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Beta-cell expression of a dominant-negative HNF-1α compromises the ability of inhibition of dipeptidyl peptidase-4 to elicit a long-term augmentation of insulin secretion in mice

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Abstract

Glucagon-like peptide-1 (GLP-1) has long-term effects on pancreatic islets by increasing the insulin secretory capacity and beta cell mass. The islet effects of GLP-1 are glucose dependent and therefore tied to glucose sensing and metabolism. We examined whether prevention of inactivation of GLP-1 by inhibiting dipeptidyl peptidase-4 (DPP-4) is sufficient to promote long-term augmentation of glucose-stimulated insulin secretion. We also explored whether a defective glucose sensing and metabolism could be overcome by DPP-4 inhibition. We administered the orally active and highly selective DPP-4 inhibitor (1-[[(3-hydroxy-1-adamantyl) amino] acetyl]-2-cyano-(S)-pyrrolidineP-4; vildagliptin; 3μmol/mouse daily) to normal, wildtype, mice and to mice with a β-cell targeted dominant-negative mutant hepato-cyte nuclear factor-1α (HNF-1α); these mice have a defective islet response to glucose. After eight weeks, vildagliptin augmented the insulin response after gastric glucose (75 mg) by 5-fold in male mice (7.3±0.8 vs 1.3±0.5 nmol/l, P<0.001) and 30-fold in female mice (26.5±5.8 vs 0.9±0.3 nmol/l, P<0.001). Furthermore, glucose-stimulated insulin secretion from isolated islets was markedly enhanced by 9 weeks treatment with vildagliptin. In contrast, in trans-genic mice, the severely suppressed insulin response was only marginally improved by vil-dagliptin in males, and not affected at all in females. We conclude that DPP-4 inhibition im-proves islet function and increases beta cell secretory responses on a long-term basis and that this is dependent on intact expression of HNF-1α.

Key words: Dipeptidyl peptidase-4, insulin secretion, glucose tolerance, mice, vildagliptin

1. **Introduction**

The incretin hormone, glucagon-like peptide-1 (GLP-1), is known to acutely stimulate insulin secretion when exogenously administered to rodents (Ahrén, 1995; Wang et al., 1995; Hargrove et al., 1996; Wang et al., 1997; Ahrén and Pacini, 1999; Parks et al., 2001). GLP-1 is also known to have long-term effects by increasing the insulin secretory capacity and islet beta cell mass, as demonstrated in rodents (Perfetti et al., 2000; Tourrel et al., 2001; Hui et al., 2002; Perfetti and Hui, 2004). The increased insulin secretion triggered by acute GLP-1 exposure in rodents is dependent on elevated glucose levels (Ahrén, 1995; Hargrove et al., 1996). This demonstrates that the molecular response of the beta cells to GLP-1 is closely tied to glucose sensing and metabolism. This is consistent with findings that GLP-1 promotes glucose sensing and beta cell expression of genes of importance for glucose actions, such as GLUT-2 (glucose-transporter-2) in association with long-term islet growth and differentiation (Perfetti et al., 2000; Abraham et al., Perfetti and Hui, 2004). An important factor for eliciting proper beta cell gene expression, especially for genes involved in establishing glucose sensing, metabolism and glucose-dependent insulin secretion, is the transcription factor hepatocyte nuclear factor (HNF)-1α (Emens et al., 1992; Dukes et al., 1998; Cha et al., 2000; Wang et al., 1998; Wang et al., 2000; Shih et al., 2001; Wang et al., 2002). However, whether this transcription factor has a key role also for the acute and long-term effects of GLP-1 on beta cell function is not known.

GLP-1 is cleaved by the enzyme dipeptidyl peptidase-IV (DPP-4) (Deacon et al., 1995; (Mentlein, 1999). Prevention of the inactivation of GLP-1 by inhibition of DPP-4 is currently being explored as a new treatment for type 2 diabetes (Deacon et al., 2004). DPP-4 inhibition has thereby been demonstrated to be anti-diabetic both in animal models of diabetes

(Pederson et al., 1998; Balkan et al., 1999; Ahrén et al., 2000; Kvist Reimer et al., 2002) and in patients with type 2 diabetes (Ahrén et al., 2002; Ahrén et al., 2004a; Ahrén et al., 2004b).

