Gender-dependent effect of Gpbar1 genetic deletion on the metabolic profiles of diet-induced obese mice

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Abstract

G-protein coupled bile acid receptor 1 (GPBAR1/TGR5/M-Bar/GPR131) is a cell surface receptor involved in the regulation of bile acid metabolism. We have previously shown that Gpbar1-null mice are resistant to cholesterol gallstone disease when fed a lithogenic diet. Other published studies have suggested that Gpbar1 is involved in both energy homeostasis and glucose homeostasis. Here, we examine the functional role of Gpbar1 in diet-induced obese mice. We found that body weight, food intake, and fasted blood glucose levels were similar between Gpbar1-null mice and their wild-type (WT) littermates when fed a chow or high-fat diet (HFD) for 2 months. However, insulin tolerance tests revealed improved insulin sensitivity in male Gpbar1+/− mice fed chow, but impaired insulin sensitivity when fed a HFD. In contrast, female Gpbar1−/− mice exhibited improved insulin sensitivity when fed a HFD compared with their WT littermates. Female Gpbar1−/− mice had significantly lower plasma cholesterol and triglyceride levels than their WT littermates on both diets. Male Gpbar1−/− mice on HFD displayed increased hepatic steatosis when compared with Gpbar1+/− males and Gpbar1−/− females on HFD. These results suggest a gender-dependent regulation of Gpbar1 function in metabolic disease.


Introduction

G-protein coupled bile acid receptor 1 (GPBAR1), also known as TGR5 (Kawamata et al. 2003) and M-BAR (Maruyama et al. 2002), is the founding member of the bile acid (BA) receptor subclass of G-protein-coupled receptors (Foord et al. 2005). GPBAR1 is a Gs-coupled cell surface receptor that is expressed on the plasma membrane, and is internalized in response to agonist activation (Kawamata et al. 2003). BA-induced cellular effects of GPBAR1 activation include stimulation of cAMP synthesis (Houten et al. 2003), and have been implicated in a range of cellular and physiological activities (Thomas et al. 2008a,b, Fiorucci et al. 2009, Wei et al. 2009).

GPBAR1 mRNA has been detected in a variety of tissues in human, rats, and mice. In mice, it is predominantly expressed in the gallbladder (Vassileva et al. 2006), but also in gastrointestinal tract, lung, heart, ovary, placenta, white adipose tissue (WAT), and brown adipose tissue (BAT; Maruyama et al. 2002, 2006, Kawamata et al. 2003, Vassileva et al. 2006, Watanabe et al. 2006). In mouse gallbladder, the expression level of Gpbar1 mRNA is 60–100 times greater than that of Gpbar1 mRNA in all other tissues analyzed, and in situ hybridization has shown that it is restricted to epithelial cells (Vassileva et al. 2006). Recently, GPBAR1 protein was localized to liver sinusoidal endothelial cells and Kupffer cells in rat (Keitel et al. 2007, 2008a), and gallbladder epithelium in human (Keitel et al. 2009). GPBAR1 is expressed in enteroendocrine cell lines, such as NCI-H716, STC-1, and GLUTag (Maruyama et al. 2002), and has been shown to stimulate the production of glucagon-like peptide 1 (GLP-1) in STC-1 cells (Katsuma et al. 2005).

BAs are known regulators of energy homeostasis and glucose metabolism that have been reported to inhibit diet-induced obesity and prevent the development of insulin resistance (Ikemoto et al. 1997, Watanabe et al. 2006, Kobayashi et al. 2007, Keitel et al. 2008b). Watanabe et al. (2006) have shown that administration of BAs to mice increases energy expenditure in BAT, preventing obesity and insulin resistance. This is achieved via the induction of the cAMP-dependent thyroid hormone activating enzyme type 2 iodosothyronine deiodinase (Dio2). Treatment of mouse brown adipocytes and human skeletal myocytes by BAs increases Dio2 activity and oxygen consumption. These effects are independent of FXR and are mediated by increased cAMP production, resulting from GPBAR1 activation by BAs. Since mouse BAT and human skeletal muscle co-express Dio2 and GPBAR1, it has been suggested that GPBAR1 regulates energy expenditure. GLP-1 secretion has been found to be induced by BAs through GPBAR1 in a murine
enteroendocrine cell line STC-1 (Katsuma et al. 2005). Olea oleracea, a natural GPBAR1 agonist isolated from olive leaves, decreased plasma glucose and insulin levels when administered to diet-induced obese C57BL/6 mice (Sato et al. 2007). These findings suggest a role of GPBAR1 in glucose homeostasis. Recently, Thomas et al. (2009) showed that activation of GPBAR1/TGR5 by a selective agonist INT-777 results in an increase in intracellular levels of cAMP, oxygen consumption, cytochrome c oxidase, and the ATP:ADP ratio, leading to an improved liver and pancreatic function and enhanced glucose tolerance in obese mice.

