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PO Box 117
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Improved insulin sensitivity and islet function after PPAR δ activation in diabetic db/db mice

Maria Sörhede Winzell¹, Erik Max Wulff², Grith Skytte Olsen², Per Sauerberg², Carsten F. Gotfredsen², Bo Ahrén¹

¹Lund University, Department of Clinical Sciences, Medicine, Lund University, Lund, Sweden, ² Novo Nordisk, Måløv, Denmark

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Corresponding author:
Maria Sörhede Winzell
Department of Clinical Sciences, Medicine
BMC, B11
SE-221 84 Lund, Sweden
Phone: +4646 2220761
Fax: +4646 2220757
E-mail: Maria.Sorhede_Winzell@med.lu.se

Abstract

The peroxisome proliferator-activated receptors (PPARs) are transcription factors belonging to the nuclear receptor superfamily. Several reports have shown that PPAR δ is involved in lipid metabolism by increasing fat oxidation and depleting lipid accumulation. Whether PPAR δ are involved in regulation of glucose metabolism is not completely understood. In this study, we examined effects of long-term PPAR δ activation on glycemic control, islet function and insulin sensitivity in diabetic db/db mice. Male db/db mice were administered orally once daily with a selective and partial PPAR δ agonist (NNC 61-5920, 30 mg/kg) for eight weeks; control mice received vehicle. Fasting and non-fasting plasma glucose were reduced, reflected in reduced HbA_{1c} ($3.6 \pm 1.6\%$ vs. 5.4 ± 1.8 in db/db controls, $P < 0.05$) and furthermore, the AUC_{glucose} after oral glucose (3g/kg) was reduced by 67% ($P < 0.05$) after long-term PPAR δ activation. Following intravenous glucose (1g/kg), glucose tolerance was improved by PPAR δ activation (K_G 1.3 ± 0.6 vs. -0.05 ± 0.7 %/min, $P = 0.048$). Insulin sensitivity, measured as the glucose clearance after intravenous glucose (1 g/kg) and insulin (0.75 or 1.0 U/kg) administration, while inhibiting endogenous insulin secretion by diazoxide (25 mg/kg), was improved (K_G 2.9 ± 0.6 vs. 1.3 ± 0.3 %/min in controls, $P < 0.05$) despite lower insulin levels. Furthermore, islets isolated from PPAR δ agonist treated mice demonstrated improved glucose responsiveness as well as cellular topography. In conclusion, PPAR δ agonism alleviates insulin resistance and improves islet function and topography, resulting in improved glycemia in diabetic db/db mice. This suggests that activation of PPAR δ receptors improve glucose metabolism and may therefore potentially be target for treatment of type 2 diabetes.

1. Introduction

In type 2 diabetes, the islets fail to compensate for the increased insulin demand caused by insulin resistance. The clinical interventions that are currently being used to treat type 2 diabetes therefore aim at increasing insulin secretion and improving insulin sensitivity. The peroxisome proliferator-activated receptors (PPARs) are transcription factors, which have been evaluated as novel targets in treatment of type 2 diabetes. The PPARs belong to the nuclear receptor super-family, which are activated by lipids and regulate the expression of genes that control glucose and lipid metabolism (Desvergne and Wahli, 1999; Lee et al., 2003). There are three different subtypes of PPARs, PPAR α , PPAR δ and PPAR γ . There is a longstanding experience with the clinical use of PPAR α and PPAR γ activators. Thus, PPAR α activators, e.g. fenofibrate and clofibrate (fibrates), are used clinically to lower plasma triglycerides (TG) and to raise HDL-cholesterol (Staels et al., 1998). Furthermore, PPAR γ activation, e.g. thiazolidinediones (TZD), are used in the treatment of type 2 diabetes (Mayerson et al., 2002; Tsuchida et al., 2005) because they improve insulin action and decrease intracellular triglyceride accumulation in both liver and skeletal muscle (Miyazaki et al., 2002; Tonelli et al., 2004). The mechanisms behind these effects are mediated by altered gene expression of critical genes (Albrechtsen et al., 2002; Cha et al., 2001).

