Increased susceptibility to diet-induced obesity in GPRC6A receptor knockout mice

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Abstract

The recently identified G protein-coupled receptor GPRC6A is activated by dietary amino acids and expressed in multiple tissues. Although the receptor is hypothesised to exert biological impact on metabolic and endocrine-related parameters, the role of the receptor in obesity and metabolic complications is still elusive. In the present study, we investigated the impact of GPRC6A deficiency in a murine model of diet-induced obesity (DIO). Male Gprc6a knockout (KO) mice and WT littermates were subjected to a high-fat diet (HFD) for 25 weeks and exposed to comprehensive metabolic phenotyping. A significant increase in body weight, corresponding to a selective increase in body fat, was observed in Gprc6a KO mice exposed to an HFD relative to WT controls. The obese phenotype was linked to subtle perturbations in energy homoeostasis as GPRC6A deficiency resulted in chronic hyperphagia and decreased locomotor activity. Moreover, diet-induced obese Gprc6a KO mice had increased circulating insulin and leptin levels relative to WT animals, thereby demonstrating that endocrine abnormalities associate with the reported disturbances in energy balance. The phenotype was further accompanied by disruptions in glucose metabolism showing that Gprc6a KO mice on an HFD display increased susceptibility to develop metabolic-related disorders. Altogether, these data suggest that the amino acid sensing receptor GPRC6A plays an important role in resistance to DIO and metabolic complications. Future studies will illuminate the underlying molecular mechanisms mediating the herein reported findings and potentially facilitate the development of novel therapeutic compounds targeting the GPRC6A receptor.

Key Words

- GPRC6A
- Obesity
- High-fat diet
- knockout
- diabetes
- energy balance

Introduction

Obesity is characterised by a state of excessive chronic fat storage and is today considered to be one of the largest human health burdens on almost all parts of the globe (McCormick & Stone 2007). Although it is widely accepted that the cause of the obesity epidemic is a consequence of rapid changes in environment and lifestyle, it is far from...
clear why some individuals are more susceptible to an obesogenic environment than others. It is believed that a heritable component predisposes some individuals to become obese; however, the short time frame in which the epidemic has evolved strongly implies that genomic alterations are unlikely to be the primary causative factor (Ryan et al. 2012). Rather, the increased incidence of obesity plausibly reflects an interaction between genotype and exposure to an obesogenic environment (Guyenet & Schwartz 2012). Rodents exposed to diet-induced obesity (DIO) are generally accepted as a valid model to mimic human obesity. This claim is based on the fact that the DIO phenotype arises as a consequence of polygenic susceptibility and the exposure to a palatable and energy-dense diet (often high in fat and/or refined carbohydrates) (Ellacott et al. 2010). Additionally, genetically modified mice have become a central tool to explore how specific genes are involved in the regulation of energy homoeostasis (Tschöp et al. 2012). Thus, monogenic mouse models exposed to DIO are frequently used to parse the mechanisms by which genes with unmapped physiological roles impact the progression of a metabolic phenotype.

Cell surface-expressed G protein-coupled receptors (GPCRs) constitute a large family of proteins whose primary function is to transduce extracellular stimuli into intracellular signals. The biological studies of GPCRs is a growing research field, the ultimate importance of which is underscored by the fact that ~40% of all marketed drugs target GPCRs (Lundstrom 2005). Therefore, the de-orphanisation of GPCRs and the subsequent studies on their pharmacology and physiological implications is the focus of intense research efforts for both academia and industry (Kroeze et al. 2003, Chung et al. 2008). The GPRC6A receptor belongs to the small subfamily C of human GPCRs, which include eight metabotropic glutamate receptors, the calcium-sensing receptor, two γ-aminobutyric acid type B receptors, three TIR taste receptors and a subset of orphan GPCRs (Wellendorph et al. 2009a).

The most potent endogenous agonists of the GPRC6A receptor are the basic L-α-amino acids: L-arginine, L-lysine and L-ornithine (Wellendorph et al. 2005), and depending on the signalling pathway studied, the receptor can be positively modulated (Kuang et al. 2005, Christiansen et al. 2007) or directly activated by divalent cations (Pi et al. 2005). Noteworthy, recent research reports have suggested that the GPRC6A receptor can also be activated by testosterone (Pi et al. 2010a) and osteocalcin (Oury et al. 2011, Pi et al. 2011). Gene expression studies have identified GPRC6A receptor mRNA in multiple tissues and organs including adipose tissue, skeletal muscle, bone, pancreas, liver and brain (Wellendorph & Bräuner-Osborne 2004, Kuang et al. 2005, Wellendorph et al. 2007), proposing that GPRC6A serves to mediate intracellular information on extracellular amino acid availability in a wide range of tissues.

