-/-}$ versus *GPR40*$^{+/+}$.

Table 1. Blood glucose, insulin, and triglyceride levels in fasted *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice fed on a control or high-fat diet

	Blood glucose (mmol/l)	Insulin (μg/l)	Triglycerides (mmol/l)
GPR40$^{+/+}$ (CD) n = 6	4.9 ± 0.3	0.2 ± 0.09	1.7 ± 0.1
GPR40$^{+/+}$ (HFD) n = 12	9.6 ± 0.3	1.4 ± 0.25	3.2 ± 0.4
GPR40$^{-/-}$ (CD) n = 10	5.5 ± 0.4	0.2 ± 0.08	1.9 ± 0.3
GPR40$^{-/-}$ (HFD) n = 9	6.0 ± 0.6	0.5 ± 0.18	1.6 ± 0.3
p (wt versus GPR40$^{-/-}$ HFD)	0.03	0.01	0.0001

Blood glucose, triglycerides, and serum insulin levels were determined in overnight-fasted *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice fed control (CD) or high-fat diet (HFD) for 8 weeks.

than increased fatty acid synthesis or decreased fatty acid oxidation. The results also provide evidence that *GPR40*, in an indirect manner, is required for the HFD-induced expression of PPARγ and the target gene FAT/CD36 in liver cells and for the development of hepatic steatosis and hypertriglyceridemia.

Transgenic overexpression of GPR40 in β cells leads to diabetes

To try to mimic sustained GPR40 stimulation and the proposed long-term negative effects of FFAs on insulin secretion (Haber et al., 2002; Zraika et al., 2002; Yaney and Corkey, 2003), we overexpressed *GPR40* under the control of the *Ipf1/Pdx1* promoter (Apelqvist et al., 1997) in transgenic mice. Transgene expression was confirmed both by in situ hybridisation and RT-PCR (Figure S4 and data not shown). The *Ipf1/GPR40* transgenic mice were born alive and lean and appeared initially healthy but soon developed overt diabetes. The *Ipf1/GPR40* mice were glucose intolerant and exhibited a loss of first-phase insulin release and a severely blunted second-phase insulin release (Figures 5A and 5B). In contrast, mice overexpressing enhanced green fluorescent protein or the Cre-recombinase under the control of the *Ipf1/Pdx1* promoter did not show any signs of hyperglycaemia or glucose intolerance when challenged with exogenous glucose (Figure S5). The diabetic phenotype observed by overexpression of *GPR40* in β cells suggests that endogenous ligand(s) exists in excess or, alternatively, that the abundance of the receptor results in ligand-independent receptor activation. The latter has been observed for a number of different receptors, including G protein-coupled receptors (Taylor et al., 1995; Wang et al., 1998; Malone et al., 2004; Hoffmann et al., 2004). Thus, overexpression of *GPR40* in β cells, potentially mimicking chronic signaling, leads to perturbed GSIS and diabetes, supporting the idea that sustained stimulation of *GPR40* is deleterious for β cell function and insulin secretion.

To begin to unravel the mechanism by which overexpression of *GPR40* leads to perturbed β cell function and insulin secretion, we examined the organization of the pancreas of 10-week-old *Ipf1/GPR40* and control mice. The pancreas from *Ipf1/GPR40* mice was morphologically normal (data not shown), indicating that expression of *GPR40* in the developing pancreatic epithelium had no gross effect on pancreatic development. Whole-mount immunostaining of the pancreas using antibodies against insulin, glucagon, and smooth muscle α-actin revealed an apparently normal organization of major blood vessels and a normal amount, distribution, and size of islets in the pancreas of the *Ipf1/GPR40* transgenic mice (Figure 5C and data not shown). Moreover, immunohistochemical analyses using antibodies directed toward activated caspase-3 failed to detect an increase in apoptosis in the pancreas of *Ipf1/GPR40* mice (data not shown), providing evidence that the impaired GSIS displayed by *Ipf1/GPR40* mice is the result of β cell dysfunction rather than β cell death. However, as observed in other diabetic animal models (Hart et al., 2000; Yamagata et al., 2002), the postnatal organization of endocrine cells within the islets was perturbed. In contrast to their normal peripheral localization, the glucagon-producing α cells were distributed throughout the islets (Figure 5C). Although the number of β cells appeared normal in the *Ipf1/GPR40* mice (data not shown), the total pancreatic insulin content was reduced by ~50% (Figure 5D). Real-time (RT) PCR analyses of insulin mRNA levels in islets derived from *Ipf1/GPR40* and control mice showed that insulin gene expression was normal in *Ipf1/GPR40* mice (Figure 5E), and, consistently, *Ipf1/Pdx1* expression was reduced by at most ~30% (Figure 6I). These results suggest that the decrease in stored insulin in these mice may be due to impaired biosynthesis of insulin protein and/or enhanced insulin secretion, resembling the situation observed in islets after long-term exposure to high levels of FFAs (Bjorklund and Grill, 1999; Furukawa et al., 1999).

