GPR119 is required for physiological regulation of glucagon-like peptide-1 secretion but not for metabolic homeostasis

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Abstract

G protein-coupled receptor 119 (GPR119) is expressed in pancreatic islets and intestine, and is involved in insulin and incretin hormone release. GPR119-knockout (Gpr119−/−) mice were reported to have normal islet morphology and normal size, body weight (BW), and fed/fasted glucose levels. However, the physiological function of GPR119 and its role in maintaining glucose homeostasis under metabolic stress remain unknown. Here, we report the phenotypes of an independently generated line of Gpr119−/− mice under basal and high-fat diet (HFD)-induced obesity. Under low-fat diet feeding, Gpr119−/− mice show normal plasma glucose and lipids, but have lower BWs and lower post-prandial levels of active glucagon-like peptide 1 (GLP-1). Nutrient-stimulated GLP-1 release is attenuated in Gpr119−/− mice, suggesting that GPR119 plays a role in physiological regulation of GLP-1 secretion. Under HFD-feeding, both Gpr119+/+ and Gpr119−/− mice gain weight similarly, develop hyperinsulinemia and hyperleptinemia, but not hyperglycemia or dyslipidemia. Glucose and insulin tolerance tests did not reveal a genotypic difference. These data show that GPR119 is not essential for the maintenance of glucose homeostasis. Moreover, we found that oleoylethanolamide (OEA), reported as a ligand for GPR119, was able to suppress food intake in both Gpr119+/+ and Gpr119−/− mice, indicating that GPR119 is not required for the hypophagic effect of OEA. Our results demonstrate that GPR119 is important for incretin and insulin secretion, but not for appetite suppression.


Introduction

Type 2 diabetes is characterized by insulin resistance with insufficient compensatory insulin secretion from pancreatic islet β-cells (Kasuga 2006). In accordance with the two components of type 2 diabetes, pharmacological treatments fall into two major categories: drugs that improve insulin sensitivity, and those that increase insulin secretion from β-cells. Agents that enhance insulin secretion in a glucose-dependent manner, such as glucagon-like peptide-1 (GLP-1) mimetics (e.g. Exendin-4 (Ex-4)), are desirable because they do not carry the risk of causing hypoglycemia (Drucker 2006). Likewise, agents that inhibit dipeptidyl peptidase IV (DPP-IV), the enzyme responsible for degrading the incretins GLP-1 and gastric inhibitory polypeptide (GIP) have also emerged as novel type 2 diabetes therapeutics (Sinclair & Drucker 2005). The successful development of drugs targeting the GLP-1 receptor has promoted more research on other G protein-coupled receptors (GPCRs) expressed in pancreatic β-cells.

The G protein-coupled receptor 119 (GPR119), first identified through a bioinformatics approach (Fredriksson et al. 2003), is predominantly expressed in pancreatic islets and β-cell lines (Soga et al. 2005, Sakamoto et al. 2006, Chu et al. 2007, 2008). The fatty acid derivatives oleoyllysophosphatidylethanolamine (LPC) and oleoylethanolamide (OEA) have been proposed as endogenous ligands of GPR119. LPC has been shown to increase cAMP and enhance glucose stimulated insulin secretion in NIT-1 insulinoma cells and perfused rat pancreas respectively, and GPR119 siRNA partially blunted the effect of LPC on glucose-stimulated insulin secretion (GSIS) in NIT-1 cells (Soga et al. 2005). Recently, our lab showed that in glucose-insensitive rat insulinoma RIN m5f cells, LPC appears to increase insulin release through both GPR119-dependent and -independent mechanisms (Ning et al. 2008). On the other hand, OEA is a naturally occurring fatty acid amide with diverse biological functions, among which are regulation of satiety and body weight (BW; Fu et al. 2003). Overton et al. (2006, 2008) identified OEA, together with two synthetic compounds, as ligands of GPR119. In a
yeast-based reporter assay, OEA displays EC$_{50}$ values of 3-2 μM for human GPR119 and 2-9 μM for mouse GPR119. They proposed that the hypoglycemic effect of OEA in rat was mediated by GPR119, but the effect of OEA on activating pancreatic GPR119 or on insulin secretion were not reported. We recently showed that OEA increases insulin release from RIN m5F cells transfected with human GPR119 and also potentiates GSIS in mouse insulinoma Min6 c4 cells (Ning et al. 2008); however, the efficacy and specificity of LPC or OEA as GPR119 ligands has not been examined in primary islets.

Recently, investigators at Arena Pharmaceuticals demonstrated that a potent GPR119 agonist (AR231453) enhanced GSIS, and improved oral glucose tolerance in lean, diabetic Leprdb/db mice (Chu et al. 2007). In a follow-up study, Chu et al. (2008) reported that GPR119 is also expressed at low levels in intestinal endocrine cells, and that its activation by AR231453 contributes to glycemic control by enhancing GLP-1 and GIP release. The specificity of AR231453 was demonstrated by the absence of effects on glucose tolerance and GLP-1 release in GPR119-deficient mice (Chu et al. 2007, 2008). Unlike OEA (Overton et al. 2006), AR231453 was observed to suppress food intake only at doses significantly higher than those required to impact glucose homeostasis (Chu et al. 2007).