Recent characterisation studies using Gprc6a knockout (KO) mice have suggested that the receptor may be implicated in mild metabolic complications (Pi et al. 2010), bone turnover (Pi et al. 2008, 2010b) and male fertility (Oury et al. 2011). By contrast, phenotyping studies of our global Gprc6a KO model revealed that the receptor is not critical for bone homoeostasis, body composition or glucose metabolism under standard housing and feeding conditions (Wellendorph et al. 2009b, Smajilovic et al. 2012). The somewhat different metabolic phenotypes between the two Gprc6a KO models suggest that their sensitivity/resistance to develop metabolic disturbances is different, proposing that our mice might require a physiological stressor to ignite a metabolic phenotype.

In the present study, we have thus subjected Gprc6a KO mice to high-fat diet (HFD) for 25 weeks while performing detailed analyses of their metabolic phenotype. We carefully examined changes in body weight and body composition while assessing energy and glucose homoeostatic parameters. To evaluate potential endocrine alterations associated with GPRC6A deficiency and HFD feeding, we also analysed an array of plasma parameters. Lastly, mRNA levels of central hypothalamic markers known to integrate both short-term and long-term energy balance regulatory factors were assessed. This provides the first study to explore the role of GPRC6A in the adaptation to obesity and the resulting metabolic complications.

Materials and methods

Animals and study design

Gprc6a KO mice were generated as previously described (Wellendorph et al. 2009b) and backcrossed into C57BL/6 background. Age-matched animals of controlled genetic background were obtained from heterozygous mating and genotypes were verified as described previously (Wellendorph et al. 2009b). To study DIO, 8-week-old male mice were given ad libitum access to an HFD (58% kcal fat, 25.5% kcal carbohydrates and 16.4% kcal protein; 5.56 kcal/g; catalogue no. D12331; Research Diets, Brunswick, NJ, USA) for 25 weeks. The animals were initially housed three to five per cage (litters from...
heterozygous breeding) in a rodent facility on a 12 h light:12 h darkness cycle under controlled temperature and humidity. To determine individual oxygen consumption, locomotor activity and food intake, all mice were single housed after 8 weeks on the HFD and throughout the experiment. Animals were killed by cervical dislocation after 6 h of food removal in the light phase, and tissues were subsequently removed, weighed and snap frozen in liquid nitrogen. To explore leptin levels in chow-fed animals, plasma from another cohort of WT and Gprc6a KO mice was used (25-week-old mice, n=9–14). A total of 24 mice (12 of each genotype) were used for these experiments. All experimental work was conducted in accordance with institutional guidelines and approved by the Animal Experiments Inspectorate in Denmark (J. No. 2008/561-1567).

Body weight and body composition

Body weights were monitored weekly throughout the study. To gain insight into potential diet-genotype interactions on fat and lean mass in Gprc6a KO and WT mice, we analysed body composition prior to the dietary intervention and after 7, 17 and 25 weeks on the HFD by quantitative MRI using EchoMRI (Echo Medical Systems, Houston, TX, USA). Fat and lean mass percentages were calculated in relation to total body weight, which was measured prior to the MRI scan. Livers from Gprc6a KO and WT mice were also scanned for post-mortem fat content using the EchoMRI system.

Indirect calorimetry

Energy expenditure (EE) and locomotor activity were assessed using a 16-chamber indirect calorimetry system (PhenoMaster; TSE Systems, Bad Homburg, Germany) as previously described (Petersen et al. 2011). In brief, after 11 weeks on HFD Gprc6a KO and WT mice were acclimatised to calorimetry cages for 5 days prior to data sampling. Oxygen consumption (VO2; ml/kg per h) and locomotor activity (>2 beam breaks) were sampled continuously for 4 consecutive days on HFD followed by 24 h of data collection without access to the diet and a 48-h re-feeding (RF) period. Oxygen consumption data for the entire period was accumulated into 12-h blocks. The RF period is additionally depicted with a higher resolution (3 data points/h). Locomotor activity is shown as cumulated activity for the whole time in which the animals were housed in the metabolic cages.