Several recent reports have suggest that also PPAR δ is involved in both lipid and glucose metabolism, because PPAR δ has been shown to increase fat oxidation and reduce lipid accumulation in adipose tissue and in other tissues (Lee et al., 2006; Reilly and Lee, 2008). PPAR δ is expressed in most metabolically active tissues and they control many genes involved in glucose homeostasis and fatty acid metabolism (Wang et al., 2003). Rodent studies have suggested that activation of PPAR δ reduces body weight, increases metabolic rate and improves insulin sensitivity, through induction of skeletal muscle fatty acid oxidation (Wang et al., 2003; Wang et al., 2008). This would suggest that also PPAR δ activation might be a target for treatment of type 2 diabetes. However, in contrast to the well-characterized actions of PPAR α and PPAR γ , the potential therapeutic role of PPAR δ activation is currently not completely established. Synthetic agonists have been developed and a recent study in over-weight humans, demonstrated improved lipid profile and improved metabolic status after a two

weeks treatment period with a PPAR δ agonist (Riserus et al., 2008). Whether PPAR δ activation also improves islet function is, however, not known. Therefore, the aim of this study was to examine both insulin secretion and insulin sensitivity after long-term activation of PPAR δ . To that end, PPAR δ was activated on a long-term basis in diabetic db/db mice using a selective and partial PPAR δ agonist (NNC 61-5920). This agonist exhibit 76 % efficacy compared to a full agonist in *in vitro* transactivation assay, with good oral pharmacokinetic properties in rats and full efficacy on FFA oxidation in vitro .

2. Material and methods

2.1 Description of the PPAR δ agonist, NNC 61-5920

The PPAR δ agonist (NNC 61-5920) is a selective, partial PPAR δ agonist (EC_{50} = 0.103 \pm 0.004 μ M; 76%) as demonstrated in *in vitro* human transactivation assays (Supplementary data, Fig S1a and Table S1). The maximum efficacy response of the full PPAR δ agonist GW501516 could be reduced by the partial agonist NNC 61-5920 to the efficacy of NNC 61-5920 it self (Supplementary data, Fig S1b). *In vitro* transactivation using mouse receptors showed similar data (mPPAR δ activity: EC_{50} = 0.51 \pm 0.32 μ M; 180 \pm 52%; GW501516: EC_{50} = 0.054 \pm 0.022 μ M, 269 \pm 39%), although potencies were slightly lower. Likewise, NNC 61-5920 had no mPPAR α or mPPAR γ activity up to 30 μ M (data not shown). In *in vitro* fatty acid oxidation in L6 muscle cells demonstrated that NNC 61-5920 was a full agonist (EC_{50} = 42 \pm 9 nM, Supplementary data, Table S2). In rats NNC 61-5920 showed high oral bioavailability, high plasma exposure, low volume of distribution and long half-life (Supplementary data, Table S3), suggesting good in vivo activity.

2.2. Animals and experimental design

Twelve-weeks old male db/db and db/+ mice (C57BL/KsBom-db/db and -db/+), purchased from Taconic Europe, Ry, Denmark, were maintained in a temperature-controlled room (22-23°C) on a 12-h light-dark cycle, and fed a standard lab chow (1324 Altromin, Im Seelenkamp, Germany) two weeks prior the experiment start. At day 0, the db/db mice were divided in two groups (20 mice per group),

and one group was dosed orally once daily with the partial PPAR δ agonist (NNC 61-5920), 30 mg/kg dissolved in 0.2% carboxymethyl-cellulose, 0.4% Tween in saline. The control mice, both db/db and db/+ received vehicle only. Body weight and 24-h food intake were measured weekly. The mice were dosed for 61 days. Seven days prior to the start of the experiment and at day 57, blood samples were taken from the retrobulbar, intraorbital capillary plexus in non-fasted, anaesthetized mice for analysis of glucose, hemoglobin A_{1C} (HbA_{1C}), insulin, high-density lipoproteins (HDL) and triglycerides. At day 0 and 57-60, oral and intravenous glucose tolerance tests were performed. At the end of the experiment, islets were isolated and examined for insulin secretory capacity as well as palmitate oxidation. Skeletal muscle was isolated and analyzed for palmitate and glucose oxidation as well as glycogen synthesis. The study was approved and conducted according to Novo Nordisk A/S animal welfare guidelines and national law.