We have previously shown that Gpbar1 plays a critical role in the formation of gallstones in mice (Vassileva et al. 2006), and that the mice with targeted deletion of this gene are protected from gallstone disease. Maruyama et al. (2006) have reported that independently generated knockout (KO) mice have smaller total BA pool size, and that female KO mice accumulate fat and gain body weight (BW) when placed on high-fat diet (HFD) for 2 months. In this study, we investigated the role of Gpbar1 in diet-induced obese mice, and found that male and female Gpbar1−/− mice respond differently to HFD challenge. There were differences between the two sexes in their plasma lipoprotein profile, response to insulin tolerance test (ITT), and liver function. These results suggest a gender-dependent regulation of Gpbar1 function.

Materials and Methods

Animals and diets

Gpbar1 KO mice in C57BL/6j background were generated as described in Vassileva et al. (2006) (Supplementary information). Mice were bred in house, and age- and gender-matched animals were used between 8 and 20 weeks of age. All mice were housed in a specific pathogen-free environment, and all experiments with animals were conducted according to the Schering-Plough ACUC guidelines for animal care.

Diets were prepared by Research Diets, Inc. (New Brunswick, NJ, USA). Mice were maintained on normal chow, containing <0.02% cholesterol, until 8 weeks of age. Wild-type (WT) and KO littermates were then fed a HFD (D12492) containing 60 kcal% fat. BWs were measured weekly. After feeding the HFD for 9 weeks, mice were killed, and their tissues were collected for further analyses.

Plasma and hepatic lipid measurements

Plasma samples were stored at 4 °C and analyzed by fast protein liquid chromatography (FPLC) to separate lipoproteins by gel permeation chromatography. The FPLC system, AKTA Basic (GE Healthcare, Piscataway, NJ, USA), comprised the following components: P-920 pumps, INV-907 and PV-908 valves, M-925 mixer, UPC-900 monitor, 100-μl sample loop, Frac-950 fraction collector, Superose 6 100/300 GL Tricorn column (C/N 0827026), and Unicorn operating system. Plasma samples manually loaded into the 100-μl loop were eluted with a degassed buffer containing DPBS without calcium and magnesium (Gibco C/N 14190) with added 10 ml/l of 5 M sodium chloride (Sigma, S-5150), 2 ml/l of 0.5 M disodium EDTA (Sigma, E-7889), and 5 ml/l of 4% sodium azide (Sigma, S8032) at a rate of 0.5 ml/min. After a column equilibration period of 10 min, the samples were injected. Subsequently, 40 0.5-ml fractions were collected beginning at 9 min after injection. Cholesterol content in each fraction was determined by a modification of the cholesterol oxidase method of Allain et al. (1974) using Wako test kits (Wako Chemicals, Richmond, VA, USA): cholesterol E (C/N 439-17501). Triglyceride (TG) concentrations were determined using the Wako L-Type TG H reagents (C/N 997-37492 and 993-37592). Forty microliters from each fraction or standards were mixed with 200 μl of the appropriate reagent and incubated in an oven at 40 °C for 15 min, and absorbance was determined in a Molecular Devices plate reader at 600 nm. Results were calculated from standard curves, and reported as micrograms of cholesterol or TGs per fraction. Quantities from fractions 15–19, 20–28, and 29–36 were summated to be reported as mg/dl of cholesterol or TGs in very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) respectively.