Food intake

Sampling of food intake was performed during two 14-day periods after 9 and 20 weeks on HFD respectively. Food intake was registered in single housed mice by manually weighing food hopper contents 3 times/week. Visual examination of the cage bottom confirmed negligible spillage of food, and we observed no apparent differences between groups, proposing that spillage has minimal impact on the herein reported data.

Glucose metabolism studies

An oral glucose tolerance test (OGTT) was performed at 23 weeks of HFD administration. Blood glucose levels were measured in blood from tail vein puncture using a glucometer (Ascensia Contour Glucometer; Bayer) at basal, 15, 30, 60, 90 and 120 min after oral gavage (3 g/kg body weight) in 6-h fasted mice (Andrikopoulos et al. 2008). OGTT area under the curve (AUC) was calculated from the individual glucose excursion curves. Glucose-induced insulin secretion was assessed after 15 weeks on the HFD. Plasma insulin levels were measured in 6-h fasted mice before and 20 min after an oral glucose load of 2 g/kg body weight. The basal blood glucose was measured at the end of the dietary intervention (25 weeks on HFD) after 6 h fasting and the homoeostasis model assessment of insulin resistance (HOMA-IR) index was calculated based on the conventional formula: HOMA-IR=(basal glucose (mmol/l)×basal insulin (mU/l))/22.5.

Plasma parameters

At the end of the study, blood was drawn from the orbital sinus of 6-h fasted mice into EDTA-coated tubes (BD Biosciences, Franklin Lakes, NJ, USA) and plasma was isolated by centrifugation and stored at −80 °C until further analysis. Insulin was measured using Ultra Sensitive Mouse Insulin ELISA Kit (Crystal Chem, Inc., Downers Grove, IL, USA). Leptin and adiponectin were measured using ELISA kits from Millipore (Billerica, MA, USA). Triglycerides were measured using Serum Triglyceride Determination Kit (Sigma–Aldrich). Cholesterol was measured using LabAssay Cholesterol Kit and free fatty acids were measured using NEFA-2HR Kit (Wako Chemicals, Neuss, Germany).

RT-quantitative PCR (qPCR)

Expression of key energy balance regulators was measured at study termination. RNA from whole hypothalamus was
extracted with RNasey lipid tissue mini kit (Qiagen), and cDNA was synthesised by RT using the ImProm-IITM reverse transcriptase (Promega). Relative mRNA levels were measured by the qPCR method using the Mx3000P from Stratagene (La Jolla, CA, USA) and SYBRPremix Ex Taq (Takara, Otsu, Japan). The relative levels of genes from different samples were compared by the ΔΔCt method using the tyrosine3-monoxygenase/tryptophan 5-monoxygenase activation protein, 𝝁 polypeptide (Ywhaz) as reference gene (Clemmensen et al. 2012). Before the ΔΔCt value was calculated, primer efficiency was validated by standard curve measurements, and primers with 95% efficiency were used. A calibrator sample was included in each assay for normalisation between runs. Primer sequences used were agouti-related peptide (Agrp), 5'-GCTGCAAGGCGAGACG-3' and 5'-GACTCTGTGGACCATACA-3'; neuropeptide Y (Npy), 5'-TTGGACTGACCCCTCTAT-3' and 5'-TGCTCATGGGCTGAATCT-3'; pro-opiomelanocortin (Pomc), 5'-AGAGAGCTGCTTCTCGGAC-3' and 5'-GCAGAGGCCGAACAGG-3'; leptin receptor b (Leprb), 5'-CTTTGTACCTTACATGCCC-3' and 5'-AGTCACAATCCATTCCTCAAT-3'; and 5'-GAAGCATT-GGGATCAAGAA-3'.

Statistical analyses

All statistical analyses and graphical presentations were performed using GraphPad Prism 5.0c (GraphPad Software, San Diego, CA, USA). Unpaired two-tailed Student’s t-test, repeated measures ANOVA or ANOVA followed by Bonferroni’s post hoc multiple comparisons were used to analyse the data. Data are presented as means ± S.E.M. and significance level is set at P < 0.05 for all analyses.

Results

Body weight and body composition

To assess the role of GPRC6A in DIO, lean Gprc6a KO and WT littermate controls were fed an HFD for 25 weeks. At the entry of study, no genotype difference in body weight was observed between the 7-week-old mice (27.5 ± 0.5 vs 28.0 ± 0.4 for WT and Gprc6a KO respectively). After 19 weeks on HFD, GPRC6A-deficient mice weighed significantly more than WT mice and at 25 weeks of HFD administration the difference was even more pronounced (43.2 ± 1.2 vs 37.6 ± 1.4; P = 0.008; Fig. 1A).

