ORIGINAL ARTICLE

Gpr40 Is Expressed in Enteroendocrine Cells and Mediates Free Fatty Acid Stimulation of Incretin Secretion

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OBJECTIVE—The G-protein–coupled receptor *Gpr40* is expressed in β-cells where it contributes to free fatty acid (FFA) enhancement of glucose-stimulated insulin secretion (1–4). However, other sites of *Gpr40* expression, including the intestine, have been suggested. The transcription factor IPF1/PDX1 was recently shown to bind to an enhancer element within the 5′-flanking region of *Gpr40* (5), implying that IPF1/PDX1 might regulate *Gpr40* expression. Here, we addressed whether *1*) *Gpr40* is expressed in the intestine and *2*) *Ipf1/Pdx1* function is required for *Gpr40* expression.

RESEARCH DESIGN AND METHODS—In the present study, *Gpr40* expression was monitored by X-gal staining using *Gpr40* reporter mice and by in situ hybridization. *Ipf1/Pdx1*-null and β-cell specific mutants were used to investigate whether *Ipf1/Pdx1* controls *Gpr40* expression. Plasma insulin, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide-1 (GLP-1), and glucose levels in response to acute oral fat diet were determined in *Gpr40* mutant and control mice.

RESULTS—Here, we show that *Gpr40* is expressed in endocrine cells of the gastrointestinal tract, including cells expressing the incretin hormones GLP-1 and GIP, and that *Gpr40* mediates FFA-stimulated incretin secretion. We also show that *Ipf1/Pdx1* is required for expression of *Gpr40* in β-cells and endocrine cells of the anterior gastrointestinal tract.

CONCLUSIONS—Together, our data provide evidence that *Gpr40* modulates FFA-stimulated insulin secretion from β-cells not only directly but also indirectly via regulation of incretin secretion. Moreover, our data suggest a conserved role for *Ipf1/Pdx1* and Gpr40 in FFA-mediated secretion of hormones that regulate glucose and overall energy homeostasis. ***Diabetes* 57:2280–2287, 2008**

Mature β-cells respond to elevated glucose levels by secreting insulin in a tightly controlled manner. The physiological response of the β-cell to elevated blood glucose levels is critical for maintenance of normoglycemia, and impaired glucose-stimulated insulin secretion (GSIS) is a prominent feature of overt type 2 diabetes. Although glucose is recognized as the major stimulator of insulin secretion from β-cells, other stimuli, such as amino acids, hormones, and free fatty acids (FFAs), also influence insulin secretion (6,7). Thus, under normal settings, insulin secretion from β-cells in response to food intake is evoked by the collective stimuli of nutrients, such as glucose, amino acids, and FFAs, and hormones like the incretins glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (6,7).

FFAs are known to influence insulin secretion from β-cells primarily by enhancing GSIS. The FFA receptor Gpr40 is preferentially expressed in β-cells and is activated by medium- to long-chain FFAs, thereby triggering a signaling cascade that results in increased levels of $[Ca^{2+}]_i$ in β-cell lines and subsequent stimulation of insulin secretion (1,3,8). *Gpr40*-deficient β-cells secrete less insulin in response to FFAs, providing evidence that Gpr40 mediates part of the FFA stimulatory effect on insulin secretion (2,4). However, loss of *Gpr40* protects mice from obesity-induced hyperglycemia, glucose intolerance, hyperinsulinemia, fatty liver development, increased hepatic glucose output, and hypertriglyceridemia (2). These data provide evidence that FFA stimulation of insulin secretion via Gpr40 contributes to obesity-induced hyperinsulinemia, which in turn is linked to fatty liver development and hepatic insulin resistance.

Lipids and FFAs also stimulate the secretion of several gut "satiety" hormones, including cholocystokinine (CCK), GLP1, and peptide YY (PYY), and the related FFA receptor Gpr120 has been suggested to mediate FFA-stimulated secretion of GLP-1 from L-cells (9). In addition, stimulation of the G-protein–coupled receptor Gpr119, the ligands of which are phospholipids and fatty acid amides, have also been shown to result in increased GLP-1 and GIP secretion (10). RT-PCR analyses have suggested that *Gpr40* is expressed in the intestine, leaving open a potential role also for Gpr40 in FFA stimulation of gut hormones (1,11).

The transcription factor IPF1/PDX1 is highly expressed in β-cells and controls key aspects of β-cell function by regulating the expression of genes involved in glucose sensing, insulin gene expression, and insulin secretion (12–14). Loss or perturbation of *Ipf1/Pdx1* function in β-cells leads to impaired GSIS and consequently diabetes or glucose intolerance in both mice and humans (12,15), highlighting the central role for *Ipf1/Pdx1* in ensuring β-cell function. Recently, IPF1/PDX1 has been shown to bind to an enhancer element within the 5′-flanking region of *Gpr40* (5), implying that *Ipf1/Pdx1* might regulate *Gpr40* expression in β-cells and thus FFA-mediated stimulation of insulin secretion.

To determine whether *Gpr40* is expressed in the intestine and whether *Ipf1/Pdx1* function is required for *Gpr40* expression, we investigated the expression of *Gpr40* in

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wild-type and *Ipf1/Pdx1* mutant mice. Here, we show that *Gpr40* is expressed in endocrine cells of gastrointestinal tract, including cells expressing the incretin hormones GLP-1 and GIP. We also show that *Ipf1/Pdx1* is required for *Gpr40* expression in β-cells and endocrine cells of the anterior gastrointestinal tract. Moreover, we show that secretion of GLP-1 and GIP is diminished in *Gpr40*-null mutant mice. Together, these data raise the possibility that *Gpr40* modulates FFA-stimulated insulin secretion from β-cells not only directly but also indirectly via regulation of incretin secretion.