Although the therapeutic potential of GPR119 has been established through activation by endogenous and pharmacological agents, its physiological function remains unclear (Laufer et al. 2008). Deletion of GPR119 appears to have no overt effect on glucose homeostasis under normal conditions. The limited characterization of the GPR119 knockout mice reported by Arena researchers showed that islet morphology and responsiveness to glucose and GLP-1 were normal, as were BW and fed/fasted glucose levels, indicating that deletion of GPR119 does not grossly impact glucose homeostasis (Chu et al. 2007, 2008). However, it is unclear if GPR119 is required to maintain lipid and glucose homeostasis under conditions of metabolic stress such as diet-induced obesity.

We carried out a systematic characterization of independently generated Gpr119$^{-/-}$ mice on the C57BL/6J background. Our results demonstrate that GPR119 is not required to maintain lipid and glucose homeostasis, but it is involved in nutrient-stimulated GLP-1 release. We also show that LPC and OEA are non-specific GPR119 agonists in terms of their insulinotropic effect and hypophagic effect respectively.

**Materials and Methods**

*Construction of targeting vector and generation of Gpr119$^{-/-}$ mice*

A gene-targeting vector was constructed using the sequence from GenBank accession number NC_000086. DNA fragments corresponding to the 5' and 3' regions of the Gpr119 locus were sub-cloned into a vector at either end of the neomycin resistance gene (neo). This targeting vector was linearized and electroporated into C57BL/6J derived ES cells. Colonies resistant to G-418 were screened for the targeted Gpr119 gene by a PCR-based strategy using one primer (5'-taagtccagatgtccgca-3'), corresponding to a region upstream of the Gpr119 gene, and another primer (5'-ccgccgctgctcggttt-3'), corresponding to the neomycin resistance gene. The predicted structure of the targeted Gpr119 locus in the PCR-positive cells was confirmed by Southern blotting, using probes that hybridize outside of and adjacent to the construct arms. Cells from several correctly targeted ES cell lines were injected into C57BL/6J-Tyr$^{-/-}$/J blastocysts to generate chimeric mice. Chimeras obtained from these clones were then bred with C57BL/6J mice to generate heterozygous (Gpr119$^{+/-}$) offspring that were further bred in order to obtain homozygous (Gpr119$^{-/-}$) mice. We used a PCR-based screening strategy with three oligonucleotide primers in a multiplex reaction corresponding to the region of homology, the neo gene, and the deleted region of the Gpr119 gene. These primers were designed to detect both wild-type (573 bp) and targeted (402 bp) alleles. Oligonucleotide sequences were as follows: Gpr119 deleted region forward 5'-ttccagcagaccacctaccat-3', Gpr119 arm of homology reverse 5'-tagtccagacgacctcaggt-3', and neo 5'-gcccccgtcctcggttt-3'.

All mice under study were weaned onto Lab Rodent Diet 20 (PMI Nutrition International, Brentwood, MO, USA LLC; 4-5% fat) and, unless specified, were group housed in polycarbonate cages in a specific pathogen-free environment on a 12 h light:12 h darkness cycle at a temperature of 22°C. For experiments exploring the effect of genotype in response to manipulation of dietary fat content, mice were fed with a semi-purified high-fat diet (HFD; 45% of calories from fat) or a semi-purified low-fat diet (LFD; 10% of calories from fat; D12451 and D12450B respectively; Research Diets, New Brunswick, NJ, USA) ad libitum unless otherwise indicated. In one cohort of mice, HFD-feeding was initiated at 70 days of age and mice remained on the diet for 36 weeks. In a second cohort of mice, the LFD- or HFD-feeding was initiated at 6-8 weeks of age and continued for 33 weeks. Blood collection after an overnight fast was performed after 31 weeks on the diet, and blood collection in the post-prandial state was performed after 33 weeks on diet. All studies were conducted in an American Association for Laboratory Animal Care accredited facility, according to protocols approved by the Schering-Plough Research Institute Animal Care and Use Committee.

In situ hybridization and immunofluorescence

Frozen pancreatic sections (10 μm) from Gpr119$^{+/+}$ and Gpr119$^{-/-}$ mice were fixed with 4% paraformaldehyde. Slides were subsequently treated with HCl and proteinase K (2 μg/ml), acetic anhydride, and dehydrated ethanol. Slides were hybridized with digoxigenin-labeled RNA anti-sense
probes generated by in vitro transcription (Roche, Cat#: 1175025) of mouse Gpr119 cDNA fragment. The cDNA fragment was generated by PCR using primers containing T7 and T3 sequences: mGPR119U29T3 AAT TAA CCC TCA CTA AAG GG tctctgtgctctaaccatctcctca; mGPR119L370T7 GTA ATCA CTC ACT ATG GCC G C tctcagccaagccctcata. Sense probes were used as hybridization controls. After hybridization, probes were recognized by an anti-digoxigenin antibody conjugated with alkaline phosphatase (Roche, Cat#: 1093274) and visualized with the substrate NBT/BCIP showing deep blue color precipitates. For immunofluorescence analysis, pancreata were fixed in 10% formalin overnight and then embedded in paraffin. Sections (5 μm) were obtained from several positions throughout the pancreas and were stored at room temperature until staining. Multiple sections from each animal were examined. Sections were blocked with 10% donkey serum (Jackson ImmunoResearch, West Grove, PA, USA) in PBS for 30 min and washed with PBS. A cocktail of primary antibodies (guinea pig anti-insulin (Sigma I-8510, 1:500), mouse anti-glucagon (Sigma G-2654, 1:100), goat anti-somatostatin (Santa Cruz SC-7819, 1:100)) was added to the slides and incubated at 4 °C overnight. Slides were washed with PBS, and then incubated with a cocktail of secondary antibodies to the respective primaries (donkey anti-guinea pig Cy3 (Jackson ImmunoResearch 706-165-148, 1:500), donkey anti-mouse FITC (Jackson ImmunoResearch 715-095-150, 1:200), donkey anti-goat Alexafluor 488 (Molecular Probes A-11055, 1:200) for 30 min at room temperature. Staining was preserved by adding a drop of Vectashield (Vector Labs, Burlingame, CA, USA) to each tissue section prior to cover slipping. Using this procedure, insulin is stained red, while glucagon and somatostatin both appear green.

