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Up-regulated insulin secretion in insulin resistance mice:

Evidence of increased islet GLP-1 receptor expression and GPR119-activated

GLP-1 secretion

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Abstract

We previously demonstrated that the incretin effect and the β-cell responsiveness to glucagon-like peptide-1 (GLP-1) are increased in insulin resistant mice. Now we examined whether this could, first, be explained by increased islet GLP-1 receptor (GLP-1R) expression and, second, be leveraged by GPR119 activation which stimulates GLP-1 secretion. Female C57BL/6J mice, fed a control (CD, 10% fat) or high-fat (HFD, 60% fat) diet for eight weeks, were anesthetized and orally given a GPR119 receptor agonist (GSK706A; 10 mg/kg) or vehicle followed after 10 min with a mixed meal (0.285 kcal). Blood was sampled for determination of glucose, insulin, intact GLP-1 and glucagon, and islets were isolated for studies on insulin and glucagon secretion and GLP-1R expression. In HFD vs. CD mice, GSK706A augmented the meal-induced increase in both AUC_{GLP-1} (81±9.6 vs. 37±6.9 pM x min, P=0.002) and AUC_{INS} (253±29 vs.112±19 nM x min, P<0.001). Also, glucagon levels were significantly increased by GSK706A (P<0.01 for both diet groups). GSK706A had no direct effects on islet hormone secretion in vitro. Glucose elimination after meal ingestion in HFD mice was increased by GSK706A (0.57±0.04 vs. 0.43±0.03 % per min, P=0.014). Islet GLP-1R expression was higher in HFD vs. CD mice (0.8±0.1 vs. 0.5±0.1, P=0.035). In conclusion, insulin resistant mice display increased islet GLP1R expression and augmented meal-induced GLP-1 and insulin responses to GPR119 activation with an increased glucose elimination. However, the concurrent increase in glucagon may decrease the antihyperglycemic response by the insulinotropic effects of GLP-1 and GPR119 agonism, resulting in only marginally improved glucose elimination.

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Introduction

In fully compensated insulin resistance, there is a sufficient up-regulation of insulin secretion whereas in glucose intolerance and type 2 diabetes this up-regulation is inadequate (Kahn, et al. 1993).Several mechanisms have been suggested to contribute to the up-regulated beta-cell function in insulin resistance, such as signals generated from nutrient metabolism, hormones and cytokines (Ahren and Pacini 2005). We previously suggested that the incretin hormones may also contribute by demonstrating in model experiments in insulin resistant mice that the incretin effect, i.e., the augmented insulin secretion seen after oral versus i.v. glucose, is increased (Ahren, et al. 2008), and that the β-cell responsiveness to intravenous glucagon-like peptide-1 (GLP-1) is augmented (Ahren et al. 2008; Simonsson and Ahren 1998).

The increased incretin effect in insulin resistance raises the question of whether GLP-1 secretion from the intestine is increased, and thus, may be therapeutically leveraged. One approach to test this hypothesis would be to activate G-protein coupled receptor 119 (GPR119). This receptor is expressed in gut enteroendocrine cells (Drucker, et al. 1994), whereby activation has been shown to result in increased release of GLP-1 (Chu, et al. 2007; Lauffer, et al. 2009). GPR119 expression is also localized to islet β-cells (Chu et al. 2007), and consequently, GPR119 activation may regulate glycemia both directly by activating islet β-cell insulin secretion (Soga, et al. 2005) and indirectly through release of intestinal incretin hormones.

In this study we explored, first, whether the increased beta-cell responsiveness to GLP-1 in insulin resistant mice is associated with increased islet GLP-1R expression. We then examined the potential to improve islet function and glucose tolerance in glucose-intolerant insulin resistant mice through GPR119 activation by administering a specific GPR119 receptor agonist. Since the effects of GPR119 activation on islet α-cells are still unknown, also glucagon release was investigated in view of the importance of glucagon for glycemic dysregulation and reduction of glucagon in therapy of type 2 diabetes (Henquin, et al. 2011). For this purpose, we used the high-fat fed insulin resistant mice, which is a well established model for glucose intolerance (Winzell and Ahren 2004), and also the recently developed stimulation of islet hormone secretion by mixed meal gavage in mice (Ahlkvist, et al. 2012).

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Material and methods

GPR119 receptor agonist

In this study we used a novel small molecule GPR119 receptor agonist (GSK2041706A, 2-[((1S)-1-{1-[3-(1-methylethyl)-1,2,4-oxadiazol-5-yl]-4-piperidinyl}ethyl)oxy]-5-[4-(methylsulfonyl)phenyl]pyrazine), (GlaxoSmithKline, Research Triangle Park, Durham, USA) (Tang et al. 2008). GSK706A is >100-fold selective for GPR119 receptor over a variety of other receptors, ion channels and enzymes, and possesses an EC_{50} = 4 nM against the human GPR119 receptor. In the acute in vivo studies, the compound was formulated in a vehicle consisting of 0.5% methocel K15M premium EP hydroxypropyl methylcellulose (HPMC; Dow Chemical Midland, MI, USA) and 0.1% Tween 80 (Fluka/Sigma-Aldrich, St. Louis, USA) in water. For the in vitro experiments, the compound was dissolved in 1% DMSO (Sigma-Aldrich, St. Louis, USA).