RESEARCH DESIGN AND METHODS

The animal studies were approved by the Institutional Animal Care and Use Committee of Umeå University and conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals. The generation of $Gpr40^{+/lacZ}$, $Ipf1/Pdx1^{-/-}$, and $Rip1/Ipf1^{\Delta}$ have been previously described (2,12,16). Briefly, $Gpr40^{+/lacZ}$ mice were generated by replacing the *Gpr40* open reading frame with the *lacZ* gene encoding *β-galactosidase* (*β-gal*). In $Ipf1/Pdx1^{-/-}$-null mice, exon 2, encoding the DNA-binding homeodomain, was deleted. The $Rip1/Ipf1^{\Delta}$ mice are generated by breeding mice in which exon 2 of the *Ipf1/Pdx1* gene is flanked by two loxP sites with mice where the Cre-recombinase is under the control of *Rat insulin 1* (*Rip1*) promoter. In the resulting $Rip1/Ipf1^{\Delta}$ mice, exon 2 of *Ipf1/Pdx1* becomes out-recombined specifically in β-cells as a consequence of Cre-recombinase expression and activity.

Glucose, insulin, GIP, GLP-1, glucagon, FFA, and triglyceride measurements. Intraperitoneal glucose tolerance tests were performed on overnight-fasted, sedated mice essentially as previously described (2). For oral glucose tolerance test, 300 μl 20% glucose solution was administered to overnight-fasted, sedated mice. For the acute, high-fat diet experiments, a paste was generated by mixing 7.5 g diet D12309 (58% kcal fat content; Research Diets) with 3 ml tap water, and 300 mg paste was then administered by oral gavage to overnight-fasted, sedated mice. Blood glucose levels were measured using a Glucometer Elite (Bayer), serum insulin levels were measured using ELISA (Mercodia), and total plasma GIP and GLP-1 concentrations were determined according to the manufacturer's instructions for the GIP-ELISA (EZRMGIP-55K; Linco Research) and the GLP-1-RIA (GLP1T-36HK; Linco Research) kit. Plasma glucagon levels were determined using the Glucagon RIA kit (GL-32K; Linco Research). FFA and triglyceride measurements were done according to the manufacturer's instructions using FFAs, Half-micro test (Roche), and Accutrend GCT Triglycerides (Roche).

In situ hybridizations, X-gal staining, and immunohistochemistry. In situ hybridization using DIG-labeled probes specific for the mouse *Gpr40* transcript was carried out on embryonic day (e) 17 embryos as previously described (2). Immunohistochemical localization of antigens, double-label immunohistochemistry, and X-gal staining on tissues and confocal microscopy were carried out as previously described (2). Primary antibodies used were rabbit anti–β-gal (Cappel), chicken anti–β-gal (AbCam), rabbit anti-GIP (Peninsula), rabbit anti–GLP-1 (Peninsula), guinea pig anti-gastrin (Euro Diagnostics), goat anti-ghrelin (Santa Cruz Biotechnology), rabbit anti-CCK (Chemicon), rabbit anti-secretin, rabbit anti-substance P, rabbit anti-PYY, rabbit anti-neuropeptide 4 (NPY), rabbit anti-serotonin (Euro-Diagnostica), rabbit anti-somatostatin (Dako), and rabbit anti-Ipf1 (17). Secondary antibodies used were Alexa 488 anti-goat, Alexa 488 anti–guinea pig, Alexa 594 anti–guinea pig, Alexa 594 anti-rabbit, Alexa 594 anti-goat (all from Molecular Probe), Cy3 anti-rabbit, and fluorescein isothiocyanate (FITC) anti-chicken (The Jackson Laboratories). The gut hormone and *Gpr40* expression analyses were performed on 2- to 3-month-old mice. For the antibody cocktail experiment, we made a two-step staining procedure: The tissue sections were first incubated with a mixture of antibodies directed against GIP, GLP-1, ghrelin, CCK, and gastrin, and for these, the corresponding Alexa 594-fluorochrome secondary antibodies were used. Next, the tissue sections were incubated with antibodies directed against β-gal for which a FITC-conjugated secondary antibody was used.

Cell counting. Pylorus and the three proximal centimetres of small intestine corresponding to the duodenum and part of the jejunum were isolated from wild-type, $Gpr40^{+/lacZ}$, and $Gpr40^{lacZ/lacZ}$ (n = 3) nonfasted mice, fixed in 4% paraformaldehyde at 4°C for 1–2 h, cryoprotected in 30% sucrose at 4°C overnight, frozen in Tissue-Tek (Sakura), and kept at −80°C. Three 8-μm-thick sections where collected on every slide with ~160 μm between sections. The sections of pylorus/duodenum were stained with antibodies against GIP, GLP-1, ghrelin, CCK, gastrin, PYY, secretin, serotonin, substance P, and β-gal and manually analyzed for distribution and colocalization of the different markers. Colocalization between β-gal and GLP-1 was also determined in the distal 3 cm of the ileum, i.e., close to the appendix, in $Gpr40^{+/lacZ}$ mice.

Quantification of mRNA expression levels. cDNA was prepared from total RNA isolated from islets (18) and from e16 pylorus/duodenum and the distal part of the ileum using NucleoSpin RNAII-kit (635990; Machery-Nagel) and Super SMART PCR (635000; Clontech). Quantitative real-time PCR analysis was performed using the ABI PRISM 7000 Sequence Detection System and SYBR Green PCR Master Mix (ABI) according to the manufacturer's recommendations. Expression of the β-2-microglobulin (*β2M*), TATA-box–binding protein (*TBP*), *β-actin*, and glyceraldhyde-3-phosphate dehydrogenase (*Gapdh*) genes was used to normalize expression levels. Primer sequences were as follows: *β-actin*, 5′-GCTCTGGCTCCTAGCACCAT-3′ and 5′-GCCACCGATCCACACAGAGT-3′; *Gapdh*, 5′-CGTGTTCCTACCCCCAATGT-3′ and 5′-TGTCATCATACTTGGCAGGTTTCT-3′; *β2M*, 5′-GCTATCCAGAAAACCCCTCAAA-3′ and 5′-CTGTGTTACGTAGCAGTTCAGTATGTTC-3′; *TBP*, 5′-GAATTGTACCGCAGCTTCAAAA-3′ and 5′-AGTGCAATGGTCTTTAGGTCAAGTT; *Ipf1/Pdx1*, 5′-TAGGACTCTTTCCTGGGACCAA-3′ and 5′-AATAAAAAGGGTACAAACTTGAGCGT-3′; and *Gpr40*, 5′-TTTCATAAACCCGGACCTAGGA-3′ and 5′-CCAGTGACCAGTGGGTTGAGT-3′.