In view of the potential of exogenous administration of GLP-1 to improve long-term beta cell function, the first aim of this study was to explore whether preservation of the endogenous GLP-1 levels by preventing GLP-1 inactivation through DPP-4 inhibition is sufficient to promote long-term augmentation of glucose-stimulated insulin secretion. To test this hypothesis, control (wild type) mice were treated chronically with the orally-active and highly selective DPP-4 inhibitor (1-[[(3-hydroxy-1-adamantyl) amino] acetyl]-2-cyano-(S)-pyrrolidine), called vildagliptin, previously called LAF237 (Villhauer et al., 2003), which previously has been shown improve glycemic control when added to metformin in subjects with type 2 diabetes (Ahrén et al., 2004b). Furthermore, the second aim of this study was to explore whether a defective glucose sensing and metabolism could be overcome by a long-term treatment with a DPP-4 inhibitor. To address this question, we treated transgenic mice with ß-cell targeted dominant-negative mutant HNF-1α (RIP-DNHNF-1α) with vildagliptin. These mice have previously been shown to exhibit pronounced hyperglycemia and glucose intolerance in association with severely blunted insulin secretion after glucose challenge (Hagenfeldt-Johansson et al., 2001; Yamagata et al., 2002; Sörhede Winzell et al., 2004).

2. Materials and methods

2.1 Animals.

The dominant negative (DN)-HNF-1α and their wildype controls were kind gifts of Dr Claes Wollheim, University of Geneva, Switzerland. As described previously, the DN-HNF-1α cDNA was inserted into a plasmid under the control of the rat insulin promotor (RIP) for construction of a RIP-DN HNF-1α transgene (Wang et al., 1998; Hagenfeldt-Johansson et al., 2001). The transgenic mice were generated by pronuclear microinjection of the construct in B6/CBAJ-F_1 x B6/CBAJ-F_1 zygotes (Hagenfeldt-Johansson et al., 2001). Transgenic and wildtype mice were transported from the animal facility of the University Medical Centre, Geneva to the In Vivo Department, Biomedical Center, Lund University, Lund after embryo transfer performed at Taconic A/S, Ry, Denmark. The animals were cross-bred for >16 generations to C57BL/6J mice. Transgenic animals were identified by polymerase-chain reaction (PCR) on genomic DNA extracted from tail biopsies using the primer 5´CTGCTAACCATGTTCATGCCT-3´ and 5´TGAATTGTGAGCCACCTCTCTC-3´. The animals were kept in a 12 h light schedule (lights on at 0600 am) and given a standard pellet diet (fat 11.4%, carbohydrate 62.8%, protein 25.8% on an energy base, total energy 12.6 kJ/g) and tap water ad libitum. Lund University Ethic Committee approved the study.

2.2. Design of the study and in vivo experiments.

When mice were 3 months of age, half of the mice were given the DPP-4 inhibitor, vildagliptin (a kind gift from Novartis Institutes for BioMedical Research, Cambridge, U.S.A.) in the drinking water (0.3mg/ml, ~3µmol villdagliptin/day/mouse; a dosing regimen previously demonstrated to provide >80-90% inactivation of plasma DPP-4 activity throughout the day). Control groups were given tap water without vildagliptin. The mice were then followed for nine weeks with regular measurements of body weight. At week 8, a glucose tolerance test was undertaken and at week 9, an in vitro experiment was undertaken

for determination of glucose-stimulated insulin secretion from isolated islets. The gastric glucose tolerance test was performed in late morning after removal of food from the cages 16 h earlier. The animals were anesthetized with an intraperitoneal injection of midazolam (Dormicum®, Hoffman-La-Roche, Basel, Switzerland, 0.2 mg/mouse) as well as a combination of fluanison (0.4 mg/mouse) and fentanyl (0.02 mg/mouse; Hypnorm®, Janssen, Beerse, Belgium). This anesthesia has previously been shown not to affect glucose-stimulated insulin secretion when compared to non-anesthetized mice (Ahrén, unpublished). Thirty min later, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinized tubes, and D-glucose (British Drug Houses, Poole, UK) was given through a gastric tube (outer diameter 1.2 mm; 75mg per mouse). Controls were given saline. The volume load was 10 µl/g body weight. At specific time points after gavage or injection, blood samples, 75 µl each, were collected. Blood was kept in heparinized tubes, immediately centrifuged whereupon plasma was separated and stored at –20°C until analysis.