Impaired expression of factors controlling glucose sensing and proinsulin processing in *Ipf1/GPR40* mice

To investigate whether the β cell dysfunction of *Ipf1/GPR40* mice mimicked that of β cells exposed to FFAs also at the molecular level, we analyzed the expression of genes and proteins that control aspects of β cell glucose metabolism and insulin biosynthesis pathways and are affected by elevated levels of FFAs. Long-term exposure of islets to elevated FFAs in vitro results in impaired expression of glucose transporter 2 (Glut2) (Gremlich et al., 1997), which, in mice, is required for normal β cell function and glucose homeostasis (Guillam et al., 1997). In the *Ipf1/GPR40* mice, the expression of Glut2 mRNA and protein in islet cells was virtually undetectable (Figures 6A, 6E, and 6I). An increased proinsulin-to-insulin (P/I) ratio is observed in many type 2 diabetics (Kahn et al., 1995; Nijpels et al., 1996; Haffner et al., 1997; Larsson and Ahren, 1999), and long-term exposure of islets to FFAs perturbs the biosynthesis and post-translational processing of prohormone convertase 1/3 (PC1/3), one of the key enzymes involved in proinsulin-to-insulin processing, leading to impaired proinsulin processing (Bjorklund and Grill, 1999; Furukawa et al., 1999). The level of PC1/3 protein but not mRNA appeared reduced in islet cells of *Ipf1/GPR40* mice (Figures 6B and 6F and data not shown); conversely, proinsulin immunoreactivity was significantly increased in β cells of these mice (Figures 6C and 6G). The reduced ex-

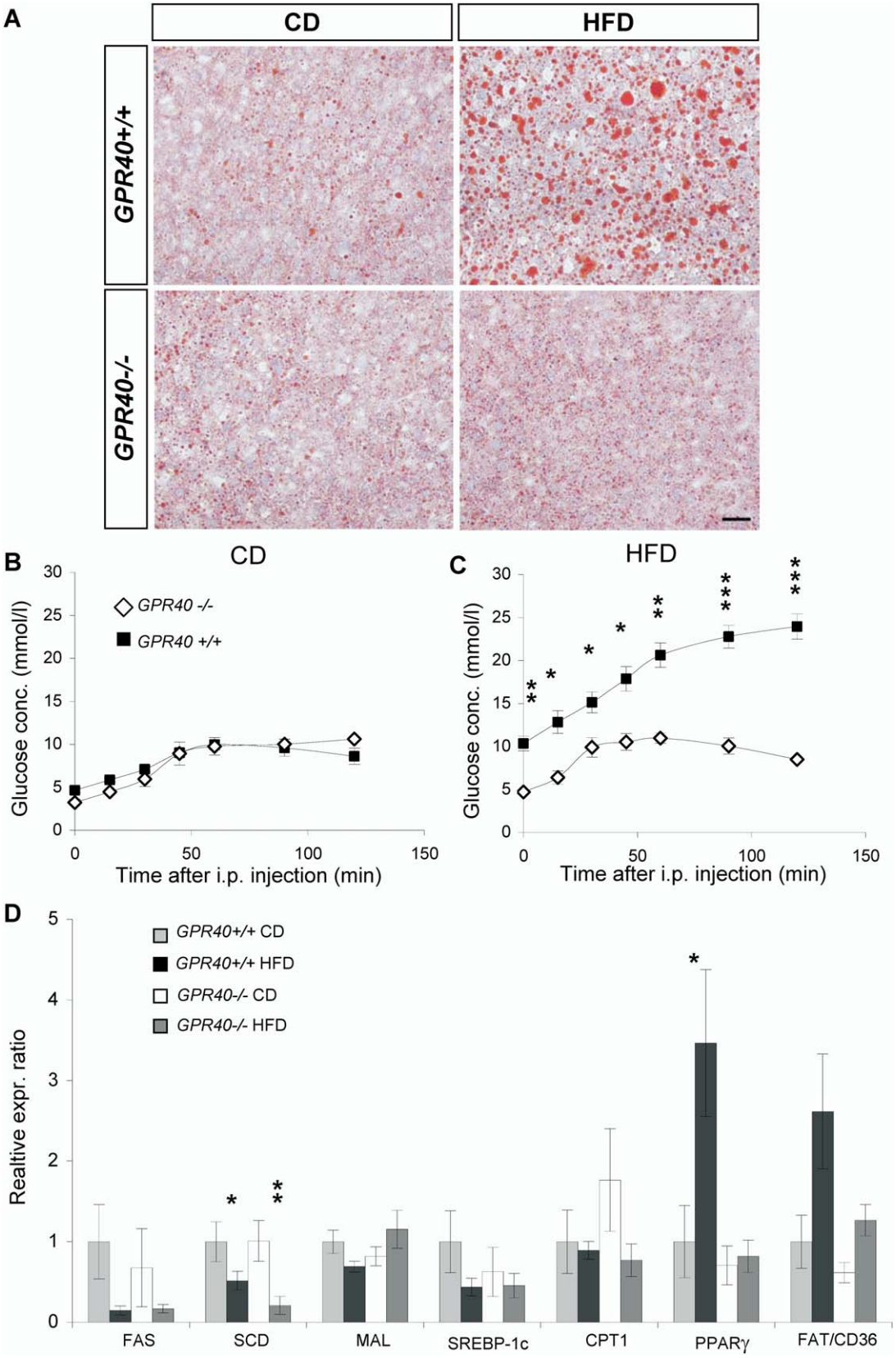

Figure 4. Hepatic steatosis and increased glucose output in high-fat-diet-fed *GPR40*$^{+/+}$ mice

A) Oil Red O staining of livers derived from *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice after control diet (CD) and high-fat diet (HFD) treatment. Scale bar, 20 μm.