Statistical analyses were performed by an unpaired Student's t test.

RESULTS

***Gpr40* is expressed in cells of the gastrointestinal tract.** *Gpr40* has been suggested to be expressed at other sites than β-cells, including in the intestine (1,11). However, these *Gpr40* expression studies build on RT-PCR analyses, and because the coding region of *Gpr40* lacks an intron, contaminating genomic DNA might give false positives in PCR analyses. To avoid such problems, we previously made use of the *lacZ* reporter gene insertion into the *Gpr40* locus of targeted $Gpr40^{+/lacZ}$ mice and showed that *Gpr40* is not expressed in brain, liver, muscle, or adipose tissue (2). We therefore extended our analyses of *Gpr40* expression using the $Gpr40^{+/lacZ}$ mice to elucidate whether *Gpr40* is expressed in the gastrointestinal tract. Distinct X-gal staining was evident in scattered epithelial cells of the gastric pylorus, duodenum, jejunum, ileum, and colon (Fig. 1*A*). The expression of *Gpr40* in the epithelium of the stomach and intestine was evident from e14.5–e15 (data not shown), i.e., coincident with the appearance of differentiated endocrine cells of the gastrointestinal tract. The expression of *Gpr40* in pylorus and duodenum was confirmed by in situ hybridization analyses using a *Gpr40* riboprobe (Fig. 1*B*). Together, these data demonstrate that *Gpr40* is expressed in scattered cells distributed throughout the gastrointestinal tract.

The $Gpr40^{+}$ cells of the gastrointestinal tract represent enteroendocrine cells. To determine the identity of the gastrointestinal cells expressing *Gpr40*, we next performed double immunohistochemical analyses of the epithelium of the gastric pylorus, duodenum, and ileum in 2- to 3-month-old mice using anti–β-gal antibodies and antibodies specific for different gastrointestinal hormones. The β-gal^{+}, i.e., *Gpr40*$^{+}$, cells were shown to express a wide variety of endocrine hormones. In the pylorus and duodenum, β-gal/*Gpr40* expression colocalized with gastrin, GIP, GLP-1, ghrelin, CCK, PYY, secretin, serotonin, and substance P expression; and in the ileum, β-gal/*Gpr40* expression colocalized predominantly with that of GLP-1 (Fig. 1*C*; data not shown). In contrast, no coexpression of β-gal/*Gpr40* and somatostatin or NPY was observed (data not shown). The degree of β-gal/*Gpr40* expression varied between ~20 and 55% for the different gastrin, GIP, GLP-1, ghrelin, CCK, PYY, secretin, and serotonin hormone-expressing cells (Table 1), and $<$1% of the substance P^{+} cells expressed β-gal/*Gpr40* (data not shown). However, immunohistochemical analyses using a cocktail of gut hormone antibodies, including GIP, GLP-1, ghrelin, CCK, and gastrin and β-gal antibodies, revealed that virtually all β-gal/