2.3. In vitro experiments.

Islets were isolated by standard collagenase digestion (Collagenase P, Roche Diagnostics GmbH, Mannheim, Germany), and subsequently handpicked under a stereo microscope. Thereafter, islets were preincubated for 30 min in HEPES balanced salt solution (HBSS; 114 mmol/l NaCl, 4.7 mmol/l KCl, 1.16 mmol/l $MgSO_4$, 20 mmol/l HEPES, 2.5 mmol/l $CaCl_2$, 0.1% BSA; pH 7.35) containing 3.3 mmol/l glucose. Then, 3 islets at a time were transferred to a multi-well plate kept on ice containing 200 µl per well of the same buffer in the presence of different concentrations of glucose. When all islets had been transferred, the plate was again placed in an incubator at 37°C; after 60 min, a sample from the buffer was removed for measurement of insulin.

2.4. Assays.

7

Insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, [^{125}I]-labelled human insulin as tracer and rat insulin as standard (Linco Research, St Charles, MO). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l and the coefficient of variation (CV) is less than 3% within assays and less than 5% between assays. Plasma glucose concentrations were determined with the glucose oxidase technique.

2.5. Calculations and statistics.

Data and results are reported as means±S.E.M. From data obtained from the gastric test, the 30 min insulin response (Δinsulin=insulin at min 30 minus insulin at basal), the suprabasal area under the 120 min insulin curve (AUC$_{insulin}$) and the suprabasal area under the 120 min glucose curve (AUC$_{glucose}$) were calculated. Statistical comparisons of data obtained from the in vivo-experiments were performed with Student´s un-paired t-tests with Bonferoni correction for multiple comparisons, whereas comparisons of data from the in vitro experiments were performed with Analysis of variance.

3. Results

3.1. Body weight.

Body weight did not differ between the wildtype and transgenic mice, and vildagliptin had no effect on body weight in neither females or males during the study period.

3.2. Gastric glucose after 8 weeks treatment (Fig. 1; Table 1).

After 8 weeks of treatment, the insulin response to gastric glucose was markedly augmented in mice treated with vildagliptin; the augmentation was 30-fold in females and 5-fold in males (P<0.001 in both genders). The augmented insulin response was associated with increased glucose tolerance in wildtype mice (P=0.038 in females and P=0.09 in males). In contrast, in transgenic female mice, vildagliptin did not increase insulin secretion or improve glucose intolerance, whereas in transgenic males, a slight increase in the Δinsulin response to gastric glucose was observed (P=0.021), although the calculation of the $AUC_{insulin}$ did not reveal any significant difference.

3.3. Insulin secretion in vitro (Fig. 2).

After 9 weeks of treatment with vildagliptin, islets were isolated and incubated for 60 min in the presence of different concentrations of glucose. A clear dose-dependent increase in insulin secretion was observed. The insulin secretion in response to glucose was augmented in islets isolated from vildagliptin-treated mice, both in females (augmented at 5.6 mmol/l glucose and above P<0.001) and in males (augmented at 8.3 mmol/l glucose and above P<0.001). In transgenic female mice, the insulin response to glucose was absent, and vildagliptin did not increase this response. Also in transgenic male mice, the insulin response to glucose was absent. However, in transgenic male vildagliptin treated mice, a significant, although slight. increase in response to glucose was seen at 22.2 mmol/l (P=0.007).