B and C) Blood glucose concentrations at the indicated time points following i.p. injection of pyruvate (2 g/kg body weight) in *GPR40*$^{-/-}$ (CD, n = 3; HFD, n = 3) and *GPR40*$^{+/+}$ (CD, n = 6; HFD, n = 7) mice. *p < 0.05, **p < 0.01, and ***p < 0.001 for *GPR40*$^{-/-}$ versus *GPR40*$^{+/+}$.

D) Quantitative real-time RT-PCR expression analyses of the indicated genes were performed using liver cDNA prepared from *GPR40*$^{+/+}$ (CD, n = 5; HFD, n = 6) and *GPR40*$^{-/-}$ (CD, n = 5; HFD, n = 7) mice on a control (CD) or high-fat diet (HFD). *p < 0.05 and **p < 0.01 for indicated animals on CD versus HFD.

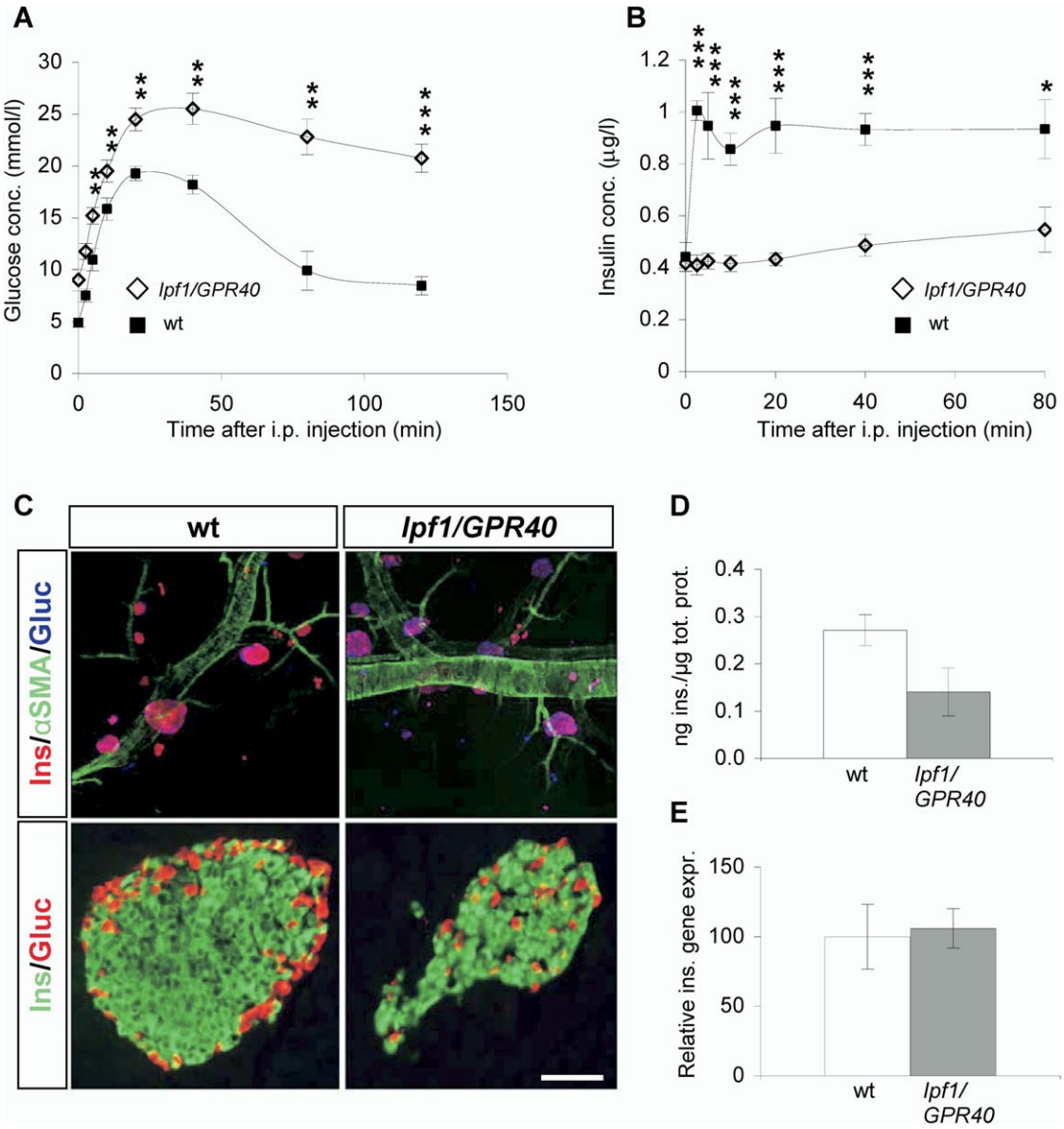

Figure 5. *lpf1/GPR40* mice are glucose intolerant and have impaired glucose-stimulated insulin secretion

A) Blood glucose was measured in *lpf1/GPR40* (◇, n = 7) and wild-type (■, n = 7) littermates at the indicated time points following i.p. injection of glucose.

B) Serum insulin levels during tolerance test in *lpf1/GPR40* (n = 7) and control littermates (n = 3). Data represent the mean value ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001 for *lpf1/GPR40* versus wild-type littermates.

C) Whole-mount immunostained adult pancreas (upper panel) from wild-type and *lpf1/GPR40* mice showing blood vessels stained for smooth muscle α-actin (green) and islets stained for insulin (pseudored) and glucagon (pseudoblue). Sectioned adult pancreas from wild-type and diabetic *lpf1/GPR40* mice stained for glucagon (red) and insulin (green). Scale bar, 20 µm.