Animals

Female C57BL/6J mice (average 22 g) were obtained from Taconic (Skensved, Denmark). After one week of acclimatisation, the mice were divided into two groups and fed either a control diet (10% fat by energy; D12450B Research Diets, New Brunswick, NJ, USA) or a high-fat diet (60% fat by energy; D12492, Research Diets) for 8 weeks. Body weight and food intake was monitored once a week. Food and water was provided *ad libitum*. The animals were housed in groups of eight per cage in a temperature-controlled (22°C) room with artificial lighting maintained on a 12h light/12h dark cycle. All experimental procedures were performed in agreement with the Animal Ethics Committee in Lund, Sweden.

GPR119 activation in vivo

Fasted (5h) mice were anesthetized with an intraperitoneal injection of midazolam (0.4 mg/mouse, Dormicum®, Hoffman-La Roche, Basel, Switzerland) and a combination of fluanisone (0.9 mg/mouse) and fentanyl (0.02 mg/mouse, Hypnorm®, Janssen, Beerse, Belgium). The mice were orally gavaged (0.25 ml) with vehicle or GSK706A (10mg/kg) 10 minutes before a new gavage with the previously described mixed

meal (Ahlkvist et al. 2012). This consisted of a mixture of glucose (60% kcal, Sigma), whey protein (20% kcal, SELF Omninutrition) and peanut oil (20% kcal, Zeta), with total caloric content of 0.285 kcal. Blood samples were collected into heparinized tubes from the retrobulbar, intraorbital, capillary plexus before and 15, 30, and 60 minutes after oral gavage for plasma glucose and insulin determination. For glucagon and intact GLP-1 measurements, blood samples were collected before and 5, 10 and 20 minutes after oral challenge into tubes containing the protease inhibitor aprotinin (Trasylol; 500 KIE/ml Bayer, Leverkusen, Germany) or a combination of aprotinin and the dipeptidyl peptidase-4 inhibitor valine pyrrolidide (0.03 mM, Novartis, Basel, Switzerland), respectively. After collection, all blood samples were immediately centrifuged (4°C) and plasma was stored (-20°C) for subsequent analysis.

GPR119 activation in vitro

Mouse islets were isolated from the pancreata of normal and high-fat diet fed mice by collagenase digestion and handpicked under the microscope. Batches of freshly isolated islets were preincubated in HEPES balanced salt solution containing 125 mmol/l NaCl, 5.9 mmol/l KCl, 1.28 mmol/l CaCl2, 1.2 mmol/l MgCl2, 25 mmol/l HEPES (pH 7.4), 5.6 mmol/l glucose and 0.1% fatty acid free BSA (Boehringer Mannheim, Mannheim, Germany) at 37°C during 60 min. Thereafter, islets in groups of three were incubated in 200 µl of the above described buffer with varying concentrations of glucose. In the first experiment, the direct effect of GSK706A on insulin secretion was determined by incubating isolated islets from mice fed the control and high-fat diet in different concentrations of glucose (2.8, 5.6, 8.3, 11.1 and 16.7 mmol/l), with or without addition of GSK706A (1 nmol/l). In a second experiment, the effect of non-glucose stimuli on GPR119 activation was determined by incubating isolated islets from mice fed the control and high-fat diet in different concentrations of glucose (2.8, 11.1 mmol/l) with or without arginine (10 mmol/l) and GSK706A (1 nmol/l). The islets were incubated for 60 min at 37°C, where after aliquots of 25 or 50 µl buffer in duplicate were collected and stored at -20°C until analysis of insulin and glucagon, respectively.

Islet GLP-1R expression

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The protein expression levels of the GLP-1R in isolated islets of control and high-fat diet fed mice were analyzed by Western blot. The islets were homogenized in a buffer containing 150 mmol/l NaCl, 2 mmol/l EDTA, 20 mmol/l Tris–HCl pH 7,5, 1% Triton X-100 and 0.2% protease inhibitor cocktail (Sigma-Aldrich, St. Louis, USA). The total amount of proteins in each sample was measured using a BCA Protein assay reagent kit (Pierce, Rockford, USA). Aliquots of tissue homogenates containing equal amounts of protein (30 μg) were separated on SDS-PAGE, and blotted onto nitrocellulose membranes (BioRad, Hercules, USA). The membranes were probed with primary antibodies against the GLP-1R (Abcam, Cambridge, UK) and actin (Abcam, Santa Cruz, USA). The secondary antibody was a horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Amersham Pharmacia Biotech, Uppsala, Sweden). The blots were developed by enhanced chemiluminescene (SuperSignal West Pico, Pierce, Rockford, USA) and the proteins were detected and quantified using a ChemiDoc XRS+ system with image lab software (BioRad, Hercules, USA).

Plasma analysis

Plasma glucose was measured with the glucose oxidase method using 2,2´-azino-bis(3-ethyl-benzothialozine-6-sulphonate) as a substrate with the absorbance measured at 420 nm on a microtiter plate reader (Fluostar/Polarstar Galaxy; BMG Labtechnologies; Offenburg; Germany). Insulin was analyzed with sandwich immunoassay technique (ELISA) using double monoclonal antibodies against insulin (Mercodia, Uppsala, Sweden). Glucagon was measured by radioimmunoassay (Millipore, Billerica, USA). Levels of intact GLP-1 were determined by sandwich immunoassay technique (ELISA) using monoclonal antibodies specific for the active form of GLP-1, whereby electrochemiluminescent labelling enables detection of voltage-mediated chemiluminescence in the SECTOR Imager plate reader (Meso Scale Discovery, Gaithersburg, USA).