Islets isolation and insulin secretion

Pancreatic islets were collected by collagenase A (1 mg/ml; Sigma–Aldrich) perfusion according to protocols described in Gotoh et al. (1985). Islets from each mouse were distributed evenly among treatment groups. Oleoyl-LPC, OEA, and Ex-4 were purchased from Sigma. Islets were washed once with PBS (Ca²⁺, Mg²⁺-free, 0-1% BSA), and pre-incubated with KREBS buffer (10 mM Hepes, 0-1% BSA, 130 mM NaCl, 5-2 mM KCl, 1-3 mM KH₂PO₄, 1-6 mM MgCl₂, 2-8 mM CaCl₂, 20 mM NaHCO₃, 2-8 mM glucose) for 30 min at 37 °C. After removing the pre-treatment buffer, islets were incubated in BSA-free KREBS buffer supplemented with desired treatment at 37 °C, 5% CO₂ for 45-60 min. After the incubation, culture media was removed and mixed in equal volume of PBS + 0-5% BSA to avoid insulin being adsorbed by the plastic wall of the plates. Insulin secretion is represented by the concentration of insulin in the culture media.

Blood analysis

Blood samples were collected from Gpr119<sup>−/−</sup> and Gpr119<sup>+/+</sup> mice by submandibular bleeding (Golde et al. 2005), or cardiac puncture in the case of terminal bleeding, into BD Microtainers blood collecting tubes. Plasma or serum samples were isolated according to BD specifications and were stored at −80 °C until analysis. To measure active GLP-1 (GLP-1 [7–36]amide), a DPP-IV inhibitor from Linco Research (Linco Cat # DP44, St Louis, MO, USA; 10 μl/ml blood) was added into pre-chilled blood collecting tubes freshly. Subsequent steps to isolate plasma were performed at 4 °C. Insulin, leptin, and GLP-1 [7–36]amide levels were measured using kits from Meso-Scale Discovery (Gaithersburg, MD, USA). The MSD mouse/rat GLP-1 [7–36]amide assay kit uses a sandwich immunoassay that detects GLP-1 [7–36]amide but also has some cross-reactivity with GLP-1 [9–36]amide (http://www.meso-scale.com). Total GIP levels were measured using a rat/mouse GIP ELISA kit from Linco Research. Glucose, free fatty acid, cholesterol, and triglyceride were measured using corresponding kits from Wako Diagnostics (Richmond, VA, USA).

Glucose and insulin tolerance test

Blood was collected after 16 h of fasting and basal glucose levels were measured using a glucose oxidase method (Glucometer Elite, Bayer). After this measure, glucose (3 g/kg BW) or insulin (0-75 mU/g BW) was administered by oral gavage or by i.p. injection respectively. Blood was collected from the tail vein at 20, 40, 60, 90, and 120 min post-dose for glucose determination.

Data analysis

Data were reported as means ± S.E.M. Analyses were performed by using two-tailed, unpaired Student t-test, or ANOVA as indicated, depending on data structures. P values <0-05 were considered statistically significant.

Results

Targeted deletion of the Gpr119 gene and generation of knockout mice

Gpr119 expression analysis in tissues of C57BL/6 mice and cell lines, measured by RT-PCR, showed that it was expressed predominantly in pancreatic islets and mouse insulinoma (Min6-C4) cells with a low expression level in intestine (data not shown), consistent with previous reports (Soga et al. 2005, Chu et al. 2007, 2008). Two independently targeted C57BL/6-derived ES cell clones were generated using the vector shown in Fig. 1A and confirmed by Southern blotting (Fig. 1B). Real-time quantitative PCR analysis showed that Gpr119 expression was not detectable in Gpr119<sup>−/−</sup> islets, while in situ hybridization confirmed that there is no Gpr119 mRNA in the islets of knockout mice (Fig. 1C). Note that in Fig. 1C, GPR119-positive signal stays across the entire area of the two islets in Gpr119<sup>+/+</sup> mice. This data, along with the high expression level of GPR119 in the Min6 pancreatic
β-cell line, suggests that GPR119 is expressed in insulin-producing β-cells, consistent with observations reported by Chu et al. (2007) but different from those of Sakamoto et al. (2006) the later of which suggested GPR119 expression in pancreatic PP-cells but not in β-cells.