D) Determination of total insulin content in isolated pancreas from diabetic *lpf1/GPR40* (gray bars) and wild-type controls (white bars); p = 0.09 for wild-type versus *lpf1/GPR40*.

E) Quantitative real-time RT-PCR of insulin mRNA from isolated islets of wild-type (white bars, n = 3) and *lpf1/GPR40* (gray bars, n = 3) mice.

pression of Glut2 and PC1/3 and accumulation of unprocessed proinsulin in β cells are likely to contribute to the development and/or progression of diabetes in the *lpf1/GPR40* mice.

Altered expression of PPARα and target genes in islets of *lpf1/GPR40* mice

PPARs act as intracellular receptors for FFAs; whereas *PPARα* expression is upregulated in isolated islets and insulinoma cells after exposure to FFAs (Zhou et al., 1998; Tordjman et al., 2002), conflicting data exist regarding the effect of FFAs on PPARγ expression in islets (Patanè et al., 2002; Lupi et al., 2004). Although the role for PPARs in β cells is not fully understood, in vitro studies suggest that PPARα stimulates fatty acid oxidation and impairs insulin secretion (Zhou et al., 1998; Tordjman et al., 2002). Hence, we next investigated the expression of *PPARα* and *PPARγ* and genes whose expression has been

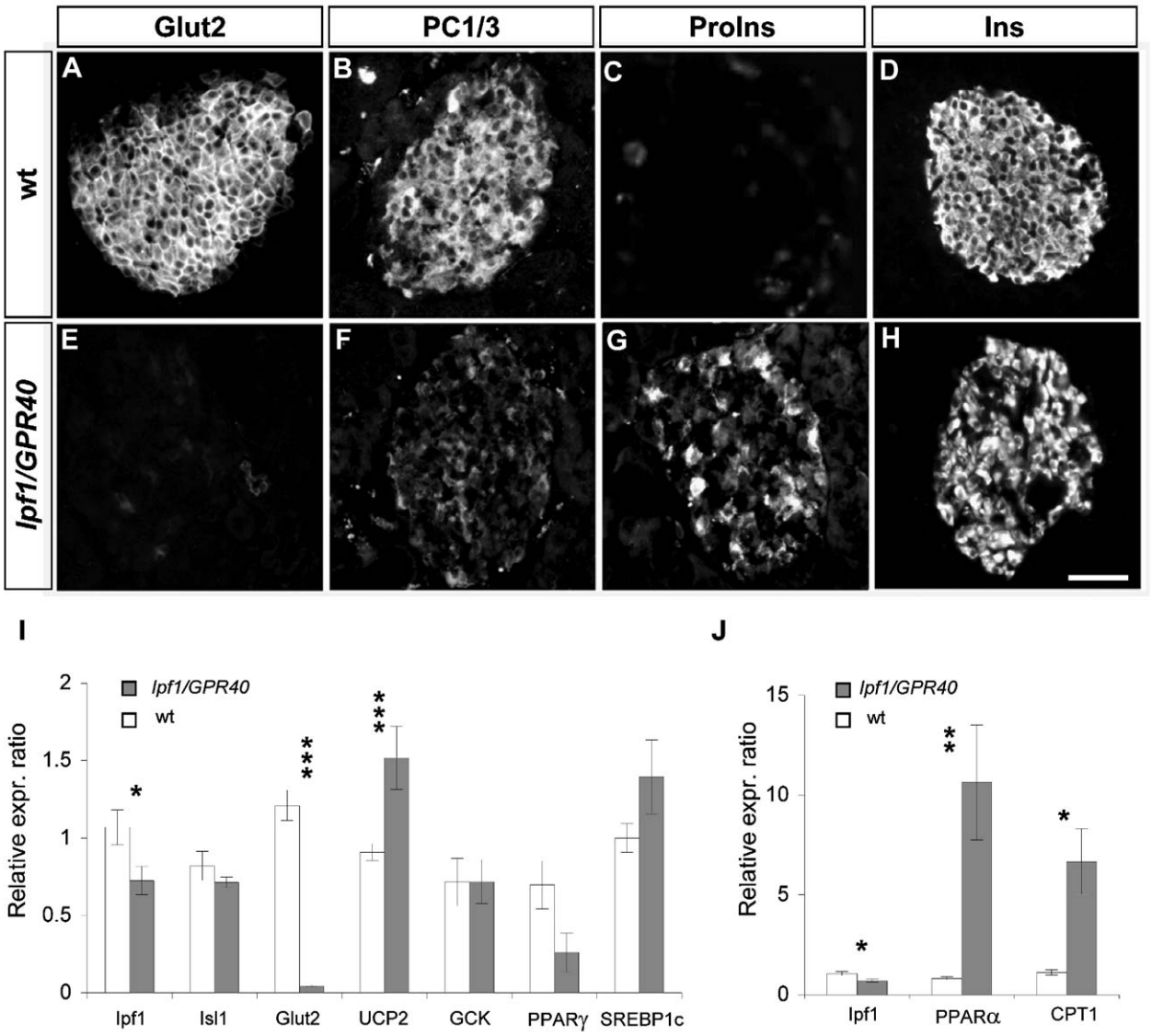

Figure 6. Perturbed expression of genes involved in GSIS and insulin exocytosis in *Ipf1/GPR40* mice

A–H) Sections of pancreas from wild-type (**A**–**D**) and diabetic *Ipf1/GPR40* mice (**E**–**H**) stained for Glut2 (**A** and **E**), PC1/3 (**B** and **F**), proinsulin (**C** and **G**), and insulin (**D** and **H**). Scale bar, 20 μm.