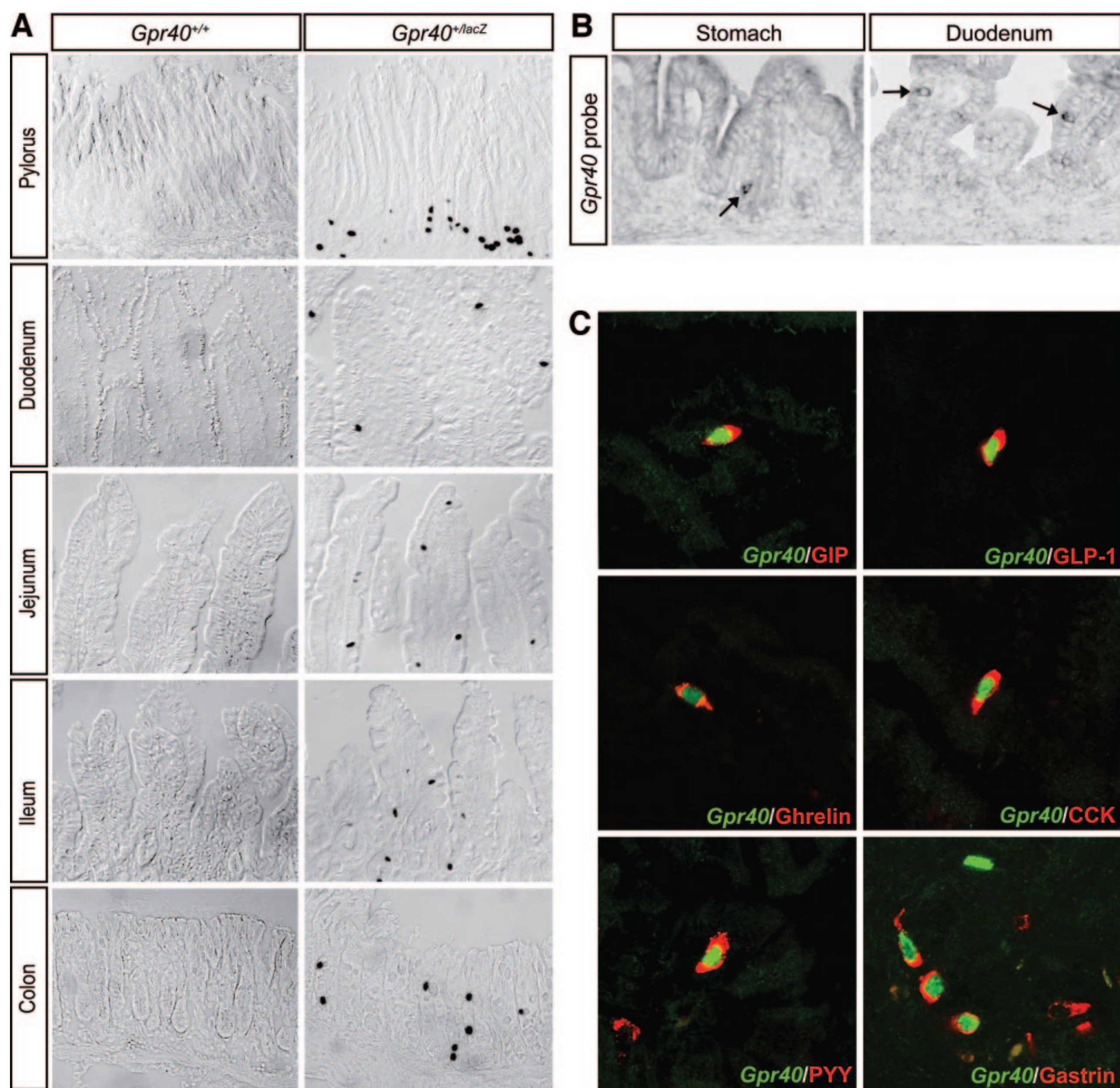

FIG. 1. *Gpr40* is expressed in gut enteroendocrine cells. *A*: X-gal staining of sections of pylorus, duodenum, jejunum, ileum, and colon in 2-month-old $Gpr40^{+/+}$ and $Gpr40^{+/lacZ}$ mice. *B*: In situ hybridization using *Gpr40*-specific probes on sections of neonatal epithelium in stomach and duodenum. Arrows indicate cells expressing *Gpr40* mRNA. *C*: Confocal sections of 2- to 3-month-old adult $Gpr40^{+/lacZ}$ pylorus and duodenum stained with anti–β-gal antibodies (green) to indicate *Gpr40* expression and antibodies specific for the indicated enteroendocrine hormones (red). (Please see http://dx.doi.org/10.2337/db08-0307 for a high-quality digital representation of this figure.)

Gpr40-expressing cells were hormone positive (data not shown). The distribution and number of enteroendocrine cells were normal in $Gpr40^{lacZ/lacZ}$ mice (Table 2; data not shown). Thus, *Gpr40* is expressed both in insulin-producing β-cells and hormone-producing cells of the gastrointestinal tract.

Impaired secretion of GIP and GLP-1 in *Gpr40*-null mutants. GIP and GLP-1 hormones are secreted from the intestinal K- and L-cells, respectively (19), and the secretion of GIP and GLP-1 hormones can be stimulated both by glucose and FFAs (20,21). The secretion of GLP-1 and GIP hormones into the circulation positively influences insulin

TABLE 1
Coexpression of gut hormones and *Gpr40*

Gastrin	GIP	GLP-1	Ghrelin	CCK	PYY	Secretin	Serotonin
52 ± 0.6	50 ± 5	55 ± 5	34 ± 3	50 ± 1	21 ± 2	30 ± 4	19 ± 3

Data are percentage of hormone-expressing cells coexpressing *β-gal*/*Gpr40*. Sections of pylorus (gastrin) and duodenum (GIP, GLP-1, ghrelin, CCK, PYY, secretin, and serotonin) from 2- to 3-month-old $Gpr40^{+/lacZ}$ mice (n = 3) were double stained for gut hormones and β-gal.

TABLE 2
Gut hormone-expressing cells in *Gpr40*$^{lacZ/lacZ}$ mice

	GIP	GLP-1	Ghrelin	CCK	Gastrin
Gpr40$^{+/+}$	490 ± 113	279 ± 38	1,114 ± 249	492 ± 40	689 ± 20
Gpr40$^{lacZ/lacZ}$	530 ± 45	246 ± 36	1,040 ± 171	383 ± 81	754 ± 52
t test	0.63	0.18	0.29	0.87	0.46

Data are *n*. Hormone-expressing cells were counted on sections from pylorus (gastrin) and duodenum (GIP, GLP-1, ghrelin, and CCK) in 2- to 3-month-old *Gpr40*$^{+/+}$ ($n = 3$) and *Gpr40*$^{lacZ/lacZ}$ ($n = 3$) mice.

secretion. This so-called incretin effect is evident when comparing oral and intravenous or intraperitoneal administration of glucose; oral glucose administration triggers a more robust insulin secretory response (19). The expression of *Gpr40* in both GIP-and GLP-1–expressing cells leaves open the possibility that Gpr40 may affect insulin secretion not only directly by virtue of its expression in β-cells, but also indirectly via regulation of incretin secretion. Consistent with the FFA but not glucose responsiveness of Gpr40, no difference in glucose clearance rates or insulin secretion was observed between *Gpr40*$^{lacZ/lacZ}$ and wild-type mice, regardless of whether glucose was administered orally or injected in the peritoneum (Fig. 2*A–D*).

We next explored the glucose and insulin response to acute, oral administration of high-fat diet in *Gpr40*$^{lacZ/lacZ}$ and wild-type mice. Plasma levels of FFAs, triglycerides, and glucagon were similar in oral high-fat diet–treated *Gpr40*$^{lacZ/lacZ}$ and wild-type mice (Supplementary Fig. 1 available in an online appendix at http://dx.doi.org/10.2337/db08-0307). In contrast, plasma insulin levels were reduced and blood glucose levels were increased at 60 min in *Gpr40*$^{lacZ/lacZ}$ compared with that of wild-type mice in response to oral high-fat diet (Fig. 3*A* and *B*), suggesting that incretin secretion in response to fat might be impaired in *Gpr40*$^{lacZ/lacZ}$ mice. Analyses of incretin levels after oral high-fat diet showed that total plasma GIP levels were reduced at 30 and 60 min and total plasma GLP-1 levels were reduced at 60 min compared with that of wild types (Fig. 3*C* and *D*). In contrast, no difference in total GIP or GLP-1 levels were observed at 30 or 60 min after oral glucose administration (Supplementary Fig. 2). Taken together, these data provide evidence for a role for Gpr40 in FFA-mediated secretion of the incretins GIP and GLP-1.