Calculations and statistical analysis

Data are reported as mean values ± SEM. Statistical significances were assessed by using Student´s t-test. Suprabasal (sAUC) areas under the curve were calculated by the trapezoidal rule for glucose and insulin data during time interval 0-60 min and for glucagon and intact GLP-1 during 0-20 min. The glucose

elimination constant (K_G) was determined as the glucose elimination rate between 15 and 60 minutes after oral challenge. Beta-cell function (0-60 min) was determined as the ratio between AUC insulin to AUC glucose.

Results

Body weight and glucose tolerance in control diet and high-fat diet fed mice

Compared to the control diet, feeding C57BL/6J mice with a high-fat diet for 8 weeks resulted in an insulin resistant phenotype, characterized with higher weight gain (11.1±0.5 versus 2.0±0.1 g, P<0.001) and elevated fasting glucose (11.0±0.2 versus 9.5±0.3 mmol/l, P=0.0014) and insulin (322±23 versus 179±11 pmol/l, P<0.001) levels in plasma (Table 1). Furthermore, we observed impaired glucose tolerance after mixed meal challenge in the high-fat compared to control diet fed mice, as indicated by higher sAUC glucose over 60 min (347±33 versus 233±22 mmol/l x min, P=0.007) and reduced K_G (0.43±0.03 versus 0.65±0.07, P=0.009) (Table 1). Basal plasma glucagon levels did not differ between control and insulin resistant mice (24.0±1.0 versus 25.9±1.3 pg/ml, NS (Table 1).

Acute effect of GPR119 activation in control diet and high-fat diet fed mice

To determine changes in intestinal GLP-1 secretion in insulin resistance, a GPR119 agonist (GSK706A, 10mg/kg) or vehicle was given orally before (-10 min) an oral liquid test meal to control and high-fat diet fed mice. In control diet fed mice, no significant difference in plasma glucose levels after meal ingestion was observed between gavage of vehicle versus GPR119 agonist (Figure 1A). There was a trend for increased insulin secretion after activation of GPR119, although not significant (Figure 1B and Table 1). Furthermore, levels of intact GLP-1 increased after GPR119 agonism compared to vehicle (Figure 1C), however significantly only after 20 min of oral meal test (0.7±0.2 versus 0.0±0.0 pg/ml, P=0.0016), and associated with a significant improvement in β-cell function compared to vehicle (513±67 versus 315±60 pg/ml x min, P=0.037) (Table 1). In insulin resistant mice, the activation of GPR119 markedly increased insulin secretion compared to vehicle (15 min: 7.8±0.6 vs. 3.3±0.3 nmol/l, P=0.00002; 30 min: 6.4±0.9 vs. 2.7±0.4 nmol/l, P=0.003) (Figure 1F), also seen with 60 min AUC data (101±13 versus 253±29 nmol/l x

min, P=0.0004) (Table 1). The insulinotrophic effect of GPR119 activation in insulin resistance was accompanied by increased levels of intact GLP-1 in plasma (5 min: 8.6±1.2 vs. 3.7±0.9 pg/ml, P=0.004; 20 min: 1.2±0.2 vs. 0.6±0.2 pg/ml, P=0.05) (Figure 1G), also seen with 20 min AUC data (34.6±4.8 versus 80.6±9.6 pg/ml x min, P= 0.0011) (Table 1). We also observed a diet-induced difference in plasma GLP-1 levels after mixed meal, with higher 20 min sAUC levels in high-fat compared to control diet feeding (34.6±4.8 versus 20.1±3.8 pg/ml x min, P=0.03) (Table 1). The glucose elimination rate was significantly higher after GPR119 activation compared to vehicle (0.57±0.04 vs. 0.43±0.03 % per min, P=0.014), although not sufficient to reduce glucose levels or AUC glucose (Figure 1E). Beta-cell function was significantly improved after GPR119 activation in HFD mice compared to vehicle (60 min $sAUC_{INS}/sAUC_{GLU}$: 760±108 versus 304±40 pg/ml x min, P=0.002) (Table 1). Concerning glucagon levels, GPR119 activation increased glucagon levels (Figure 1D and H) in both control diet (20 min sAUC: 317±69 versus 650±59 pg/ml x min, P=0.0017) and insulin resistant mice (329±52 versus 608±50 pg/ml x min, P=0.0012), compared to vehicle (Table 1).

GPR119 activation in control and high-fat diet fed mouse islets

To elucidate if GPR119 activation had any direct effect on the pancreatic islets, isolated islets from control and high-fat diet fed C57BL/6J mice were incubated with the GSK706A compound. Firstly, islets from high-fat diet fed mice had generally a higher insulin secretion at low (2.8 mmol/l), moderate (8.3 mmol/l) and at high glucose (16.7 mmol/l) compared to control diet (232±102 versus 97±44 pg/ml/islet, NS), (665±231 versus 275±90 pg/ml/islet, P=0.004) and (3355±305 versus 1731±369 pg/ml/islet, P<0.001), respectively. By incubating islets from control diet and high-fat diet fed mice with GSK706A (1 nmol/l, 100 nmol/l or 1 µmol/l) we did not observe a significant increase in insulin release at 2.8, 5.6, 8.7, 11.1 or 16.7 mmol/l glucose (data not shown). In a second experiment, the islets were incubated with arginine (10 mmol/l), a potent stimulator of both islet insulin (Weinhaus, et al. 1997) and glucagon (Franklin, et al. 2005) secretion, which induced a robust increase in insulin secretion at 11.1 mmol/l glucose (P<0.001) in both control diet and high-fat diet fed mouse islets (Fig. 2A and C). Again, no significant effect on insulin secretion was seen after GPR119 activation. Concerning glucagon secretion, GPR119 activation did not result in any significant increase in glucagon, whereas arginine induced a robust glucagon response in

islets from both control diet and high-fat diet fed mouse islets compared to vehicle at 2.8 (P<0.001 and P=0.011, respectively) and 11.1 mmol/l glucose (P<0.001 and P=0.006, respectively) (Fig. 2B and D).