I and J) Quantitative real-time RT-PCR expression analyses of the indicated genes were performed using islet cDNA prepared from *Ipf1/GPR40* (gray bars, n = 3) and wild-type (white bars, n = 3) mice. Data represent mean values ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001 for *Ipf1/GPR40* versus wild-type littermates.

linked, directly or indirectly, to *PPAR* expression and fatty acid oxidation. We also analyzed the expression of genes involved in β cell differentiation and function, lipogenesis, and cell death. The expression of *Isl1*, *glucokinase* (*GCK*), hepatocyte nuclear factor (*HNF*) *1-* and *4α*, *Forkhead box* (*Fox*) *a2*, sterol regulatory element binding protein *1c (Srebp1c)*, carbohydrate response element binding protein (*Chrep*), and *bcl-2* genes showed little or no change at the mRNA level (Figure 6I and Figure S6). The expression of the nuclear FFA receptors PPARα and PPARγ were, however, perturbed in islets of *Ipf1/GPR40* mice; *PPARα* expression was upregulated ~13-fold, whereas the expression of *PPARγ* was reduced by ~35% (Figures 6I and 6J) compared to that in control islets. The expression of the FFA-inducible gene *CPT1*, which is a target gene for *PPARα* (Assimacopoulos-Jeannet et al., 1997; Zhou et al., 1998, Rubí et al., 2002; Tordjman et al., 2002) and encodes the rate limiting enzyme in fatty acid oxidation, was upregulated ~6-fold in islets of *Ipf1/GPR40* mice (Figure 6J). FFAs, fatty acid oxidation, and direct overexpression of *PPARα* in an insulinoma cell line have also been shown to stimulate expression of uncoupling protein 2 (*UCP2*), and UCP2 levels are inversely correlated with GSIS both in vitro and in vivo (Lameloise et al., 2001; Chan et al., 1999; Zhang et al., 2001; Chan et al., 2001; Tordjman et al., 2002). *UCP2* expression was increased by ~70% in islets of *Ipf1/GPR40* mice (Figure 6I). It has been proposed that a concomitant increase in both CPT1 and UCP2 contributes to the perturbation of GSIS in β cells after exposure to FFA (Rubí et al., 2002). Thus, the increased expression of

PPARα and its target genes *CPT1* and *UCP2* in islets of *Ipf1/GPR40* mice are likely to contribute to the impaired GSIS and development of diabetes observed in the *Ipf1/GPR40* mice.

Discussion

Type 2 diabetes is strongly associated with obesity, and both conditions are increasing at an alarming pace. Obesity is often associated with elevated levels of FFAs, which promote lipid accumulation in tissues, insulin resistance, hyperinsulinemia, and hyperglycemia (Kahn, 1998; Taylor, 1999; Kahn and Flier, 2000; Kashyap et al., 2003; Moller and Kaufman, 2005). Overt diabetes develops when β cells fail to compensate for the increased demand for insulin, and sustained, elevated levels of FFAs are believed to provoke not only insulin resistance but also eventual β cell failure and hypoinsulinemia (Kahn, 1998; Taylor, 1999; Kahn and Flier, 2000; Haber et al., 2002; Zraika et al., 2002; Yaney and Corkey, 2003). Thus, obesity and elevated levels of FFAs are linked to hyperinsulinemia, insulin resistance, and glucose intolerance as well as to β cell failure, hypoinsulinemia, and overt diabetes. Still, relatively little is known about molecular mediators of FFA signaling in pancreatic β cells and how these mediators contribute to the development of hyper- and hypoinsulinemia during glucose intolerance and overt diabetes (Haber et al., 2002; Zraika et al., 2002; Yaney and Corkey, 2003). In the mouse, GPR40 is preferentially expressed in β cells and activated by FFAs, and studies in cell lines have suggested that FFAs stimulate insulin secretion from insulinoma cells through GPR40 (Briscoe et al., 2003; Itoh et al., 2003; Kotarsky et al., 2003). Our results provide evidence that GPR40 mediates both acute and long-term effects of FFAs on insulin secretion in mouse and that GPR40 signaling is linked to impaired glucose homeostasis.

Our data show that GPR40 function is not essential for glucose homeostasis under normal nutritional conditions. However, *GPR40*$^{-/-}$ mice show reduced fasting insulin levels, and isolated islets show a blunted insulin secretory response to FFAs. These results are consistent with the idea that GPR40 mediates, at least in part, the stimulatory effect of FFAs on insulin secretion. The finding that GPR40 is required for FFA-stimulated insulin secretion raises the possibility that GPR40 contributes to obesity-induced hyperinsulinemia. In support of this idea, our results provide evidence that, in contrast to *GPR40*$^{+/+}$ mice, *GPR40*$^{-/-}$ mice do not become hyperinsulimemic on a HFD. Moreover, under these conditions, *GPR40*$^{-/-}$ mice do not develop hepatic steatosis, do not show an increase in hepatic glucose output, and do not become hyperglycaemic. These results support the idea that hyperinsulinema per se contributes to hepatic steatosis, hepatic insulin resistance, and hyperglycemia (Wolfrum et al., 2004; Moller and Kaufman, 2005). Gene expression analyses suggest that increased uptake of FFAs by hepatocytes, and not increased FFA synthesis, is a major cause of HFD-induced hepatic steatosis (Kim et al., 2004). Consistent with these observations, we find that the expression of CD36, which mediates uptake of FFAs, is upregulated in livers of *GPR40*$^{+/+}$ but not *GPR40*$^{-/-}$ mice following HFD treatment, whereas the expression of genes involved in fatty acid synthesis is mainly unaffected. CD36 is a target gene for PPARγ in liver cells (Memon et al., 2000; Yu et al., 2003), and, accordingly, we find that PPARγ expression is also upregulated in fatty livers of *GPR40*$^{+/+}$ mice compared to livers of CD-fed mice and HFD-fed *GPR40*$^{-/-}$ mice. PPARγ is upregulated in livers of several obese and diabetic animal models (Chao et al., 2000; Memon et al., 2000; Bedoucha et al., 2001; Rahimian et al., 2001), and PPARγ expression has been shown to directly correlate to the development of hepatic steatosis and hepatic insulin resistance; overexpression of PPARγ in livers of mice induces hepatic steatosis (Yu et al., 2003), whereas selective inactivation of PPARγ in livers of two different mouse models results in reduced hepatic steatosis (Gavrilova et al., 2003; Matsusue et al., 2003).