Figure 1

Increased susceptibility to DIO in Gprc6a KO mice. (A) Body weight curve for WT (+/+; open circles) and Gprc6a KO mice (−/−; solid circles) during 25 weeks of HFD feeding. (B) Body fat percentage was assessed prior to HFD feeding and after 7, 17 and 25 weeks of DIO in WT (open circles) and Gprc6a KO mice (solid circles). (C) Total lean mass percentage (% Lean mass) and liver fat content were measured with an MRI scanner. Data are presented as mean ± S.E.M. (n = 10–12). *P < 0.05, **P < 0.01.

Moreover, body composition analysis measured by MRI scans demonstrated a significantly increased body fat percentage (27.6 ± 1.5 vs 21.4 ± 1.7%; P < 0.01; Fig. 1B) and lowered lean mass percentage (69.7 ± 1.4 vs 75.7 ± 1.6%, P = 0.011; Fig. 1D) in Gprc6a KO mice compared with WT.
mice after 25 of DIO. Evidently, the obese phenotype did not manifest until towards the end of the dietary intervention as body fat percentage between genotypes reached statistical significance after 25 weeks on the diet (Fig. 1B). After study termination, subcutaneous, retroperitoneal, mesenteric and epididymal fat pads were dissected and weighed (Fig. 1C). A tendency of increased subcutaneous fat in Gprc6a KO mice was observed \((P=0.079)\) whereas retroperitoneal and mesenteric fat pad compartments were significantly heavier \((P=0.011\) and \(P=0.015\) respectively) in Gprc6a KO mice compared with WT littermates. Epididymal fat pads were 56% increased in KO animals compared with WT \((P=0.009)\). Liver weights were significantly higher in the Gprc6a KO mice \((P=0.005\); Fig. 1E) and post-mortem MRI scans further revealed a twofold increase in liver fat content when compared with WT littermates \((P=0.018;\) Fig. 1F). In summary, Gprc6a KO mice display increased sensitivity to an HFD when compared with WT littermates, resulting in a clear obese phenotype.

**Food intake**

Even a small increase in energy intake and/or decrease in EE can, if chronically maintained, result in the development of obesity. To determine whether increase in food intake and/or altered EE was the main determinant of the herein observed obese phenotype, associated with GPRC6A deficiency, we sampled food intake in single housed animals. Diet consumption was carefully sampled three times/week for 14 days after 9 and 20 weeks on the HFD respectively (Fig. 2A and B) and revealed a significant

![Figure 2](http://joe.endocrinology-journals.org/C209/2013/Society%20for%20Endocrinology/DOI:10.1530/JOE-12-0550/Printed%20in%20Great%20Britain)

Assessment of energy balance regulation in Gprc6a KO mice fed an HFD. Cumulative food intake was measured over 2-week time intervals at (A) 9–11 weeks and (B) 20–22 weeks on HFD respectively. (C) \(\text{VO}_2\) (ml/h per kg) and (D) locomotor activity were sampled for 7 consecutive days for WT (white) and Gprc6a KO (black). (E) Gprc6a KO mice displayed lower oxygen consumption during the RF period (tendency; \(P=0.079\)) and significantly less locomotor activity \((P<0.001)\). Data are presented as mean \(\pm\) S.E.M. \((n=10–12)\). \(*P<0.05, **P<0.01, f, fasting; RF, re-feeding.\)
Altered insulin and leptin levels in HFD-fed Gprc6a KO mice. Levels of (A) insulin and (B) leptin were measured in chow-fed and in HFD-administered mice (25 weeks on diet) in the basal state (6 h of food removal). Data are presented as mean ± S.E.M. (n = 10–12). **P<0.01.
administration but found significantly increased levels in GPRC6A-deficient mice at the end of the study ($P<0.01$; Fig. 3A). Glucose-induced insulin secretion was assessed after 15 weeks on the HFD. Plasma insulin levels, measured in 6-h fasted mice before and 20 min after an oral glucose load of 2 g/kg body weight showed no difference in oral glucose-mediated insulin secretion (Fig. 5E). Noteworthy, fasting insulin levels after 15 weeks on the HFD were similar between genotypes. Evaluation of insulin sensitivity was accomplished by using the empirical HOMA-IR method. The method is based on fasting glucose and insulin levels and was previously validated as a reliable assessment model for insulin sensitivity in rodents (Lee et al. 2008). We found that Gprc6a KO mice on an HFD had a higher HOMA-IR score than control mice exposed to the same experimental regimen ($P<0.019$; Fig. 5D) indicating that GPRC6A deficiency associates with peripheral insulin resistance.