Liver samples (≈ 250 mg) were frozen and stored at −20 °C until analysis. Individual samples were extracted according to the procedure of Folch et al. (1957) and homogenized in 6 ml of chloroform:methanol (2:1), and 4 ml of water were added, vortexed, and centrifuged at 1000 g for 30 min. The chloroform layer was removed and dried under nitrogen. The extracted lipids were redissolved in 1 ml chloroform, and 0.1 ml was transferred into HPLC sample vials. Samples were dried under nitrogen and redissolved in 1 ml of hexane:isopropanol (98:8:1:2). Chromatography was performed essentially as described by Burrier et al. (1995) using an isocratic mobile phase containing 98.8% hexane and 1.2% isopropanol at a flow rate of 2 ml/min through a Zorbax Sil (4.6 X 25 cm) silica column (Agilent Technologies # 880952-701, Parsippany, NJ, USA). Lipids in a 5-μl injection were detected by absorbance at 206 nm and quantitated by computer integration (System Gold, Beckman, Fullerton, CA, USA) of AUCs. Cholesterol, cholesteryl ester, and TG concentrations were determined by comparison to standard curves using Non-polar Lipid Mix-B, Matrex, Inc., Pleasant Gap, PA, USA, C/N 1130. The accumulation of hepatic free cholesterol (FC) and cholesteryl esters was used as a surrogate marker of cumulative cholesterol absorption and its inhibition in mice (Salisbury et al. 1995). Data were reported as mg/g liver and mg/liver.

Blood glucose analysis

For blood glucose levels, Gpbar1−/− and Gpbar1+/+ littermate mice were fasted for 4 h. Blood was collected from the tail, and glucose was measured using glucose oxidase
method (Glucometer Elite, Bayer). For ITT, tail blood was collected after 5 h of fasting, and basal glucose levels were measured using a glucose oxidase method (Glucometer Elite, Bayer). After this measure, insulin (0-75 U/kg BW) was administered by i.p. injection, and blood was collected from the tail vein at 20, 40, 60, 90, and 120 min after the administration of dose for glucose determination.

**Histological analysis**

Mice were killed, and their livers were collected, immersion fixed in 10% neutral-buffered formalin, and paraffin embedded, and sections were stained by standard hematoxylin and eosin (H&E) methods. All tissues were examined by light microscopy and graded for severity by one investigator.

**Pancreatic islets isolation**

Pancreatic islets were isolated by collagenase digestion and discontinuous Ficoll gradient separation, a modification of the original method of Li et al. (2003). The islets were maintained in RPMI 1640 medium (11 mmol/l glucose, Invitrogen). The culture medium was supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin, and islets were incubated at 37 °C in a 5% CO₂/95% air-humidified incubator.

**mRNA expression analysis**

mRNA from various tissues was extracted utilizing the Ultraspec RNA isolation kit from Biotecx (Houston, TX, USA) following specifications from the manufacturer. cDNA was generated by reverse transcription using random hexamers (Promega) and oligo-dT primers (Life Technologies). Quantitative real-time PCR analysis was performed on an ABI 7900 sequence-detection instrument (TaqMan) following the manufacturer’s instructions. Primers sequences for the genes involved in the cholesterol, BA, fatty acid, and glucose metabolism are shown in Supplementary Table 1, see section on supplementary data given at the end of this article. Ribosomal RNA primers (PE Applied Biosystems, Foster City, CA, USA) were used as an internal control. Quantitative PCR conditions were as follows: 50 °C for 2 min; 95 °C for 10 min; 40 cycles of 95 °C for 15 s, 60 °C for 1 min. Data were analyzed using Sequence Detection Systems software version 1.7.

**Statistical analysis**

GraphPad Prism version 4 was used for graphics (GraphPad Software, San Diego, CA, USA). Values are expressed as means ± s.e.m. Statistical comparisons for significance between groups were performed using Student’s t-test. A probability value of P<0.05 was considered significant.

![Figure 1](https://example.com/f1.png)

**Figure 1** Body weights of Gpbar1−/− and Gpbar1+/+ mice. Individual values were measured from 11 to 17 mice per group on chow and HFD. Significantly higher body weights at 12, 16, and 20 weeks of age for WT males on HFD versus WT males on chow, and KO males on HFD versus KO males on chow. At 16 and 20 weeks of age, female WT mice on HFD show significantly higher BW than WT females on chow, and female KO mice on HFD are significantly heavier than the female KO mice on chow. Error bars represent ±s.e.m. *P<0.05.