Collectively, these results suggest that, under conditions of elevated levels of FFAs, GPR40-mediated insulin secretion leads to hyperinsulinemia, which in turn may contribute to the development of hepatic steatosis and increased hepatic glucose output and thereby to impaired glucose homeostasis. Additional indirect effects of hyperinsulinemia on other targets tissues cannot, however, be excluded and require further investigation. Nevertheless, our results provide evidence that loss of *GPR40* protects mice from obesity-induced hyperinsulinemia, hepatic steatosis, increased hepatic glucose output, hyperglycemia, and glucose intolerance.

Chronically elevated levels of FFAs appear to have a negative effect on insulin secretion from β cells. Forced expression of *GPR40* in β cells leads to perturbed expression of genes and proteins controlling glucose sensing, glucose metabolism, proinsulin processing, and fatty acid oxidation and to the development of diabetes, mimicking the long-term effects of FFAs on β cell function and insulin secretion (Haber et al., 2002; Zraika et al., 2002; Yaney and Corkey, 2003). Furthermore, cultured *GPR40*$^{-/-}$ islets are protected from impairment of GSIS caused by prolonged exposure to FFAs. Thus, our data suggest that GPR40 is a mediator of the long-term, negative effects of FFAs on β cell function and GSIS. PPARs act as FFA sensors and mediators of FFA signaling. Our observation that forced expression of *GPR40* in β cells leads to upregulation of *PPARα* and downregulation of *PPARγ* links GPR40 to intracellular FFA signaling in β cells. Thus, GPR40 may mediate FFA signaling in β cells both in a direct manner and also indirectly by altering the expression of PPARs.

Although the roles of PPARs in β cell function remain poorly understood, *PPARα*, as well as the target genes *CPT1* and *UCP2*, are linked to FFA-induced β cell dysfunction (Assimacopoulos-Jeannet et al., 1997; Rubí et al., 2002; Tordjman et al., 2002; Patanè et al., 2002; Chan et al., 1999; Zhang et al., 2001; Chan et al., 2001). FFAs stimulate the expression of these genes in islets and insulinoma cell lines (Assimacopoulos-Jeannet et al., 1997; Zhou et al., 1998; Lameloise et al., 2001; Li et al., 2002; Tordjman et al., 2002), and adenovirus-mediated increase of either CPT1 or UCP2 expression in vitro negatively influences GSIS (Chan et al., 1999; Rubí et al., 2002;). Moreover, both UCP2$^{+/-}$ and UCP2$^{-/-}$ mice show increased GSIS, providing further evidence for the negative correlation between UCP2 levels and insulin secretion (Zhang et al., 2001). UCP2 uncouples respiration from oxidative phosphorylation; hence, increased UCP2 expression leads to reduced production of ATP, an important stimulator of insulin secretion (Haber et al., 2002; Zraika et al., 2002; Yaney and Corkey, 2003). Increased CPT1 expression is known to stimulate fatty acid oxidation, which has been proposed to inhibit GSIS by causing a decline in pyruvate dehydrogenase activity (Zhou and Grill, 1994; Zhou and Grill, 1995; Zhou et al., 1996) that in turn will attenuate

production of malonyl-CoA, a key metabolite in stimulation of insulin secretion (Yaney and Corkey, 2003). The existence of a glucose/fatty acid oxidation or Randle cycle in β cells is, however, a matter of controversy (Segall et al., 1999; Liu et al., 1999; Boucher et al., 2004). We show that upregulation of PPARα expression in islets of *Ipf1/GPR40* mice is paralleled by elevated levels of expression of *CPT1* and *UCP2*, providing evidence for a link between GPR40 and expression of *PPARα*, *CPT1*, and *UCP2*. The combined increase in expression of both *CPT1* and *UCP2* is likely to play a significant role in the severely impaired GSIS displayed by *Ipf1/GPR40* mice.