2.3 Oral glucose tolerance test (OGTT)

OGTT was performed before and after 58 days of treatment. Over-night fasted, conscious mice received an oral glucose bolus (3 g/kg) and blood (50 μ l) was sampled from the tail vein at 0 min and 30, 60 and 120 min. Following immediate centrifugation at 4°C, plasma was separated and stored at –20°C until analysis of glucose and insulin.

2.4 Intravenous glucose tolerance test (IVGTT)

IVGTT was performed on day 58 in 4-hour fasted mice, anesthetized with 20mg/kg fluanison/ 0.8mg/kg fentanyl (Hypnorm, Janssen, Beerse, Belgium) and 10mg/kg midazolam (Dormicum, Hoffman-LaRoche, Basel, Switzerland) 30 min prior to the glucose challenge. A blood sample (50 μ l) was taken from the retrobulbar, intraorbital, capillary plexus, after which D-glucose (1g/kg; Sigma, St. Louis, MO) was injected intravenously in a tail vein (volume load 10 μ l/g). Additional blood samples were taken at 1, 5, 10, 20, 50 and 75 min after injection. Following immediate centrifugation at 4°C, plasma was separated and stored at –20°C until analysis of glucose and insulin.

2.5 Diazoxide-supplemented glucose-insulin test (DSGIT)

DSGIT was performed as previously described (Ahren and Pacini, 2006) on day 60 with PPAR δ agonist treatment. Four-hour fasted control mice and treated mice were anesthetized as described above. Thirty minutes later, diazoxide (25 mg/kg; Sigma) or saline was given as a subcutaneous injection. Ten minutes later, a blood sample was taken from the retrobulbar, intraorbital capillary plexus in heparinized tubes, and D-glucose (Sigma; 1g/kg) was given intravenously alone or together with human insulin (Actrapid[®], Novo Nordisk, Bagsvaerd, Denmark) in different doses (db/db-VEH 1 U/kg, db/db-(NNC 61-5920) 0.75 U/kg). The volume load was 10 μ l/g body weight. Additional blood samples, 60 μ l each, were collected at 1, 5, 10, 20, 50 and 75 min after the glucose injection. Blood was kept in heparinized tubes, immediately centrifuged whereupon plasma was separated and stored at -20°C until analysis.

2.6 Analysis of plasma samples

Glucose was measured using the glucose oxidase method using 2,2'-azino-bis(3-ethyl-benzothiazoline-6-sulfonate) (ABTS) as substrate. Insulin was determined radioimmunochemically using a guinea-pig anti-rat insulin antibody, ¹²⁵I-labelled human insulin as tracer and rat insulin as standard (Linco Res., St Charles, MO). Free and bound radioactivity were separated by use of an anti-IgG (goat anti-guinea pig) antibody. HbA_{1c} and hemoglobin (Hb) were measured using HbA_{1c} II (Roche/Hitachi, Mannheim, Germany) and %HbA_{1c} was calculated using the following formula: $(\text{HbA}_{1c}/\text{Hb}) * 87.6 + 2.27 = \% \text{HbA}_{1c}$ (IFCC). HDL cholesterol and triglycerides were measured by an automatic analyser Hitachi 912 (Boehringer Mannheim, Germany) using commercial kits (Boehringer Mannheim, Germany and Wako Chemicals, Germany).