GLP-1R expression in control and high-fat diet fed mouse islets

To determine the mechanism behind increased beta-cell responsiveness to GLP-1 in insulin resistance mice, diet-induced changes in islet GLP-1R expression was analyzed with Western blot. The ratio of GLP-1 receptor to actin expression in pancreatic islets was significantly higher in high-fat diet versus control diet fed mice (0.8±0.1 vs. 0.5±0.1, P<0.05) (Fig. 3).

Discussion

Insulin resistance leads to a compensatory increase of insulin secretion, which enables sufficient control of blood glucose levels (Roder, et al. 2000). If this adaptation fails the progressive deterioration of glucose tolerance and type 2 diabetes ensues. In this study we explored the role of the GLP-1 system in the compensatory up-regulation of insulin secretion during conditions of insulin resistance in mice and examined the influence of GPR119 activation, which during recent years have been explored as a potential target for the treatment of type 2 diabetes (Overton, et al. 2008). We used the well-characterized high-fat diet fed mouse model which is associated with increased body weight, hyperinsulinemia and glucose intolerance (Winzell and Ahren 2004). Our main findings are 1) that GLP-1R expression is increased in pancreatic islets during insulin resistance, and 2) that there is an increased GLP-1 secretion from the intestine after both high-fat dieting and GPR119 activation. Thus, we suggest that the GLP-1 system is up-regulated during insulin resistance, through increased responsiveness of islet β-cells to GLP-1 and intestinal L-cells to appropriate stimuli, facilitating the compensatory increase in insulin secretion during insulin resistance and this may be leveraged by GPR119 activation. We also, for the first time, demonstrate that GPR119 activation increases glucagon secretion in mice.

The GLP-1 receptor has been localized in several peripheral tissues (Baggio and Drucker 2007) and within islets the GLP-1R has been detected mainly in β-cells (Moens, et al. 1996; Tornehave, et al. 2008), although expression has also been reported in α- and δ-cells (Heller, et al. 1997). The importance of the

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GLP-1R for glucose tolerance has been demonstrated in knockout mice (Scrocchi, et al. 1998), which display an abnormal glucose response to oral glucose challenge in association with reduced insulin secretion, and is the bases for the success of incretin therapy in type 2 diabetes (Deacon, et al. 2012). In insulin resistant mice, we have previously demonstrated that the β-cell response to intravenous glucagon-like peptide-1 (GLP-1) is improved compared to control animals as a sign of increased islet responsiveness to the incretin hormone (Ahren et al. 2008; Simonsson and Ahren 1998). We here provide mechanistic bases for this effect showing that the increased target cell responsiveness to the incretin hormones is associated with increased GLP-1R expression. In line with current expression data, increased expression levels of GLP-1R mRNA and protein have also recently been found in visceral fat of insulin resistant individuals (Vendrell, et al. 2011), confirming an increase of the GLP-1R during insulin resistance also in other metabolically important tissues. Previous studies in humans report that individuals with an impaired glucose tolerance (IGT) were found to have an impaired insulinotropic effect of GLP-1 (Fritsche, et al. 2000). Also in type 2 diabetics, incretin action has been shown to be impaired in a manner caused primarily by defects in β-cell responsiveness (Holst, et al. 2011b). Hence, the ability to increase the level of GLP-1R expression in pancreatic islets during early insulin resistance may be important to facilitate the compensatory increase in β-cell insulin secretion, maintaining normal glucose tolerance.

Studies on incretin secretion in individuals with various degrees of glucose tolerance have reported conflicting results. Secretion of GLP-1 was found to be impaired in obese subjects with both NGT (Carr, et al. 2010) and IGT (Vollmer, et al. 2008), whereas, another study reported normal GLP-1 secretion in obese IGT subjects (Ahren, et al. 1997). Although defects in GLP-1 secretion have been reported in some type 2 diabetics (Toft-Nielsen, et al. 2001), a recent review by Nauck *et al* concludes that deteriorations in glucose homeostasis can develop in the absence of any impairment in incretin hormone levels (Nauck, et al. 2011). In the current study, we also tested the acute GLP-1 response during high fat feeding using the synthetic GPR119 agonist GSK706A. Since GPR119 expression has been found in both intestinal and islets endocrine cell, this receptor is an attractive target in diabetes therapy. GPR119 activation has previously been demonstrated to increase GLP-1 in wild type mice with the synthetic GPR119 agonist, AR231453 (Semple, et al. 2008), whereas this effect was lost in GPR119 -/- mice (Chu, et al. 2008). We show that the responsiveness of the intestinal L-cells to both nutrient stimuli and GPR119 activation is