**Results**

Gpbar1−/− mice fed HFD were indistinguishable by weight from their WT littermates

Gpbar1−/− males (n=15) and females (n=17) and Gpbar1+/+ littermate males (n=12) and females (n=11) were fed a HFD for 8 weeks. Similar number of Gpbar1−/− and Gpbar1+/+ control mice were fed chow. There was no difference in the food consumption between the groups (data not shown). The BWs of both male and female Gpbar1−/− mice on chow were similar to those of their WT littermates (Fig. 1). As expected, both male and female Gpbar1+/+ mice on HFD became significantly heavier than the male and female Gpbar1+/+ control mice on chow. Gpbar1−/− males and females on HFD also gained significantly more BW than the control Gpbar1−/− male and female mice on chow. The BWs of male and female Gpbar1−/− mice were not statistically different from those of their WT littermates throughout the study, both on chow and on HFD, although the Gpbar1−/− males on HFD were slightly heavier than the Gpbar1+/+ control males on HFD (Fig. 1).

Gpbar1−/− females have lower plasma lipoprotein levels

Cholesterol and TG levels in hepatic samples were measured at the end of the HFD study (Fig. 2). Gpbar1−/− males on HFD had lower FC, and Gpbar1−/− females on chow had elevated FC levels compared with their control groups. Plasma cholesterol levels in VLDL (Chol-VLDL), LDL (Chol-LDL), and HDL (Chol-HDL) fractions, as well as the total plasma cholesterol, were significantly lower in Gpbar1−/− females on both chow and HFD compared with their WT littermate controls (Fig. 3A). Male Gpbar1−/− mice on chow showed lower total plasma cholesterol and Chol-LDL (Fig. 3A). Total plasma TG levels and TG levels in VLDL (TG-VLDL), LDL (TG-LDL), and HDL (TG-HDL)
fractions were again significantly lower in Gpbar1−/− females on chow, but only TG-VLDL levels were lower in Gpbar1−/− females on HFD (Fig. 3B). In contrast, Gpbar1−/− males on both diets had TG levels comparable to those in their WT littermates with the exception of lower total TG and TG-LDL levels (Fig. 3B).

Gpbar1−/− males have improved insulin sensitivity on chow diet but impaired sensitivity on HFD

Blood glucose levels of Gpbar1−/− mice on chow were similar to those in their WT littermates at 8 and 16 weeks of age (Fig. 4A). As expected, WT mice on HFD became hyperglycemic after 8 weeks on HFD (Fig. 4A). Male Gpbar1−/− on HFD had similar blood glucose levels to those in their WT littermates. Female Gpbar1−/− mice on HFD had lower, but not significant, glucose levels than their WT littermates.

The ITT revealed improved insulin sensitivity in male Gpbar1−/− mice fed chow at all time points the blood glucose was measured after insulin administration. But when fed HFD, male Gpbar1−/− mice showed impaired insulin sensitivity at 30 and 60 min after insulin administration (Fig. 4B). Female Gpbar1−/− mice on chow were not different from their WT littermates, but when fed HFD they exhibited improved insulin sensitivity 60, 90, and 120 min after insulin administration (Fig. 4B).

Increased liver steatosis in Gpbar1−/− males on HFD

After 8 weeks on HFD, Gpbar1+/+ and Gpbar1−/− male and female mice were killed, and their livers were collected for histological analysis. As expected (Hoffler et al. 2009), Gpbar1+/+ males and females fed HFD developed hepatic lipidosis (steatosis) (Fig. 5A and C). The steatosis was in the form of microvesicular vacuolation of hepatocyte cytoplasm, characterized by small, round, clear vacuoles within the cytoplasm of hepatocytes, and consistent with the accumulation of lipids. Livers from female Gpbar1−/− mice on HFD were comparable to those from the Gpbar1+/+ littermates displaying microvesicular steatosis (Fig. 5C and D). However, when compared with their WT littermates, Gpbar1−/− males showed an elevated hepatic lipid accumulation (Fig. 5A and B). Their hepatocytes contained large, round, clear vacuoles (macrovesicular vacuolation).

Expression of genes involved in cholesterol, BA, fatty acid, and glucose metabolism in Gpbar1−/− mice on HFD

In order to investigate the molecular basis of the response of Gpbar1−/− mice to HFD challenge, we performed real-time quantitative PCR analysis of mRNAs isolated from pancreatic islets, BAT, WAT, and livers of Gpbar1−/− and Gpbar1+/+ mice fed HFD (n = 3). First, we selected 11 genes involved in the glucose homeostasis, and analyzed...
their mRNA expression levels in pancreatic islets. Glucagon levels were elevated twofold in male Gpbar1−/− mice on HFD when compared with Gpbar1+/+ littermates, and female Gpbar1−/− mice had a slightly decreased glucagon receptor (Gcgr) levels when compared with Gpbar1+/+ littermates on HFD (Supplementary Figure 1A, see section on supplementary data given at the end of this article).