2.7 Islet isolation and insulin secretion

For insulin secretion studies, islets were isolated from the pancreas by collagenase digestion and handpicked under microscope. Batches of islets were preincubated in HEPES balanced salt solution (HBSS) containing 125 mM NaCl, 5.9 mM KCl, 1.28 mM CaCl₂, 1.2 mM MgCl₂, 25 mM HEPES (pH 7.4), 3.3 mM glucose and 0.1% fatty acid free bovine albumin (Boehringer Mannheim, GmbH, Germany) for 60 min. Thereafter, islets in groups of three were incubated in 200 μ l of the HBSS with

varying concentrations of glucose and incubated for 60 min at 37°C. After incubation, aliquots of 25 µl in duplicates were collected and stored at -20°C until analysis of insulin.

2.8 Palmitate oxidation in islets

Palmitate oxidation was measured in islets isolated from mice in the different treatment groups. Batches of 30 islets in quadruplicates were incubated in a reaction mixture consisting of 0.5 mM palmitic acid complexed to 1% fatty acid free bovine serum albumin, with 0.5 µCi [1-¹⁴C]-palmitic acid (NEN, Boston, MA, specific activity 55 mCi/mmol) as tracer, 0.8 µM L-carnitine, and glucose at final concentrations of 2.8 or 16.7 mM. The reaction was terminated after 2 h, at 37°C, and the amount of released ¹⁴CO₂, trapped with benzetonium hydroxide, was determined by liquid scintillation counting.

2.9 Islet morphology

After sacrifice, the pancreas was fixed overnight in 4% paraformaldehyde. The pancreas was fractionated by the smooth fractionator method with F=½ in each of two capsules, dehydrated and embedded in paraffin in a TP1050 tissue preparation machine (Leica, Herlev, Denmark). Sections 3 µm thick were cut on a microtome at two levels 250 µm apart.

Antigen retrieval was carried out by microwave treatment in 0.01M citrate buffer, pH 6.0 followed by blocking of endogenous peroxidase, and blocking of avidin and biotin binding sites. The immunohistochemical staining for beta and non-beta cells was carried out in an Autostainer (Dako, Glostrup, Denmark) using primary antibodies to insulin and a mixture of antibodies to glucagon, somatostatin, and pancreatic polypeptide. The following stereological determination of beta- and non-beta-cell volume fractions and relative beta cell mass expressed in mg/kg body weight was carried out as described earlier with the operator blinded to the origin of the sections (Rolin et al., 2002).

2.10 Palmitate and glucose oxidation in skeletal muscle

From mice in the different treatment groups *musculus soleus* (mainly red muscle, type I oxidative fibers) and *musculus extensor digitorum longus* (EDL, mainly white muscle, type IIb glycolytic fibers)

were gently dissected and immediately transferred to pre-gassed (95% O₂-5% CO₂) Krebs-Henseleit (KRH) buffer (in mM: 118.5 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 25 NaHCO₃, 2.5 CaCl₂, 1.2 MgSO₄, and 10 HEPES) supplemented with 10 mM glucose and 2% BSA. Fatty acid oxidation was determined by incubating the muscles for 90 min in pre-gassed KRH buffer supplemented with 10 mM glucose and 0.2 mM palmitate containing 10 µCi/mL [9,10-³H]-palmitic acid (PerkinElmer; NET-043) conjugated to 2% fatty acid free BSA (Sigma; A7030) (Thompson et al., 2000). Incubations were performed at 30°C, with gentle agitation (110/min), under continuous gassing with 95% O₂-5% CO₂. The assay was terminated by addition of 10% TCA. Upon oxidation of the tritiated palmitate ³H-H₂O is generated and appears in the medium. ³H-H₂O was separated from the medium by equilibration with water in a separate container at 50°C overnight. The quantity of ³H-H₂O transferred to the water was measured by liquid scintillation counting.

Glucose oxidation was measured in the same way as fatty acid oxidation with the exception that the tritiated palmitate was replaced by 10 µCi/mL [5-³H]-D-glucose (PerkinElmer; NET-530).