enhanced in insulin resistant mice, leading to elevated levels of GLP-1 in plasma. After a meal, especially containing carbohydrates (Reimann and Gribble 2002) and fat (Lindgren, et al. 2011), GLP-1 is secreted into the portal circulation by enteroendocrine L-cells predominately located in the distal part of the intestine (Eissele, et al. 1992; Tolhurst, et al. 2009). In rats, it was recently shown that a high-fat diet results in elevated GLP-1 levels after a mixed meal challenge (Yoder 2010). Also, in another study GLP-1 levels were 3-fold higher than control in the high-fat fed canine model of obesity and insulin resistance (van Citters, et al. 2002). Thus, these previous reports support the current finding that L-cell GLP-1 release may be more responsive to nutrient stimuli when animals are fed a high-fat diet. The increased GLP-1 response to GPR119 activation in association with the increased ß-cell expression of the GLP-1 receptor explains the robust increase in insulin secretion in the insulin resistant mice. In pancreatic islets, GPR119 expression has been localized mainly in β-cells (Chu et al. 2007) and activation of this receptor has been shown to stimulate islet insulin secretion through cAMP-mediated pathways (Chu et al. 2007; Soga et al. 2005). This would suggest that the increased insulin response to GPR119 activation in insulin resistant mice may also be explained by a more profound direct islet effect of the agonist. However, the current in vitro experiments does not support such a notion. Thus, GPR119 activation had no effect on islet insulin release even at very high doses. Therefore, although minor effects on insulin secretion may have been difficult to find in these studies in incubated islets, the marked insulin response to GPR119 activation in insulin resistant mice is most likely mediated by GLP-1.

The elevated intact GLP-1 and insulin levels after GPR119 activation in the insulin resistant mice resulted in increased glucose elimination rate which underscores GPR119 activation as a potential target in glucose intolerance. However, plasma glucose levels were not reduced by GSK706A at any of the individual time points and, similarly, the AUC for glucose was not lowered. Therefore GPR119 activation seems a weak tool to improve glycemia in the setting of insulin resistance. The failure of GPR119 activation to lower glucose below baseline may be explained by the concomitant increase in plasma glucagon levels after GPR119 activation. The mechanism behind the glucagonotropic action of GPR119 activation remains to be established. As GLP-1 and insulin are potent inhibitors of glucagon secretion (Holst, et al. 2011a) and (Maruyama, et al. 1984), it is possible that the GSK706A component directly affects islet α-cells. In rodent islets, GPR119 expression has been detected in α-cells (Chu et al. 2007;

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Odori, et al. 2012), however in relatively low concentration compared to β-cells. Concordantly, the current in vitro experiments does not indicate that GPR119 activation has any direct effect on islet glucagon release, and thus, not likely the main contributor to the increased plasma glucagon levels. Since GPR119 expression is seen not only in the gut L-cells, which produce GLP-1, but also in other enteroendocrine cells, it is possible that receptor activation indirectly affects islet glucagon release via other gut hormones than GLP-1. For example, GPR119 has been found in K-cells (Parker, et al. 2009), and receptor activation has been shown to increase glucose-dependent insulinotrophic peptide (GIP) levels (Chu et al. 2008; Chu et al. 2007; Semple et al. 2008). GIP has known glucagonotropic effects, however, previously only demonstrated during fasting and eu-glycemic conditions in healthy humans (Christensen, et al. 2011; Meier, et al. 2003), and at glucose concentrations below 5.5 mM in the perfused rat pancreas (Pederson and Brown 1978). On the other hand, studies in type 2 diabetics have pointed out that there is a lack of glucagon suppression after an oral glucose load, however preserved suppression after an isoglycemic intravenous glucose infusion (IIGI) (Knop, et al. 2007; Meier, et al. 2007), pointing to gut factors as potential mediators of dysregulated postprandial glucagon secretion in diabetes. In fact, in type 2 diabetics, Lund *et al* have showed that GIP infusion during the IIGI results in hypersecretion of glucagon (Lund, et al. 2011). Hence, in the setting of insulin resistance, it can therefore not be concluded whether a GPR119-mediated increase in GIP levels is causing the rise in glucagon after the current meal challenge. As excessive α-cell glucagon secretion is an important contributor to hyperglycemia during type 2 diabetes, the ability to lower glucagon secretion is one of the main therapeutic advantages of incretin based therapy (Ahren 2011). Thus, the discrepancy between GLP-1 and glucagon plasma levels in this study suggests that GPR119-based therapy is likely most effective as part of combination therapy. Indeed, paired with a DPP-4 inhibitor, which blocks the enzymatic inactivation of GLP-1 (Deacon, et al. 1995), there is evidence to support that the glucose-lowering potential of GSK706A could be enhanced (Chu Z-L 2006). Furthermore, as antagonism of the glucagon receptor has been shown to improve islet function in mice with insulin resistance induced by a high-fat diet (Winzell, et al. 2007), GSK706A could suggestively be paired with an inhibitor of glucagon action to achieve more potent glucose-lowering effects.

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In conclusion, we suggest that the normal adaptive response of the β-cell to insulin resistance involves an up-regulation of the incretin system which involves both increased islet GLP-1 receptor expression and enhanced GPR119-activated GLP-1 secretion, resulting in improved β-cell insulin secretion. Glucose elimination is also improved by GPR119 activation, yet, with no clear glucose-lowering effects which is likely due to increased levels of circulating glucagon. Therefore it is suggested that the anti-diabetic effects of GPR119 activation that are observed in this study are best preserved in combination therapy. These results thus provide both a mechanistic basis for increased incretin effect and β-cell sensitivity to GLP-1 in insulin resistant mice and a rationale for improving islet function by GPR119 activation.