We then studied the mRNA expression levels of 43 genes involved in cholesterol, BA, fatty acid, and glucose metabolism in BAT, WAT, and livers of Gpbar1−/− and Gpbar1+/+ mice. In BAT and WAT, the mRNA levels of several genes were either elevated or decreased in male Gpbar1−/− mice compared with Gpbar1+/+ males, but none of the changes were more than twofold. There were very few changes in the female Gpbar1−/− mice on HFD when compared with their WT littermates (Supplementary Figure 1B and C). Livers from male Gpbar1−/− mice on HFD showed elevated mRNA expression levels of several genes involved in cholesterol, BA, fatty acid, and glucose metabolism when compared with those from their WT littermates, but none of the changes were greater than twofold, with the exception of Abcb4 and Cyp7a1 in KO males. Female Gpbar1−/− livers on HFD were not different from Gpbar1+/+ controls (Supplementary Figure 2, see section on supplementary data given at the end of this article). Since Gpbar1 has also been shown to be active in macrophages (Kawamata et al. 2003, Keitel et al. 2007, 2008b), we investigated the cytokine levels in the livers from

![Figure 3](https://www.endocrinology-journals.org)
and Gpbar1<sup>−/−</sup> and Gpbar1<sup>+/+</sup> mice on HFD. There was no statistically significant difference in the following cytokines: Il1a, Il1b, Il15, Il16, Il18, and Ifna. Similarly, there were no differences in the BAT and WAT cytokine profiles between Gpbar1<sup>−/−</sup> and Gpbar1<sup>+/+</sup> mice on HFD (data not shown).

**Discussion**

BAs have been implicated in the regulation of energy homeostasis and glucose metabolism (Houten et al. 2006, Keitel et al. 2008b, Nguyen & Bouscarel 2008). It has been shown recently that BAs inhibit diet-induced obesity and prevent the development of insulin resistance (Watanabe et al. 2006, Kobayashi et al. 2007). Gpbar1<sup>−/−</sup> mice on HFD, (B) Gpbar1<sup>−/−</sup> males on HFD, (C) Gpbar1<sup>+/+</sup> females on HFD, and (D) Gpbar1<sup>−/−</sup> females on HFD. Three mice per group were analyzed. Magnification ×100.
To investigate the physiological role of GPBAR1 in vivo, we placed Gpbar1<sup>−/−</sup> males and females together with Gpbar1<sup>+/+</sup> littermates on chow and HFD for 8 weeks. Our analysis suggests a gender-dependent regulation of the GPBAR1 function. There were no significant differences in the BWs between the corresponding Gpbar1<sup>−/−</sup> and Gpbar1<sup>+/+</sup> groups of mice on chow and HFD (Fig. 1). When independently generated Gpbar1 KO mice (Maruyama et al. 2006) were challenged with HFD, increased BW in female Gpbar1<sup>−/−</sup> mice was observed. One possibility for the discrepancy between the two results is the different background strain of mice, 129/svX C57BL/6 four times into C57BL/6N for Maruyama et al. (2006), and C57BL/6 for the Gpbar1 KO line described here. There were no significant differences in the hepatic lipid composition between the corresponding Gpbar1<sup>−/−</sup> and Gpbar1<sup>+/+</sup> groups of mice on chow and HFD, with the exception of lower FC in Gpbar1<sup>−/−</sup> males on HFD and higher FC in Gpbar1<sup>−/−</sup> females on chow, when compared with their WT littermates (Fig. 2). However, Gpbar1<sup>−/−</sup> females on both chow and HFD had lower plasma total cholesterol levels, as well as Chol-VLDL, Chol-LDL, and Chol-HDL, than their WT littermates. In the Gpbar1<sup>−/−</sup> males, only the total cholesterol and Chol-LDL levels were lower than those in the Gpbar1<sup>+/+</sup> littermates (Fig. 3A). Female Gpbar1<sup>−/−</sup> mice on chow had lower plasma total TG, TG-VLDL, TG-LDL, and TG-HDL levels than Gpbar1<sup>+/+</sup> females on chow, but only TG-VLDL levels were lower when on HFD. Male Gpbar1<sup>−/−</sup> mice had lower total TG and TG-LDL levels than their WT littermates when fed chow, but no differences were observed when fed HFD (Fig. 3B).