Effects of LPC and OEA on insulin secretion are not GPR119-specific

There was no gross difference in islet morphology between Gpr119+/+ and Gpr119−/− mice as reflected by glucagon/somatostatin/insulin staining patterns (Fig. 2A). There was no genotypic difference in insulin content at 10 weeks, 6 months, and 12 months of age. The total insulin content (ng insulin/mg pancreas protein) of 12-month old mice are as follows: Gpr119+/+-male: 81.9±16.3 (N=8); Gpr119−/− male: 79.6±10.0 (N=9); Gpr119+/+-female: 136.3±34.3 (N=7); Gpr119−/− female: 122.1±27.5 (N=11). To characterize the role of GPR119 in mediating the effects of LPC and OEA on insulin secretion, we isolated islets from 5 Gpr119+/+ and 5 Gpr119−/− mice. While animal to animal variability was noted, there was no effect of genotype on islet yield. Islets from individual mice were evenly distributed into the following treatment groups: 2.8 mM glucose, 2.8 mM glucose +10 μM LPC, 2.8 mM glucose +10 μM OEA, 16.7 mM glucose, 16.7 mM glucose +10 nM Ex-4, 16.7 mM glucose +10 μM LPC, 16.7 mM glucose +10 μM OEA. We chose to use 10 μM ligands because in pilot experiments, we have treated pooled islets from Gpr119+/+ or Gpr119−/− mice with OEA up to 50 μM in 16.7 mM glucose and we have failed to observe an enhancement on GSIS (data not shown). All animals displayed normal GSIS, and 10 nM Ex-4 further increased GSIS by approximately threefold in both Gpr119+/+ and Gpr119−/− islets. LPC and OEA did not change basal insulin secretion, and the effect of these agents on GSIS was modest and variable in both Gpr119+/+ and Gpr119−/− islets (Fig. 2B and C). Using the same experimental conditions, we also studied islets from Gpr119+/+ and Gpr119−/− mice after HFD-feeding (Fig. 2D and E). The mice still display GSIS. While Ex-4 increased GSIS approximately threefold in both Gpr119+/+ or Gpr119−/− mice, the effects of LPC and OEA on GSIS were not significant.

Gpr119−/− mice are viable and develop normally, and had normal metabolic parameters under basal conditions

Gpr119−/− mice appeared healthy, fertile, and no abnormalities were detected upon gross examination. To determine whether the absence of GPR119 results in abnormalities in major tissues, we performed a complete necropsy on two male and two female Gpr119−/− mice and one male and one female Gpr119+/+ mouse. All tissues/organs were collected and examined histologically. There were no consistent abnormalities detected in any of the organs examined from the Gpr119−/− mice.

**Figure 1** Generation of Gpr119−/− mice. (A) Top: wild-type Gpr119 locus; black rectangle represents the exon, transcription is from left to right. Middle: targeting vector; thick lines represent regions of homology to Gpr119, shaded rectangle represents neo cassette. The restriction enzyme sites: X, Xhol; S, SalI; N, Nhe. Bottom: Gpr119 targeted locus; the positions of the oligonucleotide primers used to screen targeted ES cells are indicated with black arrowheads. (B) DNA probes (black rectangles in A) from 5’ upstream and 3’ downstream homologous regions of Gpr119 gene and neo were used to screen the ES cells. An 8.5 kb and a 1.7 kb Xhol/EcoRV fragment were expected for the wild-type Gpr119 locus and for the targeted locus respectively, when the 5’ probe was used. An 8.5 kb and a 7.3 kb Xhol/EcoRV fragment were expected for the wild-type Gpr119 locus and for the targeted locus respectively, when the 3’ probe was used. Since the Gpr119 gene is chromosome X-linked, and the ES cell line used in the study was male, all targeted clones do not show a wild-type allele. (C) In situ hybridization of pancreas sections from Gpr119+/+ and Gpr119−/− mice. Frozen sections were hybridized with digoxigenin-labeled RNA probes targeting Gpr119 mRNA sequence. Probes are recognized by an anti-digoxigenin antibody conjugated with alkaline phosphatase. Positive signals show deep brown color.
mice that could be attributed to the genotype. Gpr119+/+ and Gpr119−/− mice were assessed in a SHIRPA protocol including the full Irwin and locomotor activity (LMA) assays (Rogers et al. 2001). We tested 15 male Gpr119+/+ mice and 14 male Gpr119−/− mice. Testing commenced when animals were between 9 and 10 weeks and was completed when animals were 12–13 weeks of age. The Irwin pinpointed only mild differences in grip strength and limb tone and a marginal difference in tail clip response latency. The LMA showed that Gpr119+/+ and Gpr119−/− mice explored an open arena in an identical manner. The automated LMA also confirmed the LMA and number of rears scores from the Irwin further establishing the similarity of the genotypes. On this basis, Gpr119−/− mice appear to be behaviorally normal.

Serum samples were collected from a total of 72 chow-fed mice ranging from 66 to 72 days of age (N=19 for