**Discussion**

To uncover the role of the GPRC6A receptor in obesity and its associated metabolic complications the current study explores, for the first time, the impact of GPRC6A deficiency in a mouse model of DIO. Here, we show that ablation of the GPRC6A receptor predisposes mice to develop obesity when exposed to an obesogenic environment (standard housing and ad libitum access to a HFD). The obese phenotype was associated with impairments in glucose metabolism, subtle perturbations in energy balance regulation, hepatic steatosis and increased plasma levels of insulin and leptin. We propose that the herein reported DIO phenotype linked to GPRC6A deficiency is a consequence of small – but chronic – differences in energy balance.

In contrast to the metabolic phenotype published by Pi et al. (2008) our global Gprc6a KO mice do not suffer from body weight, body composition or metabolic abnormalities under standard laboratory conditions (rodent chow diet) (Wellendorph et al. 2009b, Smajilovic et al. 2012). Here, we demonstrate that GPRC6A deficiency is linked to a delayed-onset increase in sensitivity to DIO when KO animals are compared with WT littermates. Thus, after 25 weeks of HFD feeding the Gprc6a KO mice...
display significantly higher body weight and alterations in body composition (increased fat mass) compared with WT animals. Moreover, dissection analysis revealed that visceral compartments exhibit a marked increase in fat accumulation evidenced by a 56% increase in epididymal fat in Gprc6a KO mice. These observations suggest that the increased sensitivity to DIO, coupled to GPRC6A deficiency, is a consequence of a diet-genotype interaction, hence metabolic characterisation studies using chow-fed Gprc6a KO and WT mice failed to identify a phenotype (Wellendorph et al. 2009b, Smajilovic et al. 2012).

However, we cannot rule out the possibility that the phenotypic traits arise from multiple factors (e.g. age, stress and diet) interacting with the genotype. Yet, the delayed-onset obesity linked to GPRC6A deficiency is unlikely to be exclusively associated with stress from handling or ageing, as we previously did not detect any body weight differences in 38-week-old animals on a regular chow diet (Wellendorph et al. 2009b).

Here, we identified that DIO Gprc6a KO mice suffer from subtle energy homeostatic perturbations. The KO mice were hyperphagic, less active and exhibited a trend towards dysfunctional adaptation in oxygen consumption when the HFD was re-introduced after a period of fasting. Interestingly, Gprc6a KO animals displayed elevated plasma insulin and leptin levels, but exhibited no differences in plasma lipid markers or adiponectin levels. To investigate if the elevated circulating levels of leptin in DIO Gprc6a KO mice were associated with parallel changes in hypothalamic gene expression, we examined the expression of neuropeptides involved in feeding behaviour (Schwartz et al. 2000). We identified increased Pomc mRNA expression in the hypothalami from Gprc6a KO mice, indicating that GPRC6A deficiency and HFD feeding associate with transcription changes in the hypothalamus. Generally, Pomc mRNA levels are increased by central leptin and insulin (Schwartz et al. 1997, Benoit et al. 2002), which in turn serves to reduce feeding and increase EE. Further, leptin promotes the transcription of Socs3, which subsequently provides negative feedback to terminate Leprb downstream signalling (Bjorbaek et al. 1998, Bjorbaek et al. 2000). However, the expression of Socs3 and Leprb were unaltered in KO relative to WT mice in our study. Collectively, the increased Pomc mRNA expression associated with GPRC6A deficiency suggests that leptin amply induces the anorexigenic precursor, but also that the KO animals may be resistant to the effects of POMC products, hence the excessive eating and unaltered negative feedback response. Nevertheless, this discussion is speculative, as mRNA levels for a small selected group of neuropeptides examined at study termination do not provide a definitive readout of leptin sensitivity (Myers et al. 2010). Thus, whether GPRC6A plays a direct role in how peripheral anabolic signals impact hypothalamic energy balance regulation remains elusive and additional studies are required to untangle if these changes play a direct role in the progression of the obese phenotype or whether they are compensatory responses to the differences in adiposity.