2.11 Muscle insulin sensitivity

Muscle insulin sensitivity was determined in EDL muscle as insulin-stimulated versus basal glycogen synthesis. Thus, isolated muscle were incubated in KRH buffer supplemented with 5 mM D-[U-¹⁴C]-glucose and 0.1 % BSA with or without 10 nM insulin for 30 minutes. The muscles were frozen in liquid nitrogen, weighed, and boiled in 1N NaOH for 30 minutes. Glycogen was precipitated overnight at -20°C in ethanol after addition of 0.35 mg/ml unlabeled glycogen (Sigma). After centrifugation (20 min, 2800xg), the glycogen pellet was washed in ice-cold ethanol, solubilized in water, and radioactivity was measured by liquid scintillation counting (Packard, TRI-CARP 1500). Fold over basal was then calculated as a measure of muscle insulin sensitivity for each mouse.

2.12 Statistical analysis

All data are presented as mean±SEM. In the IVGTT, the acute insulin response (AIR) to intravenous glucose was calculated as the mean of suprabasal 1 and 5 min values, and the glucose elimination was quantified using the glucose elimination constant, K_G, calculated as the slope of the logarithmic

transformation of circulating glucose between 5 and 20 min after the glucose bolus. Area under the curve (AUC) was calculated with the trapezoid rule. Multiple comparisons between the different groups were performed by one-way ANOVA. Dunnett's multiple comparison test or Bonferroni's post hoc test were used to calculate statistical differences between the groups. Significant statistical difference was considered at $P<0.05$.

3. Results

3.1 Body weight and basal plasma parameters

Diabetic db/db mice were administered with the PPAR δ agonist (NNC 61-5920) for 61 days. After 57 day of treatment, HDL-cholesterol levels were increased (3.4 ± 0.8 vs. 2.6 ± 0.8 mM, $P<0.001$), and plasma triglyceride levels were reduced (0.8 ± 0.2 vs. 1.1 ± 0.5 mM, $P<0.05$) (Table 1). There was no significant elevation of plasma triglyceride in the non-treated db/db mice compared to the normal mice (db/+), and the reduction in triglyceride levels was different also from the db/+ control, which was 1.0 ± 0.5 mM ($P<0.05$). Fasting blood glucose levels were elevated in db/db compared to db/+, while there was significantly reduced glycemia in PPAR δ agonist treated mice (9.0 ± 2.8 vs. 13.3 ± 7.1 mM in db/db controls, $P<0.001$), which also was reflected as reduced HbA_{1c} levels (3.6 ± 1.6 vs. 5.4 ± 1.8 %, $P<0.05$). There was also a tendency to reduced basal insulin levels, although this was not significant ($P>0.05$). There was no significant difference in body weight between the vehicle or the PPAR δ agonist treated mice throughout the 8-week study (Fig 1A). Food intake was measured during the first 6 weeks of the study, with no differences between the groups (Fig 1B).

3.2 In vivo glucose challenge

An OGTT performed prior to the treatment with the PPAR δ -agonist demonstrated severely impaired glucose tolerance in db/db mice compared to the db/+ mice (Fig. 2A). The AUC_{glc} from 0 to 120 min after the glucose bolus was 1020 ± 160 mM*min in db/+ mice and 3360 ± 360 mM*min in db/db mice ($P<0.001$). After 60 days of PPAR δ activation, glucose excursion in the OGTT had improved significantly (AUC_{glc} 2030 ± 940 vs. 2850 ± 660 mM*min in db/db control mice, $P<0.05$, Fig. 2B).

Insulin levels tended to be lower after PPAR δ agonist treatment compared to the db/db control, however this did not reach statistical significance ($P>0.05$, Figs 2 C and D).