Figure 2 Effects of LPC and OEA on insulin secretion are not GPR119-specific. (A) Immunofluorescent staining of pancreatic islets from Gpr119+/+ and Gpr119−/− mice respectively. Red stains for insulin-expressing β-cells (Cy3); green stains for glucagon-expressing α cells (FITC), and somatostatin-expressing δ cells (Alexafluor 488). (B and C) Effects of LPC (10 μM), OEA (10 μM) and Ex-4 (10 nM) on insulin secretion in islets of chow-fed mice at low (2.8 mM) and high (16.7 mM) glucose conditions. (D and E) Effects of LPC, OEA, and Ex-4 on insulin secretion in islets of high-fat fed mice. The experimental conditions are identical to those in B and C. Insulin values within each genotype and dietary treatment were normalized to those under low glucose. The P values of unpaired, two-tailed t-tests were posted above the bars of high-glucose group. The 16.7 mM glucose group was compared with the 2.8 mM glucose group to test for GSIS. The Ex-4, LPC and OEA groups were compared with the 16.7 mM glucose group to test for effects on GSIS.
Gpr119+/+ females, N=17 for Gpr119+/+ males, N=15 for Gpr119−/− females, and N=21 for Gpr119−/− males). The mice were group housed (2–4 mice per cage) and were fasted for 4 h prior to blood collection. There were no genotypic differences in BW or plasma leptin, insulin, glucose, free fatty acids or triglyceride within each sex (Table 1). Of note, the difference in glucose levels in male mice between the genotypes were not significant (P=0.10, pair-wise t-test). Given the relatively large sample size, we conclude that both Gpr119+/+ and Gpr119−/− mice have normal metabolic parameters under chow-fed conditions. Additionally, a complete blood count and chemistry tests on a smaller number of Gpr119+/+ and Gpr119−/− mice (N=4 for Gpr119+/+ females, N=6 for Gpr119+/+ males, N=5 for Gpr119−/− females, and N=5 for Gpr119−/− males) did not reveal a statistically significant genotypic difference. Similarly, no differences were observed in the urine analysis, including glucose, bilirubin, ketones, specific gravity, blood, pH, protein, urobilinogen, nitrite, and leukocytes.

Gpr119+/+ and Gpr119−/− mice show a similar metabolic response to prolonged HFD-feeding

To investigate the role of GPR119 under metabolic stress, 25 male Gpr119+/+ and 13 age-matched male Gpr119−/− mice (2–4 mice per cage) were fed a HFD (45% kcal as fat) starting at 10 weeks of age. Both Gpr119+/+ and Gpr119−/− mice gained weight similarly during the 36 weeks of HFD feeding (Fig. 3A). Fasting insulin levels increased with HFD feeding from 0.5 ng/ml at week 0 to 10–15 ng/ml at week 36 (Fig. 3B), while glucose levels remained relatively constant (Fig. 3C), suggesting the development of whole body insulin resistance but not overt diabetes in both Gpr119+/+ and Gpr119−/− mice. The insulin levels appeared to be lower in knockout mice for the later time points, but the within-group variability also increased, and the differences did not reach statistical significance. Both Gpr119+/+ and Gpr119−/− mice developed severe hyperleptinemia with HFD feeding (Fig. 3D), but they maintained normal fatty acid levels (Fig. 3E), and they did not develop hypertriglyceridemia (Fig. 3F). Gpr119−/− mice did, however, exhibit lower triglyceride levels after 28–36 weeks on HFD (Fig. 3F).

Gpr119−/− mice had lower BW and lower post-prandial GLP-1 levels on LFD

To identify any genotypic difference that might have been missed in the above HFD study with group-housed mice, we performed another study using individually housed mice fed with a semi-purified LFD (10% kcal as fat) or HFD (45% kcal as fat) ad libitum beginning at 6–8 weeks of age. After ~20 weeks, significant effects of both diet and genotype on BW and BW changes were observed, principally for mice on LFD (Fig. 4A, C and D; Table 2). On HFD, the difference in BW between genotypes did not reach statistical significance, similar to observations from the group housed animals (Fig. 3A). On LFD, Gpr119−/− mice had lower BW compared with the Gpr119+/+ mice; this was driven by significantly less fat mass gain in Gpr119−/− mice (Table 2), suggesting that adipose tissue was the affected organ. Oral glucose tolerance (evaluated 14 weeks after initiation of dietary treatment) was not significantly different between genotypes in mice fed LFD (Fig. 5A) or HFD (Fig. 5B). Similarly, no significant genotypic effect on insulin tolerance (evaluated 19 weeks after initiation of the dietary treatment) was observed in these mice fed LFD (Fig. 5C) or HFD (Fig. 5D).

In the fasted state, HFD-fed mice had higher plasma glucose and insulin levels relative to LFD-fed mice. However, there was no genotypic difference (Table 3). In the post-prandial state, there was a significant effect of diet on plasma leptin (Table 3), consistent with the diet-induced differences in adiposity. There was also a significant effect of diet on fed plasma insulin and plasma cholesterol levels (Table 3). There were no significant differences in plasma adiponectin, triglyceride or free fatty acid levels between groups (Table 3).

Since GPR119 is expressed in intestinal enteroendocrine cells (Chu et al. 2008, Reimann et al. 2008, Parker et al. 2009), we measured GLP-1[7–36]amide and total GIP levels in these animals. There was a significant effect of diet on fasting plasma GLP-1[7–36], with higher levels observed in HFD fed mice (Table 3). In the post-prandial state, there was a significant

<table>
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<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
<th>P value</th>
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<tbody>
<tr>
<td>Genotype</td>
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<td>Gpr119−/−</td>
<td>Gpr119+/+</td>
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<td>17</td>
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<td>19</td>
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<tr>
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<td>Leptin (ng/ml)</td>
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<td>Insulin (ng/ml)</td>
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<td>Glucose (mg/dl)</td>
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<td>TG (mg/dl)</td>
<td>41±0.3±2.3</td>
<td>39.0±2.3</td>
<td>23.5±2.0</td>
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BW, body weight; FFA, free fatty acid; TG, triglyceride; NS, not significant (P>0.05).

Table 1 Serum parameters of Gpr119+/+ and Gpr119−/− mice maintained on a chow diet. Mice were fed chow diet and were fasted for 4 h prior to blood collection.