***Ipf1/Pdx1* is required for the expression of *Gpr40* in β-cells.** Recent data show that the transcription factor IPF1/PDX1 can bind to an enhancer element within the *Gpr40* 5′-flanking region (5), leaving open the possibility that IPF1/PDX1 might regulate *Gpr40* expression. *Ipf1/Pdx1*-null mutant mice fail to form a pancreas and thus die at the neonatal stage (16), precluding any analyses of a role for IPF1/PDX1 in the regulation of *Gpr40* expression in β-cells. We have, however, previously generated β-cell–

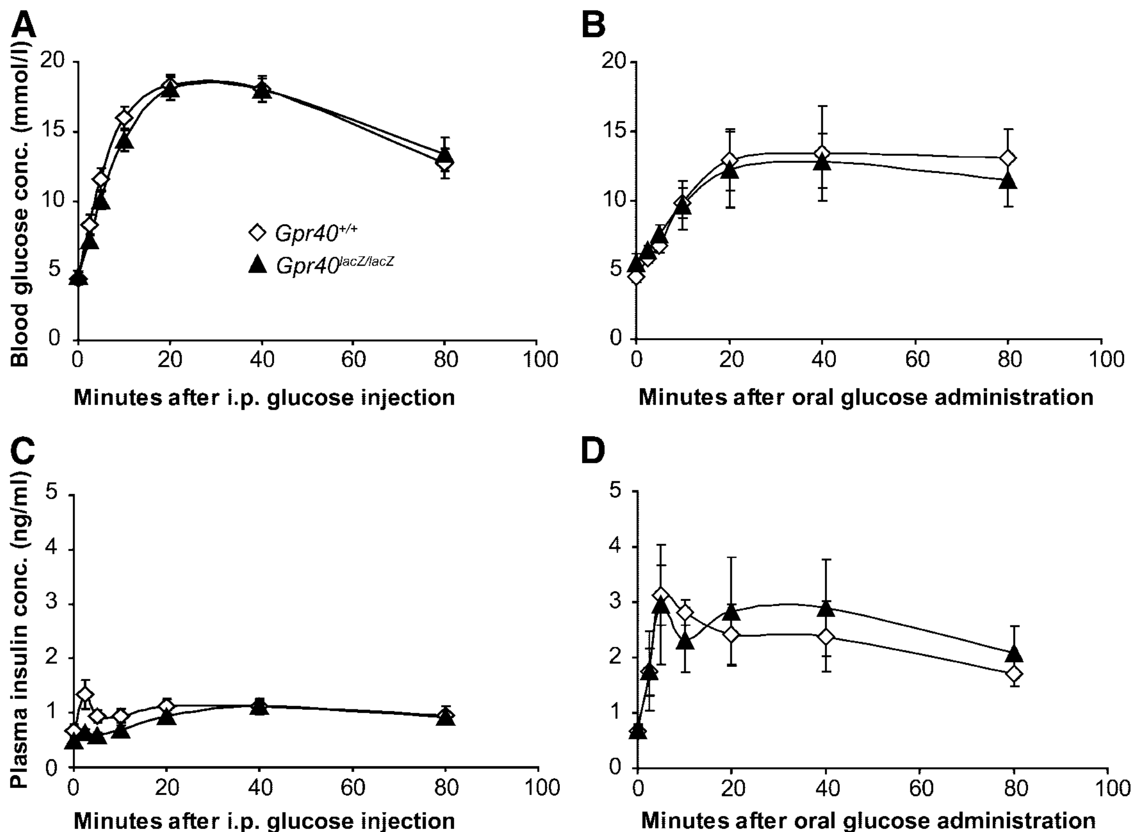

FIG. 2. Oral glucose tolerance is normal in *Gpr40*$^{lacZ/lacZ}$ mice. Blood glucose (*A* and *B*) and plasma insulin (*C* and *D*) levels in 2- to 3-month-old *Gpr40*$^{+/+}$ (◇) and *Gpr40*$^{lacZ/lacZ}$ (▲) mice after intraperitoneal glucose injections (*A* and *C*) and oral glucose administration (*B* and *D*). *Gpr40*$^{+/+}$ ($n = 16$) and *Gpr40*$^{lacZ/lacZ}$ ($n = 16$) for the intraperitoneal glucose injections. *Gpr40*$^{+/+}$ ($n = 6$) and *Gpr40*$^{lacZ/lacZ}$ ($n = 5$) for the oral glucose administration test.