Declaration of interests

All authors have approved the final version of the article. L. Ahlkvist and B. Ahrén have no conflicts of interest to disclose. K Brown is employed by GlaxoSmithKline.

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Author contributions

L Ahlkvist contributed with the design of the study, undertaking of the experiments, analyses and statistics, interpretation and discussion of the data and writing the manuscript. K Brown contributed with the design of the study and gave comments to the manuscript. B Ahrén contributed with design of the study, interpretation and discussion of the data and writing the manuscript.

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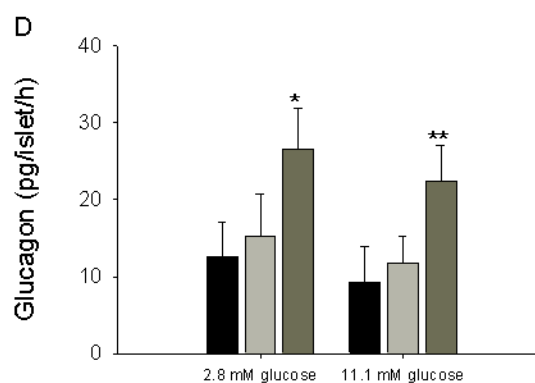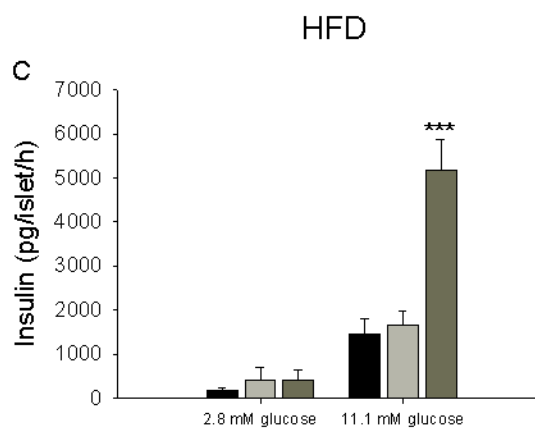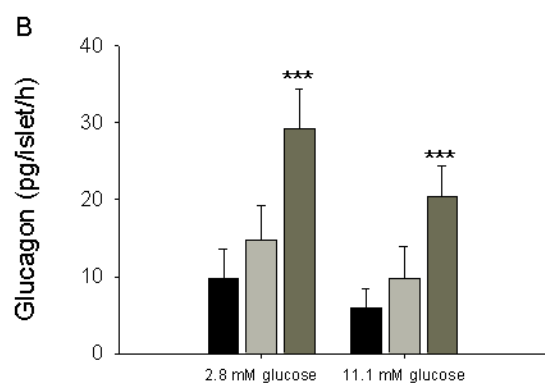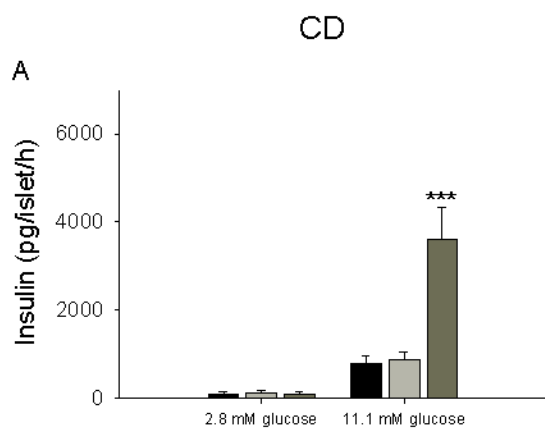
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Fig. 1. Plasma glucose (A, E), insulin (B, F), intact GLP-1 (C, G) and glucagon (D, H) levels after vehicle (●) versus GSK706A (10 mg/kg, ○) in control diet (CD) and high-fat diet (HFD) fed C57BL/6J mice following oral meal challenge. Means ± S.E.M. are shown, n=9-13 mice per group. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Fig. 2. Insulin (A, C) and glucagon (B, D) levels after incubation of isolated islets from control diet (CD) and high-fat diet (HFD) mice in vehicle (black bars), GSK706A (1 nmol/l, light bars) or arginine (10 mmol/l, green bars). Means ± S.E.M. are shown, n=4 mice per group. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Fig. 3. The ratio of GLP-1 receptor to actin protein levels (A) and representative western blot (B) in pancreatic islets in control diet (CD, black bar) versus high-fat diet (HFD, grey bar) fed mice. Means ± S.E.M. are shown, n=4 mice per group. *$P<0.05$.