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genotypic effect on GLP-1[7–36] levels (Table 3), which was attributed largely to lower levels in \textit{Gpr119}\(^{\text{K/K}}\) mice under LFD-feeding. To test the hypothesis that GPR119 participates in physiological regulation of GLP-1 release, we collected blood samples from fasted chow-fed \textit{Gpr119}\(^{\text{C/C}}\) and \textit{Gpr119}\(^{\text{K/K}}\) mice and 30 min after an oral glucose load (3 g/kg). As shown in Fig. 6, the glucose load resulted in a threefold increase in GLP-1[7–36] in the \textit{Gpr119}\(^{\text{C/C}}\) mice (\(P!0.01\), \(t\)-test after Bonferroni correction); by contrast, the response was completely absent in the \textit{Gpr119}\(^{\text{K/K}}\) mice (Fig. 6A). The total GIP levels after the glucose load was not significantly different between genotypes (Fig. 6B). Insulin levels 30-min post-glucose load were significantly lower in the \textit{Gpr119}\(^{\text{K/K}}\) mice (Fig. 6C). However, the lower GLP-1 and insulin levels did not translate into lower plasma glucose levels (Fig. 6D), consistent with the results from the glucose tolerance test (GTT; Fig. 5) and previous reports (Chu et al. 2007).

\textit{Gpr119} is not required for the hypophagic action of OEA

\textit{Gpr119}\(^{\text{K/K}}\) mice treated with OEA (30 mg/kg) decreased their food intake (Fig. 7), as previously described for both rats and mice (Fu et al. 2003). Similarly, \textit{Gpr119}\(^{\text{K/K}}\) mice also

\textbf{Figure 3} \textit{Gpr119}\(^{\text{+/+}}\) and \textit{Gpr119}\(^{\text{−/−}}\) mice show a similar metabolic response to prolonged HFD-feeding. Mice were switched from chow to HFD when they reached 70 days of age. Serum samples were collected from 4 h fasting animals periodically, and the values were plotted by genotype. Data were presented as mean±S.E.M. (A) Body weight. (B) Insulin. (C) Glucose. (D) Leptin. (E) Free-fatty acid (FFA). (F) Triglyceride. *Significant difference between genotypes (\(P<0.05\)).

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displayed hypophagia in response to OEA, and the effect was of similar magnitude to Gpr119$^{+/+}$ mice during the first 6 h. OEA-treated Gpr119$^{+/−}$ mice ate less than vehicle treated mice 24 h after dosing. Overall, these results suggest that, under this experimental condition, the hypophagic actions of OEA do not require GPR119 activation.

**Discussion**

Prior work has demonstrated that GPR119 is expressed in pancreatic β-cells and intestinal L-cells, and that signaling via this receptor augments glucose-stimulated insulin release and GLP-1 release (Soga et al., 2005, Overton et al., 2006, 2008, Chu et al., 2007, 2008, Reimann et al., 2008, Parker et al., 2009). Additionally, reports have shown that mice deficient in GPR119 appear normal under chow-fed conditions, but fail to respond to a small molecule GPR119 agonist (Chu et al., 2007). We have extended these observations by evaluating the effect of high-fat feeding on growth and metabolic parameters in an independently generated line of GPR119-deficient mice. The major finding of this study was that there was no significant effect of Gpr119 ablation on glucose homeostasis, as

### Table 2

<table>
<thead>
<tr>
<th>Measure</th>
<th>Gpr119$^{+/+}$</th>
<th>Gpr119$^{−/−}$</th>
<th>Gpr119$^{+/+}$</th>
<th>Gpr119$^{−/−}$</th>
<th>Diet</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-fat diet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial body weight (g)</td>
<td>20.0±0.3</td>
<td>20.0±0.3</td>
<td>19.9±0.6</td>
<td>19.3±0.4</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Terminal body weight (g)</td>
<td>39.8±1.3</td>
<td>35.4±1.3</td>
<td>44.8±2.0</td>
<td>42.3±0.8</td>
<td>&lt;0.001</td>
<td>0.022</td>
</tr>
<tr>
<td>Body weight change (g)</td>
<td>19.3±1.0</td>
<td>15.5±1.1</td>
<td>25.0±1.8</td>
<td>23.1±0.8</td>
<td>&lt;0.001</td>
<td>0.047</td>
</tr>
<tr>
<td>Initial fat mass (g)</td>
<td>2.2±0.1</td>
<td>2.0±0.1</td>
<td>2.2±0.2</td>
<td>2.1±0.1</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Terminal fat mass (g)</td>
<td>13.8±0.8</td>
<td>10.7±1.0</td>
<td>18.4±1.4</td>
<td>16.9±0.5</td>
<td>&lt;0.001</td>
<td>0.013</td>
</tr>
<tr>
<td>Fat mass change (g)</td>
<td>11.6±0.8</td>
<td>8.6±0.9</td>
<td>16.3±1.2</td>
<td>14.8±0.5</td>
<td>&lt;0.001</td>
<td>0.019</td>
</tr>
<tr>
<td>Initial lean mass (g)</td>
<td>16.7±0.5</td>
<td>16.2±0.2</td>
<td>16.3±0.4</td>
<td>15.8±0.3</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Terminal lean mass (g)</td>
<td>23.1±1.0</td>
<td>22.2±0.4</td>
<td>24.0±0.7</td>
<td>22.8±0.5</td>
<td>NS</td>
<td>0.028</td>
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<tr>
<td>Lean mass change (g)</td>
<td>6.4±0.4</td>
<td>6.0±0.3</td>
<td>7.7±0.6</td>
<td>7.1±0.5</td>
<td>0.010</td>
<td>NS</td>
</tr>
</tbody>
</table>
measured by plasma glucose and insulin levels, and GTT and insulin tolerance test (ITT), under basal and diet-induced obesity. There was, however, an effect of \( \text{Gpr119} \) ablation on BW and body composition, and on circulating GLP-1\([7–36]\).