Ipf1/GPR40 mice also show altered expression of other genes coupled to β cell function and insulin secretion. The expression of *Glut2* mRNA and protein was severely reduced in islets of *Ipf1/GPR40* mice. Although not rate limiting, Glut2 expression is essential for efficient uptake of glucose into the β cell, and Glut2 null mutant mice are glucose intolerant and lack first-phase insulin secretion in response to glucose (Guillam et al., 1997). Glut2 expression is also perturbed in several diabetic animal models and in islets exposed to FFAs (Efrat, 1997; Gremlich et al., 1997). Thus, the impaired expression of Glut2 in *Ipf1/GPR40* mice may contribute to the impaired GSIS observed in these mice. Exposure of islets to FFAs has little effect on levels of PC1/3 mRNA but leads to impaired biosynthesis of PC1/3 protein, leading to perturbed proinsulin processing (Furukawa et al., 1999). Accordingly, mRNA levels of *PC1/3* were not affected in β cells of *Ipf1/GPR40* mice, but PC1/3 immunoreactivity was reduced, accompanied by accumulation of proinsulin in the β cells.

Collectively, our data provide evidence that GPR40 mediates both acute and chronic effects of fatty acids on β cells. Thus, GPR40 may play a key role at multiple stages in the development of type 2 diabetes. In the early phase, characterized by obesity, hyperinsulinemia, and normoglycemia, elevated FFAs lead to excessive stimulation of GPR40 signaling, resulting in hyperinsulinemia, hepatic steatosis, and impaired glucose tolerance. These actions of GPR40 are revealed by the phenotype of the *GPR40*$^{-/-}$ mice. In the later phase, chronic overstimulation of GPR40 signaling eventually leads to impaired β cell function, hypoinsulinemia, and overt diabetes, as seen in the *Ipf1/GPR40* transgenic mice. Since human GPR40 is also activated by FFAs and is expressed selectively (but not exclusively) in β cells (Briscoe et al., 2003; Kotarsky et al., 2003), it seems plausible that it plays a similar role as in mouse. Hence, GPR40 antagonists may represent a useful therapeutic strategy for the prevention and treatment of obesity-associated type 2 diabetes.

Experimental procedures

Generation of transgenic and mutant *GPR40* mice

A 930 bp XbaI-BglII *GPR40* cDNA fragment was cloned behind the *Ipf1* promoter and used for the generation of transgenic mice (Apelqvist et al., 1997; Hogan et al., 1994). The primers used for genotyping were the following: 5′-GGGAAGAGGAGATGTAGACTT-3′ (*Ipf1/Pdx1* primer) and 5′-GTAGAGGGGAGCAAAGTG-3′ (*GPR40* primer). A targeting cassette including IRES-*nlslacZ* (Arber et al., 1999), an SV40 polyadenylation signal, and a lox-flanked pgk-neo cassette was used to replace the entire GPR40 coding domain (Figure 1B; Hogan et al., 1994). A 493 bp 3′ HinDIII–EcoRI fragment was used to screen for ES cell recombinants (Figure 1B). *GPR40*$^{-/-}$ mice were genotyped by PCR.

Oil Red O staining

Livers from four mice of each group were fixed in 4% PFA in 0.1 M PBS for 2 hr at 4°C, frozen, sectioned, stained with Oil Red O, and counterstained with Meyer's hematoxylin (DAKO).

Islet cultivation

Islets were isolated by collagenase digestion of pancreas (Ahren et al., 1997), incubated 14 hr at 37°C in RPMI-1640 (GIBCO 21875-034 + 5.5 mM glucose), and equilibrated in UB buffer (3.3 mM glucose) for 1 hr. Islets were stimulated for 2 hr in UB buffer with low (3.3 mM) and high (20 mM) glucose ± palmitic acid (final concentration, 0.5 mM; Sigma P9767-5G). For long-term exposure of islets to FFAs, islets were incubated at 37°C in RPMI-1640 (GIBCO 21875-034 + 5.5 mM glucose) ± palmitic acid (final concentration, 0.5 mM) for 48 hr prior to glucose stimulation. UB buffer (10×) was prepared as follows: NaCl 14.6 g, KCl 880 mg, $CaCl_2 \times H_2O$ 376 mg, $MgCl_2 \times 6H_2O$ 488 mg, and HEPES 11.9 g. Dissolve in 200 ml H_2O. Upon dilution, set pH 7.35 and add 0.1% BSA (ICN 105033, fatty acid free).

Mouse diet

Mice were fed ad libitum with a standard mouse chow (Lactamin, Stockholm, Sweden) and kept under a light-dark cycle of 12 hr. When stated, 8-week-old *GPR40*$^{+/+}$ and *GPR40*$^{-/-}$ mice were put on a high-fat diet (58 kcal % fat) or control diet (10.5 kcal % fat) for 8 weeks (D12310 and D12309, respectively, New Brunswick, NJ). Body weight and nonfasted blood glucose levels (Glucometer Elite, Bayer Inc.) were monitored every second week.

Glucose, insulin, and triglyceride measurements

Glucose tolerance and insulin secretion were measured in overnight-fasted (12–16 hr) mice following intraperitoneal injection with 1 g/kg (dose/kg body weight) glucose. Blood glucose levels were measured using a Glucometer Elite (Bayer Inc.), triglycerides (TG) with Accutrend GCT (Roche), and serum insulin levels were using ELISA (Mercodia). Total pancreatic insulin was extracted using acid ethanol (75% EtOH, 0.2 M HCl) and measured using a Sensitive Rat Insulin RIA Kit (Linco). Total pancreatic protein concentration was determined using Bio-Rad protein assay (Bio-Rad).