In the IVGTT performed after 58 days treatment, glucose tolerance was reduced in db/db mice compared to db/+ mice (K_G -0.05 ± 0.7 vs. 3.1 ± 0.3 %/min in db/+, $P=0.003$; Fig. 2E) in association with impaired acute insulin response (AIR 221 ± 90 vs. 820 ± 154 pM in db/+, $P=0.015$, Fig. 2F). PPAR δ -agonist treatment improved glucose elimination (K_G 1.3 ± 0.6 vs. -0.05 ± 0.7 %/min in db/db control, $P=0.048$), without increasing insulin secretion (AIR 300 ± 4 vs. 221 ± 90 pM in control db/db mice, Fig. 2F).

3.3 Insulin sensitivity measured with the DSGIT

Insulin sensitivity was evaluated using a novel approach where endogenous insulin secretion was inhibited by intraperitoneal injection of diazoxide 10 min prior to the IVGTT (Ahren and Pacini, 2006). Diazoxide markedly reduced the glucose-stimulated insulin secretion in db/db control mice (AIR in the IVGTT 221 ± 74 pM vs. -506 ± 269 pM in the DSGIT, $P<0.05$), and this was associated with reduced glucose tolerance ($P<0.05$, Figs. 3A and C). Similar effects were seen in PPAR δ -agonist treated mice (Fig. 3B and D). Db/db control mice were administered with 1 U/kg insulin and the PPAR δ -agonist treated mice with 0.75 U/kg insulin (Actrapid®). The insulin doses resulted in different insulin levels with AUC_{ins} 252 ± 55 nM*min in control mice and 46 ± 13 nM*min in the treated mice ($P<0.001$). The insulin dose was 25% lower in the PPAR δ -agonist treated mice compared to the control db/db mice, yet the plasma insulin levels in the treated mice was 82% lower compared to vehicle administered db/db mice. Despite lower circulating insulin levels, K_G was significantly improved in PPAR δ -agonist treated mice ($K_{G5-20\text{ min}}$ 2.9 ± 0.6 vs. 1.3 ± 0.3 %/min in control db/db mice, $P<0.05$, Figs. 3A and B). Plotting the mean glucose elimination against the mean insulin peak, demonstrated that despite a lower insulin peak, glucose elimination was much improved in PPAR δ -agonist treated mice (Fig. 3E).

3.4 In vitro insulin secretion and islet palmitate oxidation

Insulin secretion in isolated islets after 61 days of treatment with the PPAR δ agonist demonstrated improved islet glucose sensitivity (Fig. 4A). Insulin secretion at 3.3 mM glucose was increased in islets from both treated and non-treated db/db mice compared to db/+ islets. There was a significant increase over basal insulin secretion at 5.6 mM glucose after PPAR δ activation, while at high glucose concentrations (16.7 mM) there was no difference between the treated and the control groups.

Islet palmitate oxidation at low glucose was elevated in db/db islets compared to db/+ islets, and PPAR δ activation further increased islet fat oxidation, supporting increased fat oxidation as one mechanism of action also in pancreatic islets (Fig. 4B). At high glucose, palmitate oxidation was similar in islets from db/db controls and after PPAR δ activation.

3.5 Islets morphology

Figure 4C shows the staining pattern and morphology of islets in representative sections of pancreas from the three groups. Lean db/+ mice had small, compact islets with high insulin staining intensity beta cells in the core and well stained non-beta cells in the mantle (Fig. 4C). Diabetic db/db mice had much bigger islets with weakly and variably stained beta cells in the core and with well stained non-beta cells in the mantle and in the core. The islets of mice treated with the PPAR δ agonist showed an intermediate morphology, where the insulin staining intensity was higher and less variable and the proportion of non-beta cells in the core was lower than in the vehicle treated diabetic mice (Fig. 4C). The mass of both beta and non-beta cells was more than twice as much in vehicle treated diabetic mice as in the db/+ control group (Fig 4D). PPAR δ agonism did not change the mass of beta or non-beta cells.