There was no difference in the fasting blood glucose levels between male and female Gpbar1<sup>−/−</sup> mice and their WT littermates on chow and HFD at the beginning and at the end of the study (Fig. 4A). However, the ITT performed at the end of the HFD feeding revealed improved insulin sensitivity in Gpbar1<sup>−/−</sup> males on chow, but impaired insulin sensitivity in Gpbar1<sup>−/−</sup> males on HFD at 30 and 60 min after insulin administration, when compared with their WT littermates. In contrast, the female Gpbar1<sup>−/−</sup> mice fed HFD showed improved insulin sensitivity at 60, 90, and 120 min after insulin administration (Fig. 4B). These results are in agreement with the analysis of another independently generated Gpbar1 KO line, where the authors have reported on impaired glucose tolerance of Gpbar1<sup>−/−</sup> males, and improved glucose tolerance of transgenic mice overexpressing Gpbar1/TGR5, after 8 weeks on HFD (Thomas et al. 2009). Currently, the molecular mechanism behind the sex difference in the response to ITT is not clear. However, one can speculate that the fact that Gpbar1<sup>−/−</sup> males on HFD are slightly heavier than their WT littermate controls may be a contributing factor to the impaired insulin sensitivity. The improved insulin sensitivity of the Gpbar1<sup>−/−</sup> males on chow and of the Gpbar1<sup>−/−</sup> females on HFD needs further investigation.

At the end of the HFD study, both male and female Gpbar1<sup>−/−</sup> mice displayed liver steatosis. But Gpbar1<sup>−/−</sup> males showed a higher degree of liver lipidosis when compared with their WT littermates or the Gpbar1<sup>−/−</sup> females on HFD (Fig. 5). These results are in agreement with recent reports that treatment of diet-induced obese C57BL/6J mice with the GPBAR1/TGR5-selective agonist, INT-777, led to reduction of liver steatosis (Thomas et al. 2009). Expression profile of 11 genes involved in glucose homeostasis in pancreatic islets (Supplementary Figure 1A) and of 43 genes involved in the BA, cholesterol, fatty acid, and glucose homeostasis in the BAT, WAT, and livers of Gpbar1<sup>−/−</sup> and Gpbar1<sup>+/+</sup> mice on HFD did not reveal more than 2.5-fold changes between KO and WT control mice (Supplementary Figures 1B, C and 2).

Sexual dimorphism in HFD-induced obesity and metabolic alterations, and hepatic transport of taurocholate have been reported in mice and rats (Simon et al. 1999, Nishikawa et al. 2007, Hwang et al. 2010, Grove et al. 2010). In humans, key characteristics of obesity such as pattern of fat accumulation, energy homeostasis, and sensitivity of the brain to adiposity hormones, insulin and leptin, have been shown to be influenced by gender (Woods et al. 2003, Regitz-Zagrosek et al. 2007). It has been reported that male C57BL/6J mice on HFD gain more BW than the females, although the underlying mechanism of this sex differences remains unclear (Hwang et al. 2010). In this study, we used Gpbar1 KO mice on C57BL/6J genetic background and compared them to their WT littermates. In agreement with the published data, we observed that the males on HFD gained more weight than the females on HFD. But the fact that male Gpbar1<sup>−/−</sup> mice responded to HFD challenge differently than Gpbar1<sup>−/−</sup> females when compared with their WT littermates points to a gender-dependent regulation of Gpbar1 function. At this point, it is unclear why male and female Gpbar1<sup>−/−</sup> respond differently to HFD challenge. It is possible that the KO mice on HFD undergo sex-related hormonal changes that may contribute to the observed differences. It is important to note that GPBAR1 KO is a genetic deletion, and developmental effects may play a role.

In conclusion, our study extends the analysis of Gpbar1 KO mice on HFD and demonstrates that the deletion of GPBAR1 has differential effect in males and females. Recently, GPBAR1 has emerged as a promising new target in metabolic disease. Although the relevance of our data to human GPBAR1 biology is not yet established, our findings of a sexual dimorphism in GPBAR1 function may have important implications for designing of GPBAR1-based therapeutic strategies.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1677/JOE-10-0009.
Declaration of interest

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