In situ hybridizations and immunohistochemistry

GPR40 in situ hybridizations and immunohistochemistry were carried out as described elsewhere (Apelqvist et al. [1997] and www.mshri.on.ca/rossant/protocols/DpERK%20Immunohistochem.html). At least 20 unique islets were analyzed from three independent individuals from wild-type and *Ipf1/GPR40* mice for the immunohistology expression analyses. Primary antibodies used were the following: guinea pig anti-insulin (Linco), mouse anti-Pro-insulin (O.D. Madsen, Gentofte, Denmark), guinea pig anti-glucagon (Linco), rabbit anti-glucagon (EuroDiagnostica), rabbit anti-glucose transporter 2 (B. Thorens, Lausanne, Switzerland), rabbit anti-prohormone convertase 1/3 (D. Steiner, Chicago), rabbit anti-cleaved caspase 3 (Cell Signaling), rabbit anti-Foxa2 (T.M. Jessel, New York), FITC-conjugated mouse anti-α-SMA (SIGMA), and rabbit anti-α-MSH (National Hormone and Pituitary Program, NIDDK). Secondary antibodies used were the following: ALEXA 488 anti-guinea pig, 594 anti-guinea pig (Molecular Probe), Cy3 anti-rabbit, and Cy3-anti-rat (Jackson Laboratory).

Quantification of mRNA expression levels

cDNA was prepared from total RNA isolated from islets (Ahren et al., 1997) and livers using NucleoSpin RNAII-kit (635990, Machery-Nagel) and Super SMART PCR (635000, Clontech). RT-PCR analysis was performed using the ABI PRISM 7000 Sequence Detection System and SYBR Green PCR Master Mix (ABI) according to the manufacturers' recommendations. Expression of the 18S RNA (*18S*), β-2-microglobulin (*b2M* and *TBP*), was used to normalize expression levels. Primer sequences were the following: *18S*: 5′-CACACGCTGAGCCAGTCAGT-3′, 5′-AGGTTTGTGATGCCCTTAGATGTC-3′; *b2M*: 5′-GCTATCCAGAAAACCCCTCAAA-3′, 5′-CTGTGTTACGTAGCAGTTCAGTATGTTC-3′; *Bcl2*: 5′-ACCGGCATCTGCACACCT-3′, 5′-CACAAAGGCATCCCAGCC-3′; *TBP*: 5′-GAATTGTACCGCAGCTTCAAAA-3′, 5′-AGTGCAATGGTCTTTAGGTCAAGTT; *GCK*: 5′-GCTTTTGAGACCCGTTTTGTG-3′, 5′-GAGTGCTCAGGATGTTAAGGATCTG-3′; *Glut2*: 5′-TCCTCGTGGCGCTGATG-3′, 5′-CTGGTTGAATAGTAAAATATCCCATTGAT-3′; *Ipf1/Pdx1*: 5′-TAGGACTCTTTCCTGGGACCAA-3′, 5′-AATAAAAAGGGTACAAACTTG

AGCGT-3′; *Isl1*: 5′-ATTGCCGCAACCAACACA-3′, 5′-CATGGGCGATCCA CCAAA-3′; *Ins*: 5′-CCACCCAGGCTTTTGTCAA-3′, 5′-TCCCCACACACC AGGTAGAGA; *PC1/3*: 5′-TGGAGGCAAACCCAAATCTTAC-3′, 5′-GAG TACGACCCATTGGCCAG-3′; *PPARα*: 5′-GACAAGGCCTCAGGGTACCA-3′, 5′-GCCGAATAGTTCGCCGAAA-3′; *PPARγ*: 5′-AAGGCGAGGGCGAT CTTG-3′, 5′-ATCATTAAGGAATTCATGTCGTAGATGAC-3′; *UCP2*: 5′-CAG GTCACTGTGCCCTTACCA-3′, 5′-AGGCATGAACCCCTTGTAGAAG-3′; *CPT1*: 5′-GGCTCATTTCCGGGAACAAA-3′, 5′-GGTACAGGAACGCACA GTCTCA-3′; *SREBP1*: 5′-TGCGTGGTTTCCAACATGAC-3′, 5′-TGGCCT CATGTAGGAATACCCT-3′; *FAS*: 5′-GGAGTTCTCAGGCCGGGATA-3′, 5′-GGGTACATCCCAGAGGAAGTCA-3′; *SCD*: 5′-GGCCTGTACGGGATCATA CTG-3′, 5′-GGTCATGTAGTAGAAAATCCCGAAGA-3′; *MAL*: 5′-GCTGGC TAATATTCAGGAAGTTTCTG-3′, 5′-GGGTACCGGAAGGCCATTT-3′; *FAT/CD36*: 5′-TCATATTGTGCTTGCAAATCCAA-3′, 5′-GCTTTACCAAAGATGT AGCCAGTGT-3′; *HNF1α*: 5′-CAGCACCAGTCCCACAGTGT-3′, 5′-GGCT GTGCCCGTTGGA-3′; *HNF4α*: 5′-ACGTGCTGCTCCTAGGCAAT-3′, 5′-GAT GGACACACGGCTCATCTC-3′; *Foxa2*: 5′-GAGCCATCCGACTGGAGCA-3′, 5′-GGAATGAGCCCGTCGCTAG-3′; *GPR40*: 5′-TTTCATAAACCCGGACC TAGGA-3′, 5′-CCAGTGACCAGTGGGTTGAGT-3′; *Chrebp*: 5′-ACTCAGG GAATACACGCCTACAG-3′, 5′-TCTTGGTCTTAGGGTCTTCAGGAA-3′.

Supplemental data

Supplemental Data include six figures and Supplemental Experimental Procedures and can be found with this article online at http://www.cellmetabolism.org/cgi/content/full/1/4/245/DC1.

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