3.6 Palmitate and glucose oxidation in skeletal muscle

Palmitate oxidation of isolated EDL muscle at day 61 of treatment with PPAR δ agonist was significantly increased (Fig. 5A). A similar tendency was observed in soleus muscle (Fig. 5B). On the contrary, the capacity of glucose oxidation was not affected in either muscle types (Fig. 5C and D). The increased palmitate oxidation was accompanied by a tendency, although not significant, towards

restoration of the decreased muscle insulin sensitivity observed as decreased insulin-stimulated glycogen synthesis in the db/db versus control db/+ mouse (Fig. 5E).

4. Discussion

In this study we demonstrate that PPAR δ activation for 61 days in diabetic db/db mice reduces glycemia in association with improved insulin sensitivity and improved islet function. Furthermore, skeletal muscle fat oxidation was enhanced, which is in agreement with other studies on PPAR δ activation (Wang et al., 2003; Wang et al., 2008). PPAR δ is widely expressed (Braissant et al., 1996) and is an important regulator in adipose tissue, in skeletal muscle, in the heart and in the liver (de Lange et al., 2008; Tanaka et al., 2003). Our current findings suggest additionally that PPAR δ activation has important effects on insulin sensitivity and islet function. These effects are independent from reduced food intake or reduced body weight.

We found that PPAR δ activation improved glycemia both in the fed and in the fasted state of diabetic db/db mice. There was a tendency to reduced basal insulin levels although this was not significant. The reduced glucose together with the lower insulin levels suggested improved insulin sensitivity as a primary effect. This was supported by the results obtained from the oral and intravenous glucose tolerance tests, where PPAR δ agonist treatment lead to improved glucose tolerance, without any significant increase in insulin secretion. We also showed an increased insulin sensitivity in db/db mice treated with the PPAR δ agonist using a novel method for estimation of insulin sensitivity, the DSGIT. In this method, mice are treated with diazoxide prior to injection of glucose and insulin is used for direct estimation of insulin sensitivity in diabetes models (Ahren and Pacini, 2006). Diazoxide blocks the endogenous insulin secretion by opening of K_{ATP}-channels, thereby inhibiting the depolarization of the plasma membrane and blocking the influx of Ca²⁺ (Hansen et al., 2004; Henquin, 2000). This method was recently validated in comparison with insulin sensitivity estimated from IVGTT data using minimal modeling (Ahren and Pacini, 2006; Pacini et al., 2001). The DSGIT has the advantage over the regularly used insulin tolerance test, the interpretation of which is complicated due to counter-regulatory responses occurring during hypoglycemia.

The improved insulin sensitivity by the PPAR δ agonist is in line with a recent study showing that PPAR δ deficient mice have reduced insulin sensitivity and that activation of PPAR δ in db/db mice improves insulin sensitivity (Lee et al., 2006). Hence, PPAR δ activation seems to be similar to PPAR γ activation in its effect in improving insulin sensitivity (Tonelli et al., 2004). However, the improved insulin sensitivity observed after PPAR δ activation may occur through a different mechanism compared to PPAR γ activation. TZD activation of PPAR γ results in reduced circulating fatty acids and increased levels of adiponectin but increased body weight while PPAR δ activation instead promotes a metabolic shift, which reduces hepatic glucose production, stimulates fat burning, which together enhance insulin sensitivity.

The different PPARs seem to have different roles in lipid metabolism. While PPAR α stimulates hepatic fatty acid oxidation and lipid accumulation and PPAR γ regulates lipid synthesis in the adipocytes, PPAR δ increase glycolysis and lipogenesis in the liver while stimulating fatty acid oxidation in skeletal muscle. A mouse model with transgenic overexpression of PPAR δ demonstrated that constitutive PPAR δ activation resulted in a lean phenotype, which was resistant to diet-induced obesity (Wang et al., 2003). In skeletal muscle, PPAR δ is expressed at a higher level in soleus muscle. Overexpression of PPAR δ in skeletal muscle resulted in a switch to muscle type 1, which is more oxidative, with increased numbers of mitochondria and increased expression of genes involved in fat oxidation (Luquet et al., 2003). Thus, one important mechanism of PPAR δ activation may occur via improved muscle fat oxidation. This is in agreement with our findings that PPAR δ agonism resulted in increased fat oxidation in EDL and with a tendency to increased oxidation in soleus muscle. There was no effect on glucose oxidation, suggesting that the main effect in skeletal muscle is to increase fat oxidation.