4. Discussion

Vildagliptin (previously called LAF237) is an orally active highly selective inhibitor of DPP-4 (Villhauer et al., 2003). Vildagliptin shows antidiabetic action in subjects with type 2 diabetes and is therefore currently explored as a new treatment for this disease (Ahrén et al., 2004a; Ahrén et al., 2004b). Like other DPP-4 inhibitors, vildagliptin stimulates insulin secretion in normal mice in a manner dependent upon the function of both GLP-1 and glucose-dependent insulinotropic peptide (GIP) receptors, indicating that incretin action is the most probable source of pharmacological effects in mice (Hansotia et al., 2004). In this study, we show that administration of vildagliptin improves glucose tolerance and the insulin response to gastric glucose over an eight-week period. In fact, the augmentation of the insulin response to gastric glucose by vildagliptin was marked, with an approximately 20-fold augmentation in female mice. The glycemia was reduced by approximately 50% in female mice in response to this large increase in insulinemia; in male mice vildagliptin augmented the insulin response only 6-fold and this was associated with a reduction of the glycemic response by 25%. The reduction in the glycemia was observed at 30 min after gastric glucose, whereas the insulin response was augmented at 15 min. This suggests that the influx of glucose from the gut after the gastric gavage is not affected by vildagliptin, and thus that the glucose reducing effect is due mainly to the augmented insulin response. Moreover, glucose-stimulated insulin secretion from isolated islets was increased by 9 weeks of treatment with vildagliptin. This suggests that prevention of GLP-1 inactivation seems to induce sufficient increase in endogenous incretin levels allowing for a stimulated insulin secretion over a long period of time. This is of importance when developing DPP-4 inhibition as a mode for treatment of type 2 diabetes, because long-term treatment would be anticipated to induce a progressively increased effect. Long-term treatment with DPP-4 inhibition might result in changes in beta-cell function, which may be in line with beta-cell effects induced by GLP-1 (Perfetti et al., 2000; Tourrel et

al., 2001; Abraham et al., 2002; Hui et al., 2002; Perfetti and Hui, 2004). We found a gender difference in the magnitude of the insulin response to vildagliptin, since the DPP-4 inhibitor augmented the insulin response to gastric glucose in female mice by approximately 20-fold whereas the corresponding augmentation in male mice was approximately 6-fold. The basis for this difference is not known, and has not been observed in other mouse strains (data not shown).

In this study, we also examined whether the effects of DPP-4 inhibition would overcome defects in beta cell function, which is associated with defective glucose sensing and metabolism by studying whether vildagliptin improves glucose tolerance also in mice subjected to β-cell overexpression of a dominant negative form of the transcription factor HNF-1α. These mice have defective function in the HNF-1α (Hagenfeldt-Johansson et al., 2001; Yamagata et al., 2002; Sörhede Winzell et al., 2004) that results in a maturity-onset diabetes type 3 (MODY3)-like diabetes. MODY3 is the most common form of MODY and is caused by heterozygous mutations in the gene encoding the homeodomain-containing transcription factor hepatocyte nuclear factor-1α (HNF-1α) (Yamagata et al., 1996). The defective beta-cell HNF-1α results in markedly suppressed insulin response to glucose (Hagenfeldt-Johansson et al., 2001; Yamagata et al., 2002; Sörhede Winzell et al., 2004). We show here that the efficiency of DPP-4 inhibition is markedly reduced in this type of diabetes because vildagliptin did not increase insulin secretion in female mice and only slightly augmented insulin secretion in male transgenic mice. The study therefore suggests that intact glucose sensing and metabolism in the beta cells are of profound importance for the efficacy of DPP-4 inhibition. It will be of interest to assess whether human subjects harbouring mutations in the HNF-1α will also have a diminished efficacy of DPP-4 inhibition.

In summary, this study shows that DPP-4 inhibition by vildagliptin improves glucose tolerance on a long-term basis in normal mice, which is accompanied by increased glucose-stimulated insulin secretion, suggesting that preservation of endogenous incretin hormones is sufficient to improve glucose-dependent insulin secretion. The study also shows that similar to mice doubly deficient in GLP-1 and GIP receptors (Hansotia et al., 2004), mice subjected to beta-cell overexpression of a dominant negative form of HNF-1α are largely unresponsive to vildagliptin. These data suggest that proper glucose sensing and metabolism are important in mice for the efficacy of DPP-4 inhibition mediated by enhanced GLP-1 and/or GIP activities. Our findings further indicate that intact function of beta-cells (in a manner dependent on HNF-1α) is required for the long-term augmentation of glucose-stimulated insulin secretion. Based on these results we conclude that incretin augmentation by DPP-4 inhibition improves islet function and increases beta cell secretory responses on a long-term basis and that this is dependent on intact expression of HNF-1α.

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Table 1 30 min insulin response (Δinsulin), the suprabasal area under the 120 min insulin curve ($AUC_{insulin}$) and the suprabasal area under the 120 min glucose curve ($AUC_{glucose}$) for data obtained from the gastric glucose tolerance test in female and male wildtype C57BL/6J mice and mice subjected to β-cell overexpression of DN-HNF-1α with or without a 8 week treatment with the DPP-4 inhibitor, vildagliptin in the drinking water (controls given plain water). There were 6 animals in each group. Means±S.E.M. are shown. Asterisks indicate the probability level of random difference between controls and vildagliptin-treated animals within each group ([a]$P<0.05$; [b]$P<0.01$; [c]$P<0.001$).