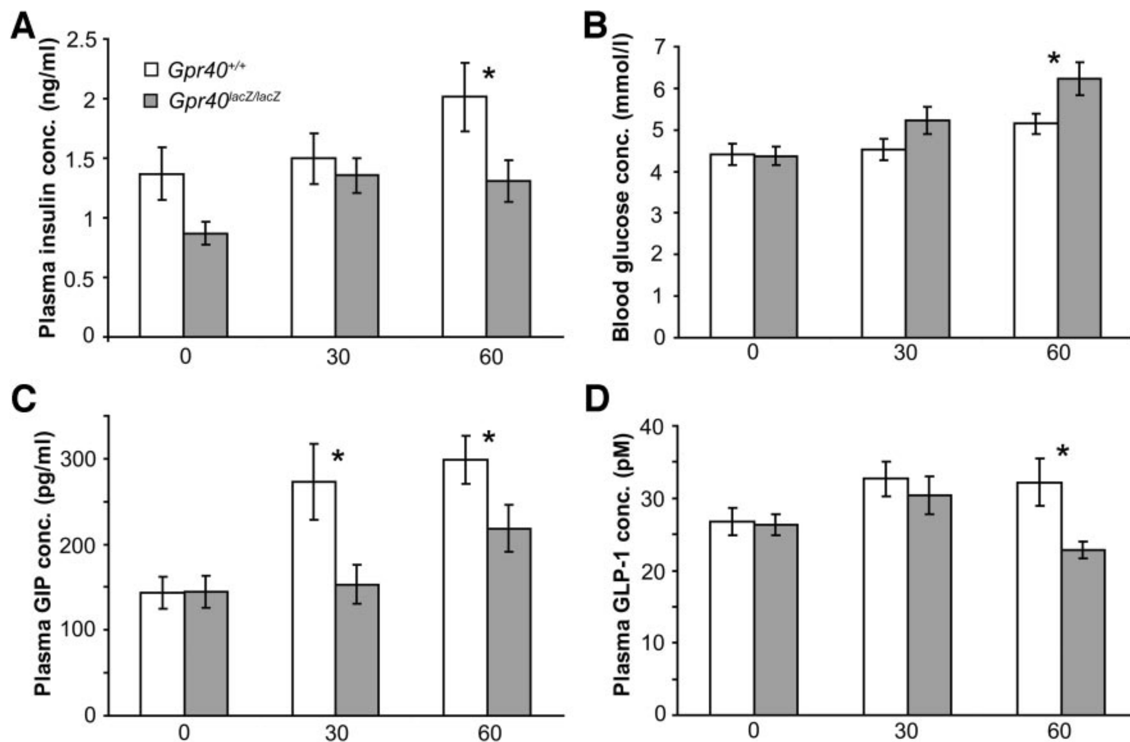

FIG. 3. Reduced plasma levels of GIP and GLP-1 in *Gpr40*$^{lacZ/lacZ}$ mice in response to fat diet. Plasma insulin, GIP (total), GLP-1 (total), and blood glucose levels (*A–D*) were determined in 2- to 3-month-old *Gpr40*$^{+/+}$ (*n* = 10–16, □) and *Gpr40*$^{lacZ/lacZ}$ (*n* = 8–20, ▨) mice after oral high-fat diet administration. *x*-axis indicates minutes after oral gavage. Data are means ± SE. **P* < 0.05 for *Gpr40*$^{+/+}$ vs. *Gpr40*$^{lacZ/lacZ}$.

specific *Ipf1/Pdx1* mutants, denoted *RIP/Ipf1*$^{\Delta}$ mice, using the Cre-LoxP system (12). The conditional inactivation of *Ipf1/Pdx1* in β-cells of *RIP/Ipf1*$^{\Delta}$ mice results in β-cell dysfunction due to reduced expression of key β-cell components, including insulin, glucose transporter type 2, and PC1/3, and the mice consequently show severely impaired insulin secretion and develop diabetes (12). To elucidate whether *Gpr40* expression was regulated by *Ipf1/Pdx1* in adult β-cells, we bred the *Gpr40*$^{+/lacZ}$ allele into the *RIP/Ipf1*$^{\Delta}$ background. The *Gpr40*$^{+/lacZ}$ mice carry the *lacZ* gene targeted into the *Gpr40* locus, thus allowing monitoring of *Gpr40* expression by X-gal staining. In *RIP/Ipf1*$^{\Delta}$ mice, the conditional inactivation of the *Ipf1/Pdx1* gene in β-cells occurs progressively after birth, and the mice develop overt diabetes when *Ipf1/Pdx1* has been inactivated in ~80% of the β-cells (12).

In 5-week-old *Ipf1/Pdx1*$^{+/+}$;*Gpr40*$^{+/lacZ}$ control mice, strong, uniform X-gal staining was observed in the β-cells (Fig. 4*A*). In contrast, the majority of the β-cells in islets of age-matched, glucose-intolerant but not overt diabetic *RIP/Ipf1*$^{\Delta}$;*Gpr40*$^{+/lacZ}$ mice were X-gal^{-}, providing evidence that conditional inactivation of *Ipf1/Pdx1* in β-cells results in a loss of *Gpr40* expression (Fig. 4*A*). Quantitative RT-PCR of cDNA prepared from islets confirmed the decreased expression of *Gpr40* in β-cells of *RIP/Ipf1*$^{\Delta}$ mice and showed that *Gpr40* expression was reduced to a similar extent to that of *Ipf1/Pdx1* in isolated islets (Fig. 4*B*). Together, these data provide evidence that *Ipf1/Pdx1* (directly or indirectly) is required for *Gpr40* expression in β-cells.

***Gpr40* expression in endocrine cells of the anterior gastrointestinal tract requires *Ipf1/Pdx1*.** Apart from β-cells, IPF1/PDX1 is expressed also in hormone-producing cells of the gastric pylorus and duodenum, where it has been shown to be required for the expression of several hormones (22). The similar expression profiles observed for IPF1/PDX1 and *Gpr40* raised the possibility that the expression of *Gpr40* in endocrine cells of the anterior gastrointestinal tract, like that in β-cells, is dependent on *Ipf1/Pdx1*. To explore a potential role for IPF1/PDX1 in regulating *Gpr40* expression, we bred the *Gpr40*$^{+/lacZ}$ allele into the *Ipf1/Pdx1*-null mutant (16) background and analyzed the intestinal expression of *Gpr40* by X-gal staining. Because *Ipf1/Pdx1*-null mutants die at the neonatal stage, *Gpr40* expression analyses were performed on late-stage, embryonic gastrointestinal tissue. In contrast to control *Ipf1/Pdx1*$^{+/+}$;*Gpr40*$^{+/lacz}$ mice, no X-gal^{+}, i.e., *Gpr40*-expressing, cells were observed in the gastric pylorus and duodenum of *Ipf1/Pdx1*$^{-/-}$;*Gpr40*$^{+/lacz}$ embryos (Fig. 5*A*). Quantitative RT-PCR on cDNA isolated from the gastric pylorus and duodenum of wild-type and *Ipf1/Pdx1*$^{-/-}$ embryos confirmed that expression of *Gpr40* in these regions of the gastrointestinal tract is dependent on *Ipf1/Pdx1* (Fig. 5*B*). In the more distal regions of gastrointestinal tract, including the ileum, where IPF1/PDX1 is not expressed, β-gal/*Gpr40* expression was unaffected in *Ipf1/Pdx1*-null mice (data not shown). Together, these data provide evidence for a conserved role for *Ipf1/Pdx1* in regulating *Gpr40* expression in pancreatic β-cells and endocrine cells of the gastric pylorus and duodenum.