Previously, we hypothesised a role for GPRC6A in L-arginine-induced insulin release but failed to detect altered L-arginine-induced insulin secretion in GPRC6A-deficient mice exposed to standard housing and feeding conditions. Further, glucose tolerance and insulin sensitivity experiments revealed that the GPRC6A receptor does not play a key role in glucose homeostasis in chow-fed animals (Smajilovic et al. 2012). Another research group has reported mildly impaired glucose metabolism in their global Gprc6a KO mouse model when administered a chow diet (Pi et al. 2008). These somewhat contradictory observations indicate a difference in sensitivity/resistance to develop metabolic disturbances between the two reported Gprc6a KO models. Noteworthy, Pi et al. (2008) reported their chow-fed 16-week-old male mice to exhibit 25–30% body fat. By contrast, we observe only 6% body fat in 16-week-old male mice (independent of genotype) (Smajilovic et al. 2012). Thus, the initially reported perturbations in glucose metabolism associated with GPRC6A deficiency may potentially be secondary to the obese phenotype (Pi et al. 2008). Nevertheless, whether these reported differences in adiposity and glucose homeostasis reflect variations in experimental and/or environmental settings, or whether they relate to differences in the engineering of the Gprc6a KO mouse models remains to be determined. However, it has become increasingly accepted that disruption of standard physiological conditions occasionally is required for a subtle phenotype to emerge (Ellacott et al. 2010, Tschöp et al. 2012). Thus, as we previously failed to identify differences in glucose- and energy metabolism between chow-fed Gprc6a KO and WT mice we decided to introduce a dietary stressor in the present study. The HFD proved sufficient to increase obesity in Gprc6a KO relative to WT mice, signifying that these animals are predisposed to develop metabolic complications. Therefore, despite differences in experimental regimes and mouse KO models with putative differences in sensitivity to metabolic complications, a growing consensus that the GPRC6A receptor plays a role in whole body metabolism is thus evolving.
In the present study we detected an array of metabolic complications associated with GPRC6A receptor KO and HFD feeding. At the end of the HFD intervention, i.e. the obese phenotype had already emerged; GPRC6A-deficient mice demonstrated increased basal plasma glucose levels, impaired oral glucose tolerance and insulin resistance, emphasising that glucose homeostatic disruptions had surfaced. However, as we did not observe any differences in basal insulin levels or glucose-induced insulin secretion after 15 weeks of HFD administration the impaired glucose metabolism is likely to be secondary to the increased adiposity linked to GPRC6A deficiency and HFD feeding. In support of this explanation, our previous metabolic characterisation study of GPRC6A chow-fed animals failed to detect any metabolic genotype abnormalities (Smajilovic et al. 2012), implying that disruption in glucose metabolism is not a driving factor of the metabolic phenotype. Noteworthy, the decreased locomotion and the inability to adjust in energy usage following a food stressor (fasting and RF) may be independent of the hyperphagia-induced obese phenotype associated with ablation of GPRC6A. Further, given that eating and locomotion are both rewarding and evoke hedonic responses (Garland et al. 2011), it suggests that GPRC6A-related actions in the mesolimbic system may play a role for the herein reported findings. Although increasing evidence indicates that energy homeostatic disturbances are linked to interrelated changes in reward pathways (Adam & Epel 2007) this remains highly speculative and merely serves as an alternative hypothesis left for future studies to uncover. Thus, exploring these ideas (e.g. by employing behavioural experiments and/or HFD pair-feeding regimes) is urgently needed to delineate how disruption of GPRC6A signalling is linked to the development of obesity and the associated metabolic disturbances.

In summary, we have shown that genetic disruption of the GPRC6A receptor accelerates the development of obesity and associated complications when animals are exposed to an obesogenic environment. Our previous characterisation studies, on chow-fed animals, failed to detect any abnormalities associated with GPRC6A deficiency and thus support that the dietary stressor is responsible for the progression of the herein identified phenotype. Though the mechanisms by which GPRC6A exerts its metabolic actions remain to be determined, our findings add the receptor to a growing list ofGPCRs reported to affect energy balance. The therapeutic potential of targeting GPRC6A is obvious but definitely not trivial, as key questions on GPRC6A biology are left unanswered.

Because GPRC6A is expressed in many tissues, the DIO phenotype potentially arises as a consequence of the receptor being unable to provide intracellular information on circulating amino acids in several of these. Future studies are highly warranted to illuminate the underlying cellular and molecular mechanisms mediating the herein reported findings as they hold the potential to facilitate the development of novel therapeutic compounds targeting the GPRC6A receptor.


Received in final form 5 February 2013
Accepted 21 February 2013
Accepted Preprint published online 21 February 2013