	Δinsulin (nmol/l)	$AUC_{insulin}$ (nmol/l 120 min)	$AUC_{glucose}$ (mmol/l 120 min)
Females			
Wildtype controls	0.9±0.3	42.5±12.9	676±48
Wildtype vildagliptin	26.6±5.8[c]	847.5±138.6[c]	385±39[a]
Transgenic controls	0.25±0.11	18.6±5.6	1,425±317
Transgenic vildagliptin	0.21±0.04	17.5±3.5	1,402±278
Males			
Wildtype controls	1.3±0.5	51.5±15.6	469±39
Wildtype vildagliptin	7.3±0.8[c]	387.9±89.4[c]	305±48[b]
Transgenic controls	0.07±0.02	8.5±1.2	1,562±81
Transgenic vildagliptin	0.12±0.04[a]	9.3±1.6	1,326±108

Legends to the Figures

Fig. 1 Plasma levels of insulin and glucose during gastric glucose tolerance test in female wildtype mice and transgenic mice subjected to beta-cell overexpression of DN-HNF-1α with or without an 8 week treatment with the DPP-4 inhibitor, vildagliptin in the drinking water (controls given plain water). There were 6 animals in each group. Means±S.E.M. are shown. Asterisks indicate probability level of random difference between the groups (*P<0.05; **P<0.01).