DISCUSSION

The FFA-responsive G-protein–coupled receptor Gpr40 is expressed in pancreatic β-cells where it contributes to FFA-mediated enhancement of glucose-induced insulin secretion (1–4), and *Gpr40* mutant mice do not develop hyperinsulinemia on a high-fat diet (2). Here, we show that *Gpr40* is expressed also in hormone-producing cells of the gastrointestinal tract, including GIP^{+} and GLP-1^{+} cells. FFAs are known to stimulate the secretion of both GIP and GLP-1 incretin hormones (20,21), and other G-protein–

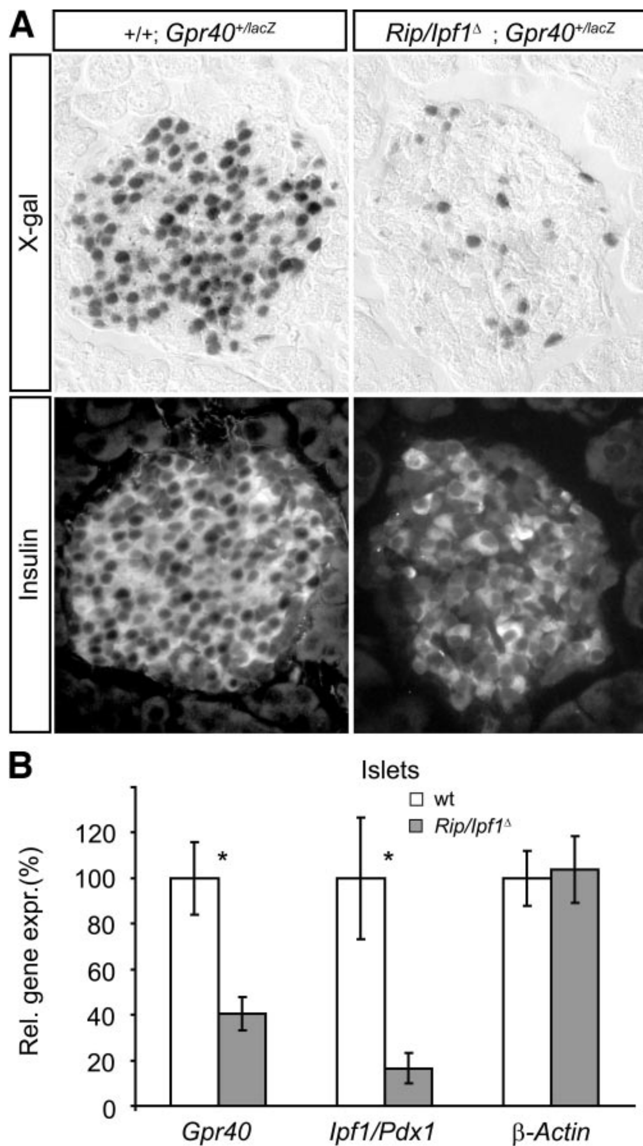

FIG. 4. *Gpr40* expression in β-cells cells requires *Ipf1/Pdx1*. *A*: X-gal–stained sections (*top panel*) of 5-week-old adult islets from *Ipf1/Pdx1*$^{+/+}$;*Gpr40*$^{+/lacZ}$ and *RIP/Ipf1*$^{\Delta}$;*Gpr40*$^{+/lacZ}$ mice counterstained with anti-insulin antibodies (*bottom panel*). *B*: Quantitative real-time RT-PCR expression analyses of islet cDNA from *Ipf1/Pdx1*$^{+/+}$ (□, n = 4) and *RIP/Ipf1*$^{\Delta}$ (▒, n = 6) mice. Data are means ± SE. *$P < 0.05$ for *Ipf1/Pdx1*$^{+/+}$ vs. *RIP/Ipf1*$^{\Delta}$ islets.

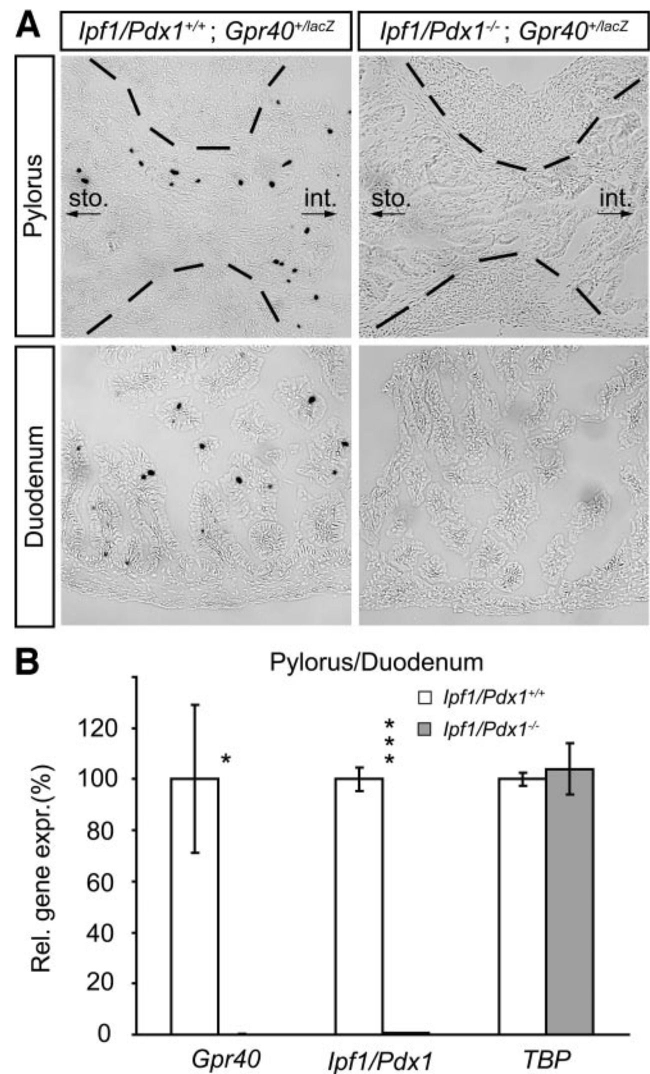

FIG. 5. *Gpr40* expression in enteroendocrine cells requires *Ipf1/Pdx1*. *A*: X-gal–stained sections of the pyloric sphincter and in duodenum of e17 *Ipf1/Pdx1*$^{+/+}$;*Gpr40*$^{+/lacZ}$ and *Ipf1/Pdx1*$^{-/-}$;*Gpr40*$^{+/lacZ}$ embryos. *B*: Quantitative real-time RT-PCR expression analysis of cDNA isolated from pylorus/duodenum of *Ipf1/Pdx1*$^{+/+}$ (□, n = 3) and *Ipf1/Pdx1*$^{-/-}$ embryos (▒, n = 3). Data are means ± SE. *$P < 0.05$ and ***$P < 0.001$ for *Ipf1/Pdx1*$^{+/+}$ vs. *Ipf1/Pdx1*$^{-/-}$. Brackets in *A* indicate the border of the smooth muscle layer surrounding the lumen of the gut tube. sto., stomach; int., intestine.