CD

A

HFD

E

B

F

C

G

D

H
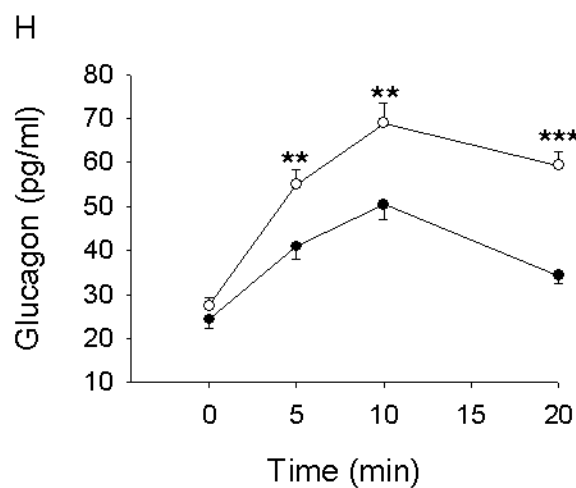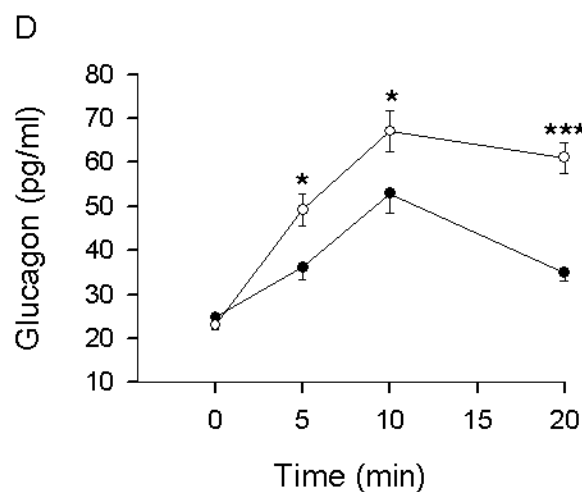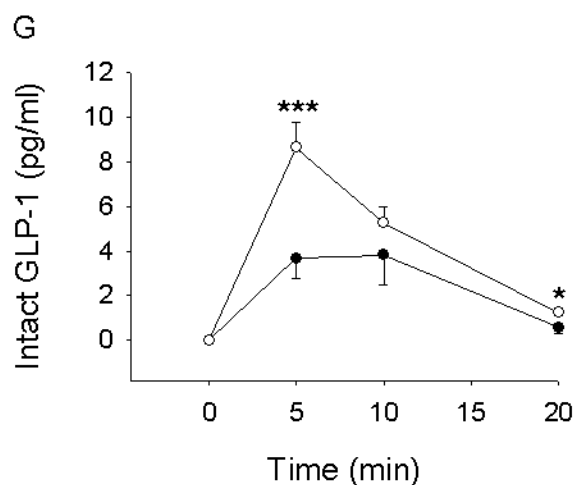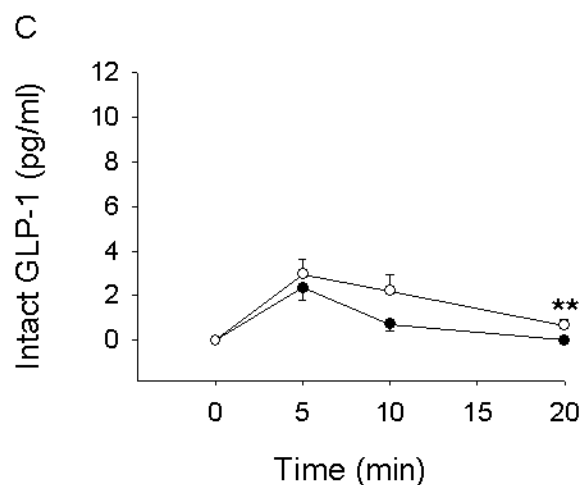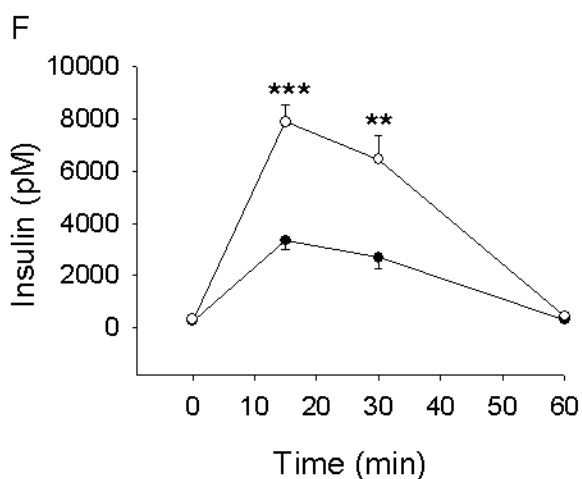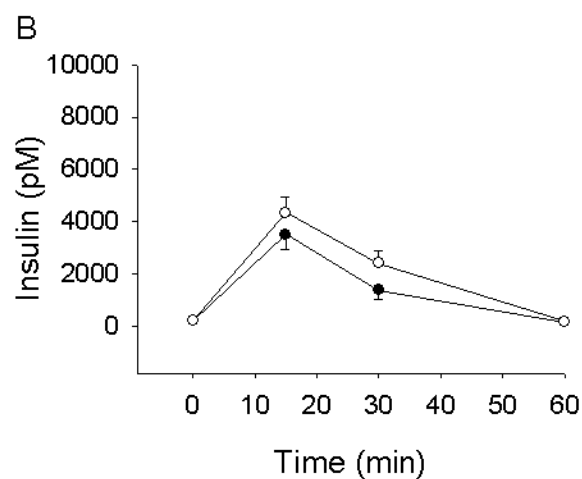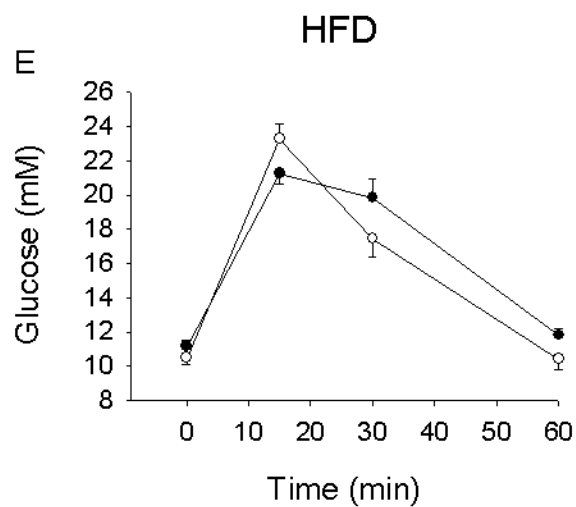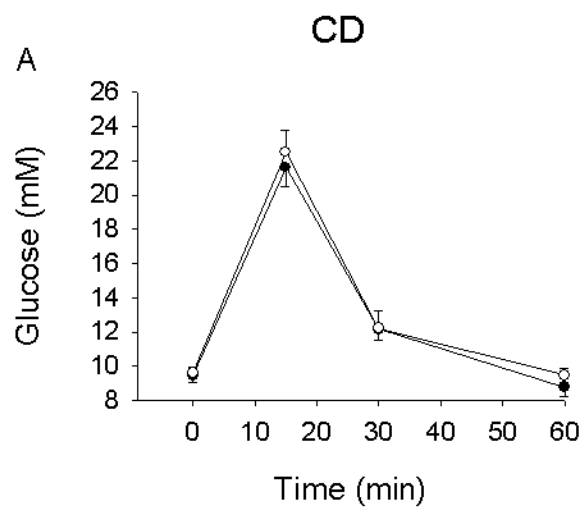
- ● Vehicle
- ○ GSK706A (10 mg/kg)