Additionally, \( \text{Gpr119}^{+/+} \) mice demonstrated a hypophagic response to OEA, suggesting that GPR119 is not required for this response.

Although studies on pharmacological activation of GPR119 by synthetic and endogenous agonists have been reported, the physiological functions of GPR119 are only Table 3

<table>
<thead>
<tr>
<th>Measure</th>
<th>Low-fat diet</th>
<th>High-fat diet</th>
<th>( P ) value</th>
<th>Diet</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Gpr119}^{+/+} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{Gpr119}^{-/-} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fasted state</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>111±4</td>
<td>107±5</td>
<td>0.0002</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>1.6±0.1</td>
<td>1.2±0.1</td>
<td>0.012</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>GLP-1[7–36]amide (pg/ml)</td>
<td>2.8±0.1</td>
<td>3.4±0.2</td>
<td>0.0005</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td><strong>Post-prandial state</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>145±5</td>
<td>152±9</td>
<td>0.011</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>3.9±0.4</td>
<td>3.7±0.5</td>
<td>0.006</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>GLP-1[7–36]amide (pg/ml)</td>
<td>14.7±1.7</td>
<td>8.4±0.9</td>
<td>0.004</td>
<td>NS</td>
<td></td>
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<tr>
<td>Total GIP (pg/ml)</td>
<td>278±53</td>
<td>250±31</td>
<td>0.001</td>
<td>NS</td>
<td></td>
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<tr>
<td>Leptin (ng/ml)</td>
<td>53.4±6.9</td>
<td>46.5±8.0</td>
<td>0.004</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>22.8±1.1</td>
<td>23.2±1.1</td>
<td>0.011</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>95.1±7.1</td>
<td>68.7±6.4</td>
<td>0.006</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>44.5±8.5</td>
<td>46.1±6.7</td>
<td>0.011</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Free fatty acids (mM)</td>
<td>0.42±0.03</td>
<td>0.44±0.02</td>
<td>0.006</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>
partially understood (Lauffer et al. 2008). A recent study showed that pharmacological activation of GPR119 is sufficient to increase GLP-1 release and improve glucose tolerance (Chu et al. 2008); however, it did not directly address the role of GPR119 in a physiological setting. Our results provide evidence that GPR119 signaling is required for physiological regulation of GLP-1 secretion after food ingestion: 1) in the fasted state, GLP-1 levels were lower in Gpr119<sup>K−/−</sup> mice under HFD feeding (Table 3), 2) Gpr119<sup>K−/−</sup> mice had lower GLP-1[7–36] levels in the post-prandial state compared with Gpr119<sup>C+/+</sup> mice, especially on LFD (Table 3), and 3) when mice were given an oral glucose load, Gpr119<sup>K−/−</sup> mice clearly showed a deficiency in elevating GLP-1 levels compared with wild-type animals (Fig. 6A). The lower plasma insulin levels in Gpr119<sup>K−/−</sup> mice 30 min after an oral glucose load (Fig. 6C) may have been due to a lack of GLP-1 stimulation rather than a lack of GPR119 signaling in pancreatic β-cells because Gpr119<sup>K−/−</sup> islets had normal GSIS (Fig. 2). Since we did not detect a reduction in the intestinal expression of the preglucagon gene in Gpr119<sup>K−/−</sup> mice (data not shown), GPR119 is most likely involved in regulation of GLP-1 secretion. The intracellular mechanisms controlling nutrient-stimulated GLP-1 secretion from intestinal endocrine cells are poorly understood, but both endocrine and neural factors are believed to be involved (Drucker 2006). Several GPCRs, including FFAR1/GPR40, GPR120, and GPR119 are found to be expressed in intestinal entoendocrine cells and have been implicated in the regulation of GLP-1 secretion (Hirasawa et al. 2005, Shapiro et al. 2005, Chu et al. 2008, Lauffer et al. 2008, Reimann et al. 2008, Parker et al. 2009). Since glucose is not a direct ligand of GPR119, GPR119 is likely involved in GLP-1 secretion at a step downstream of glucose uptake or metabolism. Further studies are needed to dissect the signaling mechanisms from nutrient sensing to GLP-1 secretion, and to address how GPR119 is involved in the process.

Although GPR119 plays a role in physiological regulation of GLP-1 secretion, it does not appear to be required to maintain metabolic homeostasis under both basal and diet-induced obesity. In addition, the reduced GLP-1 and insulin levels in Gpr119<sup>K−/−</sup> mice did not seem to result in a significantly higher blood glucose level in these mice 30 min after an oral glucose load (Figs 5 and 6). Interestingly, we observed in Gpr119<sup>K−/−</sup> mice a lower BW under LFD-feeding (Table 2), and a lower triglyceride level after prolonged HFD-feeding (Fig. 3F). The BW and fat mass phenotype cannot be explained by alterations in GLP-1 or
functions, may affect multiple targets in the variety of cell bioactive fatty acid derivatives with diverse physiological high enough local concentrations of LPC and OEA to the One possible explanation is that we were not able to deliver independent effects (Ning et al. 2008), we were not able to observe a significant effect of these two agents on insulin secretion in isolated primary islets after repeated attempts. Note that these islets display normal response to glucose and Ex-4 (Fig. 2), similar to what was previously reported by Chu et al. (2007), suggesting that at least in pancreatic islets there is unlikely to be an increased GLP1R sensitivity. The observation remains an interesting topic for further research.