Fig. 2 Medium insulin concentrations after 60 min incubation of islets isolated from female or male transgenic mice with a beta-cell targeted overexpression of a dominant-negative HNF-1α and their wildtype counterparts after a 9 week treatment with the DPP-4 inhibitor, vildagliptin in the drinking water (controls given plain water). There were 8-16 incubations with three islets in each incubation for each experimental group. Means±S.E.M. are shown. The results in males are shown twice with different scale on the Y-axis.
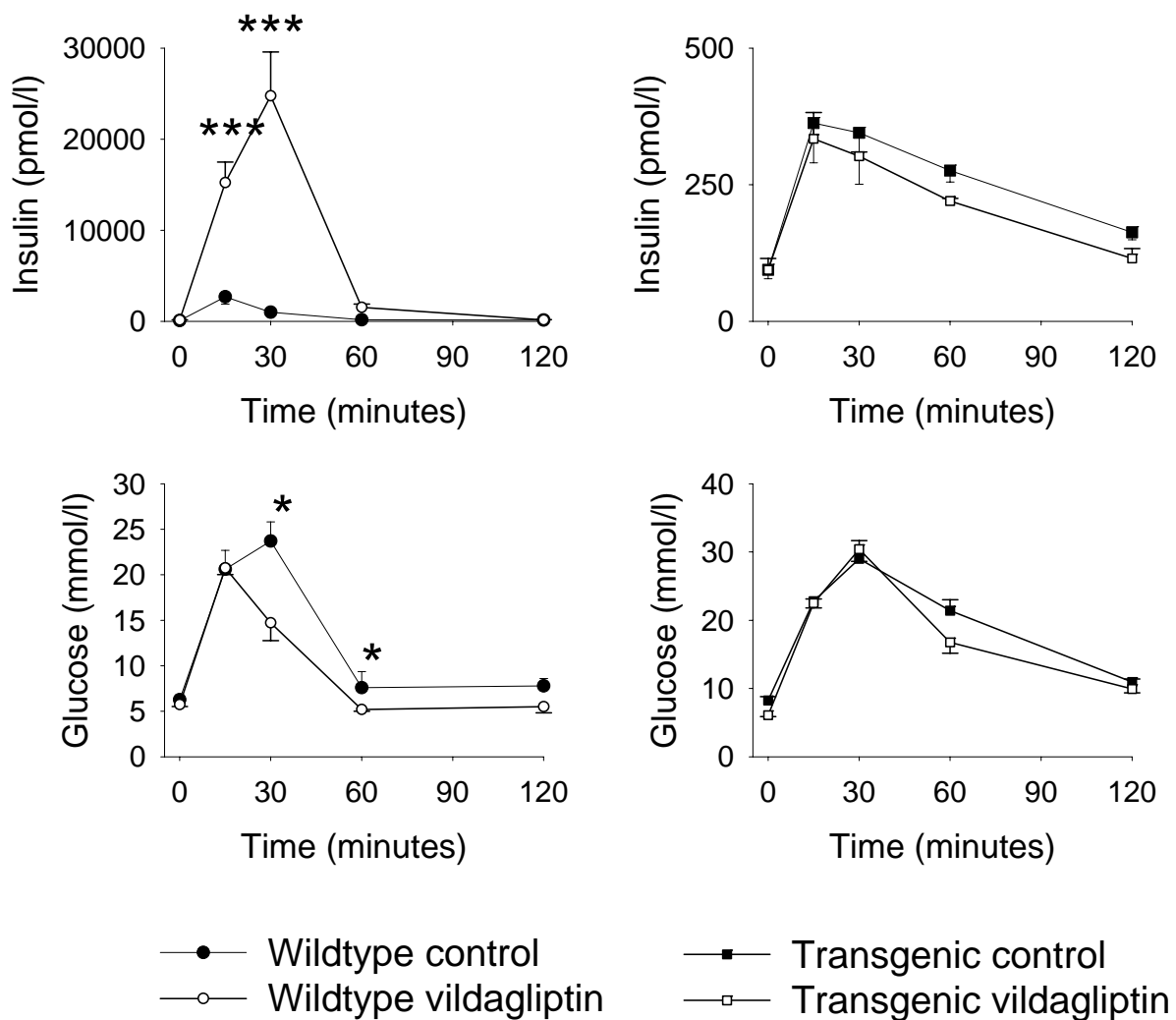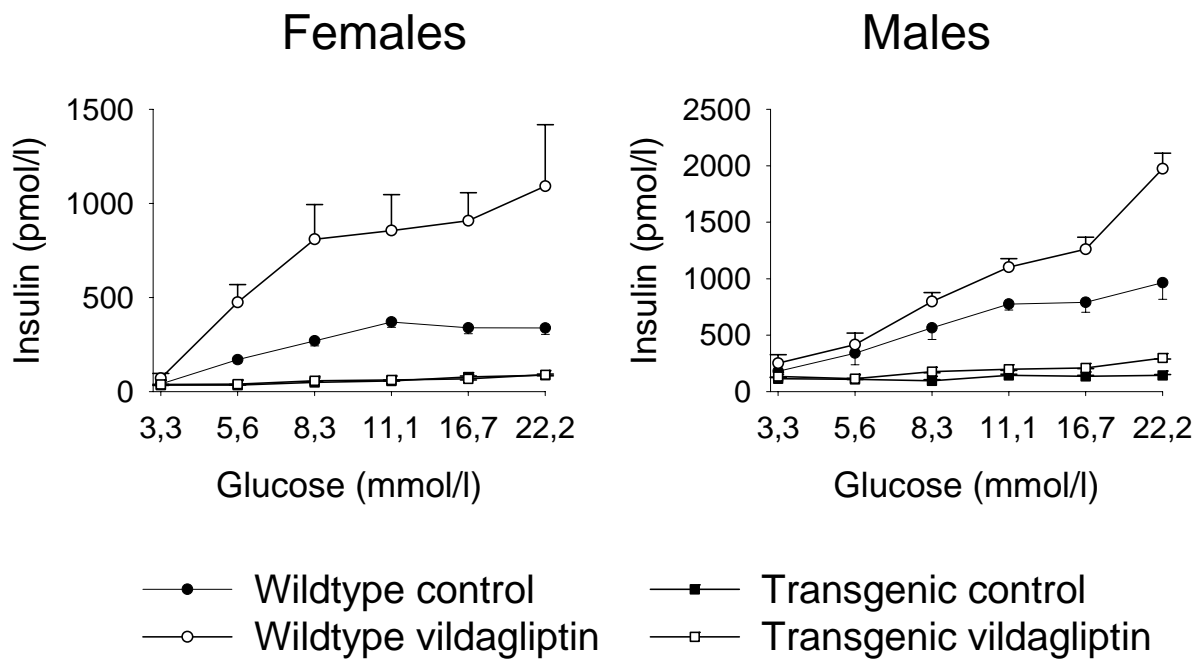
Fig. 1

Fig. 2