coupled receptors, such as Gpr120 and Gpr119, have been implicated in the secretion of incretin hormones (9,10). Activation of Gpr120 by α-linolenic acid, docosahexaoienic, or palmitoleic acid in STC-1 cells promoted GLP-1 secretion (9), and upon an oral load of the Gpr119 agonist AR231453, plasma concentrations of GLP-1 and GIP increased in control animals but not in *Gpr119*-deficient mice (10). In this study, we found that *Gpr40*-null mice show impaired secretion of both GIP and GLP-1 in response to acute, oral fat diet administration with a concomitant reduction in insulin secretion and glucose clearance. Together, these findings provide evidence for a role for Gpr40 in FFA stimulation of incretin secretion. The expression of *Gpr40* in endocrine cells of gastrointestinal tract leaves open the possibility that Gpr40, as a component of the entero-insular axis, may regulate insulin secretion in response to fatty acids at several levels. Thus, apart from directly influencing insulin secretion from β-cells via circulating FFAs, Gpr40 may indirectly stimulate GSIS from β-cells by modulating the secretion of the incretin hormones GIP and GLP-1 in response to FFAs present in the gastrointestinal lumen (23,24).

The gut hormones ghrelin and CCK play important and opposing roles in regulating food intake; ghrelin is considered to be an appetite hormone, and CCK a satiety hormone. The release of these two hormones is regulated by food intake, especially fat. Ghrelin levels in blood circulation are reduced by long-chain fatty acids (25), whereas CCK levels are increased by medium- to long-chain fatty acids (26). However, *Gpr40* mutant mice show a normal growth rate on both control and high-fat diet and do not present with any apparent signs of perturbed food intake patterns (2,4). Ghrelin has also been suggested to influence insulin secretion, but the data are conflicting; both stimulatory and inhibitory effects on insulin secretion have been reported (27–31). The role, if any, for Gpr40 in

mediating secretion of ghrelin and CCK in response to FFAs will have to await future analyses.

In β-cells, IPF1/PDX1 regulates the expression of several genes that ultimately ensure proper GSIS and thus β-cell function (12–14,17). Relatively little is known about the regulation of *Gpr40* expression in β-cells. A recent study suggests, however, that IPF1/PDX1 and the basic-helix-loop-helix transcription factor NeuroD/β2, which also is expressed in β-cells, bind to an enhancer element within the 5′-flanking region of *Gpr40* (5). Here, we show in vivo that loss of *Ipf1/Pdx1* in β-cells impairs *Gpr40* expression, providing evidence not only that IPF1/PDX1 can bind to the *Gpr40* 5′-flanking region (5) but that IPF1/PDX1 is required for *Gpr40* expression in β-cells. IPF1/PDX1 is expressed also in endocrine cells of the gastric pyloric antrum and duodenum (22,32). In *Ipf1/Pdx1*-null mutant mice, the expression profile of several gut hormones is changed; fewer gastrin$^+$ but more serotonin$^+$ cells were, for example, observed in the antrum of these mice (22). In this study, we show that the expression of *Gpr40* in endocrine cells of the anterior gastrointestinal tract is lost in *Ipf1/Pdx1*$^{-/-}$ mice. Thus, like *Ipf1/Pdx1*, *Gpr40* is expressed in both β-cells and endocrine cells of the anterior gastrointestinal tract, and *Ipf1/Pdx1* function is essential for *Gpr40* expression in both of these cell types. Taken together, these data suggest a conserved role for *Ipf1/Pdx1* in cells that secrete hormones in response to food intake. However, IPF1/PDX1 is not expressed in the more distal part of the gastrointestinal tract, and *Gpr40* expression at these sites is not affected in *Ipf1/Pdx1*-null mice. Thus, *Gpr40* expression in posterior enteroendocrine cells is *Ipf1/Pdx1* independent. However, the identity of transcription factors regulating *Gpr40* expression in endocrine cells of the posterior gastrointestinal tract remains unknown.

Like other cells of the gastrointestinal epithelium, enteroendocrine cells undergo constant renewal involving stem cell division, differentiation, and cell death. *Gpr40*$^+$ cells are more abundant in the gastric pyloric antrum and duodenum than in the more posterior ileum and colon. In the gastric pyloric antrum, *Gpr40* is predominantly expressed in gastrin$^+$ cells close to the crypts of pyloric pits. In the intestine, *Gpr40* expression was evident in endocrine cells expressing ghrelin, GIP, GLP-1, CCK, PYY, substance P, serotonin, and secretin. Although virtually all *Gpr40*-expressing cells were hormone positive, only ∼20–55% of the individual hormone expressing cells also expressed *Gpr40*. Whether this reflects the maturation process of the cycling enteroendocrine cells, i.e., that *Gpr40* is expressed only at a specific stage of differentiation or that only a subpopulation of the individual enteroendocrine cells expresses *Gpr40*, which in turn would indicate functional differences, remains an open question.

By virtue of its contribution to FFA-enhanced insulin secretion from β-cells, GPR40 is a link between obesity and type 2 diabetes. FFA stimulation of insulin secretion from β-cells is reduced in *Gpr40* mutant mice, and these mice do not develop hyperinsulinemia on a high-fat diet (2). The expression of *Gpr40* in GLP-1$^+$ and GIP$^+$ cells and the impaired secretion of these hormones in Gpr40-null mice in response to acute, oral fat diet leaves open the possibility that the difference in insulin levels in control and *Gpr40*-null mice on high-fat diet results from combined direct, i.e., β-cells, and indirect, i.e., incretin cells, effects of FFA on insulin secretion. The expression of *Gpr40* in endocrine cells expressing hormones that control food intake is suggestive of a role for Gpr40 in the secretion of also these hormones. Increased knowledge of the role for Gpr40 in β-cells and endocrine cells of the gastrointestinal tract may therefore be of great therapeutic relevance not only for obesity-associated diabetes but also for obesity itself but will have to await the generation of β-cell– and enteroendocrine cell–specific *Gpr40* mutant mice.

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