A

Ratio GLP-1R/Actin

B

| kDa | CD | HFD |
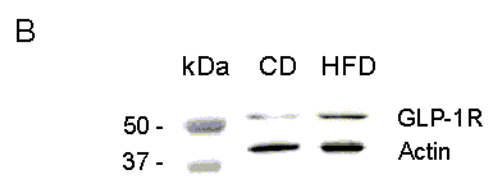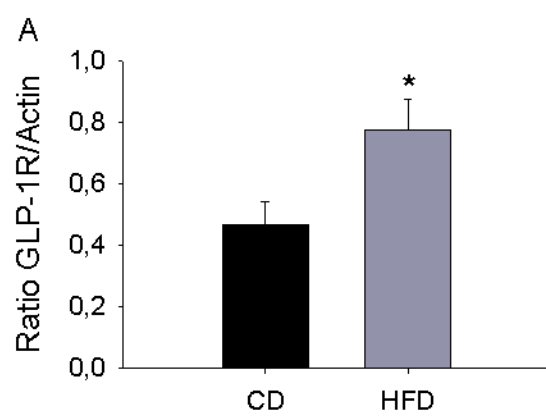| 50 - | | | GLP-1R
| 37 - | | | Actin

Table 1. Weight gain, fasting and dynamic intact GLP-1, islet hormones and β-cell function after vehicle (A) versus GPR199 activation (B) in control diet (1) and high-fat diet (2) fed C57BL/6J mice following oral mixed meal challenge.

	CD vehicle [1A]	CD GSK706A [1B]	HFD vehicle [2A]	HFD GSK706A [2B]
Weight gain (8 weeks, g)	2.0 ± 0.1		11.1 ± 0.5 $^{P<0.001}$	
Fasting glucose (mmol/l)	9.5 ± 0.3		11.0 ± 0.2 $^{P=0.0014}$	
Fasting insulin (pmol/l)	179 ± 11		322 ± 23 $^{P<0.001}$	
Fasting glucagon (pg/ml)	24.0 ± 1.0		25.9 ± 1.3 ^{NS}	
K_G (% per min)	0.65 ± 0.07	0.61 ± 0.06 ^{NS}	0.43 ± 0.03 $^{(†\ 1A/2A)}$	0.57 ± 0.04 $^{P=0.014}$
sAUC $_{GLUCOSE\ x\ 60\ min}$ (mmol/l)	233 ± 22	251 ± 35 ^{NS}	355 ± 33 $^{(†\ 1A/2A)}$	347 ± 25 ^{NS}
sAUC $_{INSULIN\ x\ 60\ min}$ (nmol/l)	76 ± 16	112 ± 19 ^{NS}	101 ± 13	253 ± 29 $^{P<0.001}$
sAUC $_{GLP-1\ x\ 20\ min}$ (pg/ml)	20.1 ± 3.8	36.7 ± 7.9 ^{NS}	34.6 ± 4.8 $^{(\#\ 1A/2A)}$	80.6 ± 9.6 $^{P=0.0014}$
sAUC $_{GLUCAGON\ x\ 20\ min}$ (pg/ml)	317 ± 69	650 ± 59 $^{P=0.0017}$	329 ± 52	608 ± 50 $^{P=0.0017}$
sAUC $_{INS}$/sAUC $_{GLU\ x\ 60\ min}$	315 ± 60	513 ± 67 $^{P=0.037}$	304 ± 40	760 ± 108 $^{P=0.002}$

Means ± S.E.M. are shown, n=9-13 mice per group (A and B). K_G is defined as the glucose elimination rate 15-60 minutes during oral challenge. sAUC: suprabasal area under the curve. Beta-cell function is represented by the ratio AUC insulin to AUC glucose. # P <0.05, † P < 0.01