Although LPC and OEA have been previously reported to mediate insulin secretion in certain insulinoma cell lines (Soga et al. 2005, Ning et al. 2008), we were not able to observe a significant effect of these two agents on insulin secretion in isolated primary islets after repeated attempts. Note that these islets display normal response to glucose and Ex-4 (Fig. 2). One possible explanation is that we were not able to deliver high enough local concentrations of LPC and OEA to the β-cells within an islet. It is also possible that LPC and OEA, as bioactive fatty acid derivatives with diverse physiological functions, may affect multiple targets in the variety of cell types within the islet, such that the net effect on β-cell insulin secretion is not as apparent as in β-cell lines. In fact, we have previously observed that LPC has both GPR119-dependent and independent effects (Ning et al. 2008). Mouse GPR119 has high-constitutive activity (Chu et al. 2007) that may reduce the effects of ligand-driven receptor activation in islets. The low potency of LPC and OEA to GPR119 could also be an explanation. In isolated islets, the recently reported GPR119 agonist AR231453 enhances GSIS to an extent similar to 25 nM GLP-1. AR231453 has an EC50 of 4.7 nM in a cAMP assay and an EC50 of 3.5 nM in insulin secretion in HIT-T15 cells, which is about 1000 times more potent than LPC and OEA. LPC and OEA were not used in the Chu et al. (2007) study. At the time of the present study, we did not have access to AR231453, and as a result we cannot directly compare the effects of LPC and OEA with this compound. Nevertheless, it is reasonable to assume that AR231453 is a potent and selective GPR119 agonist, while LPC and OEA are weaker and non-specific agonists.

Interestingly, while this manuscript was in revision, a paper published online in Diabetes had similar observations about OEA effect on insulin secretion (Lauffer et al. 2009). The effect of OEA on GLP-1 secretion peaked at 10 μM and dropped off at higher concentrations. Intriguingly, when rat islets were exposed to 5 mg/kg OEA i.v. in 13 mM glucose for 60 min, no effect on insulin secretion was observed. The authors concluded that circulating OEA does not cause significant increases in insulin release from pancreatic β-cells. They also thought that the lack of effect of OEA on insulin secretion could be due to an insufficient local delivery of ligand to the β-cells within an islet. On the other hand, a 200-fold lower dose of intraluminal OEA was effective in triggering GLP-1 release from gut L-cells, presumably through GPR119 (Lauffer et al. 2009). The change in GLP-1 from intraluminal OEA administration resulted in an increase in insulin, however, it did not translate into a difference in plasma glucose levels, similar to our findings in Fig. 6.

The role of GPR119 in mediating the hypophagic effect of OEA remains controversial. Overton et al. (2006) reported that OEA and the synthetic agonist PSN632408 can reduce food intake in Sprague–Dawley rats after acute dosing at 30 and 100 mg/kg respectively. Sub-chronic oral administration of the synthetic compound also reduced food intake in HFD-fed rats. They therefore proposed GPR119 as a novel target for obesity and related metabolic disorders (Overton et al. 2006). The involvement of GPR119 activation on food intake in mice was not confirmed by recent studies using a more potent and specific GPR119 agonist AR231453 (Chu et al. 2007, 2008), although the authors did mention that small-molecular weight GPR119 agonists, when given at doses significantly higher than that required to improve glucose tolerance, did produce modest hypophagia (Chu et al. 2008). At higher doses, however, it is hard to conclude that such effects are GPR119-specific. Under our experimental conditions, GPR119 is not required for the hypophagic action of 30 mg/kg OEA in mice (Fig. 7). It is possible that species differences in brain GPR119 expression may be responsible for the discrepancy in the literature (Overton et al. 2006, 2008). Since OEA is a bioactive molecule with pleiotropic function, it is possible that the hypophagic effect of OEA in mice could be mediated by factors other than GPR119. Indeed, Fu et al. (2003) had previously reported that OEA regulates feeding and BW through activation of the nuclear receptor PPARγ, showing that OEA administration causes metabolic changes similar to those produced by PPARγ agonists and that the hypophagia in response to OEA administration was absent in PPARγ null mice.

Figure 7 The effect of OEA (30 mg/kg i.p.) or vehicle on 24 h food intake in chow-fed Gpr119+/+ (N=20) and Gpr119−/− (N=22) mice. Following on overnight food deprivation, mice aged 13–16 weeks were administrated 30 mg/kg OEA (Sigma–Aldrich) or vehicle (5% PEG 400, 5% Tween in saline) by i.p. injection. One hour after dosing, two pellets of pre-weighed chow were provided and food intake was measured 1, 2, 4, 6, and 24 h later. Values are means ± S.E.M. *Significant difference between genotypes (P<0.05).
While the reported data in mice do not reject a role for GPR119 signaling in OEA-mediated effects, they do indicate that these effects, if present, are subtle. Finally, the effects of GPR119 signaling on the release of GLP-1 (Chu et al. 2008, Lauffer et al. 2009) suggest a possible role for this receptor in BW regulation; however, the relationship between GLP-1 release by small-molecule agonists, or undiscovered endogenous agonists, and their effects on food intake remain to be further investigated.

Declaration of interest

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