Type 2 diabetes is accompanied by dyslipidemia with increased triglyceride levels elevated LDL cholesterol and reduced HDL cholesterol, which has been treated through PPAR α activation by fibrates for many years (Staels et al., 1998). A recent study demonstrated that the specific PPAR δ agonist GW501516 resulted in significantly elevated HDL levels after 4 weeks treatment of obese

rhesus monkeys (Oliver et al., 2001). At the same time both triglyceride levels and small-dense low-density lipoproteins were reduced. It was suggested that PPAR δ activation promotes reverse cholesterol transport, partly by increasing the expression of the ABCA1 reverse cholesterol transporter in macrophages. In the present study, the activation of PPAR δ , using NNC 61-5920, resulted in significantly improved lipid profile in diabetic db/db mice, as shown by reduced triglyceride levels as well as increased HDL levels.

A main novel finding in this study is that PPAR δ agonist treatment improves islet function in db/db mice. Thus, it was found that the insulin response to glucose was improved in islets isolated from treated mice. The islet effects caused by PPAR δ activation may be both direct and indirect and at present the mechanism is not completely understood. PPAR α is expressed in islets and activation of the receptor has been shown to enhance beta cell function in insulin resistant rodents (Koh et al., 2003). However, islets from PPAR α deficient mice exhibited similar glucose-stimulated insulin secretion and thus a peripheral signal may be more important for the observed islet effect (Guerre-Millo et al., 2001). Also PPAR γ activation has been demonstrated to improve islet function (Diani et al., 2004). Islets from db/db mice are much larger than normal islets and also contain more insulin. It has been suggested that diabetic islets contain more fat and that reduction of intracellular fat improved islet function (Shimabukuro et al., 1998). We observed that islets from db/db mice have significantly increased palmitate oxidation than control db/+ mice. PPAR δ activation increased the fat oxidation, which is a potential mechanism by which the treatment improved the islet function. We also showed improved islet structure in PPAR δ agonist treated db/db mice. Thus, in control db/db mice, non-beta cells are scattered in the islets while control db/+ mice have non-beta cells located to the mantle of the islets. Activation of PPAR δ in db/db mice partly restored the islets structure to resemble normal islets. It is however possible that this is not a direct effect on islet but a secondary effect due to the improved insulin sensitivity and glucose tolerance. Similar findings have been observed after PPAR γ agonism using pioglitazone, which also showed improved islet structure with greater beta cell granulation (Diani et al., 2004).

In conclusion, this study showed that long-term PPAR δ activation in diabetic db/db mice improves insulin sensitivity and islet function along with improved lipid profile. This profile of effects suggests that PPAR δ activation is of interest to explore further in the search for novel targets for improving islet dysfunction in type 2 diabetes.

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Table 1. Basal plasma parameters before and after 57 days of treatment of db/db mice with the PPAR δ agonist NCC 61-5920.

	Day 0			Day 57		
	db/+	db/db veh	db/db	db/+	db/db veh	db/db
	NCC61-5920			NCC61-5920		
Glucose (mM)	7.2 \pm 1.1	15.8 \pm 4.6	15.2 \pm 5.0	7.0 \pm 1.5	20.4 \pm 5.5	14.6 \pm 6.9**
(Non-Fasted)						
Glucose (mM)	5.2 \pm 0.9	11.7 \pm 2.2	11.9 \pm 3.2	5.6 \pm 0.6	13.3 \pm 7.1	9.0 \pm 4.4***
(Fasted)						
Insulin (pM)	67 \pm 28	1790 \pm 1120	1440 \pm 830	148 \pm 12	1370 \pm 1340	890 \pm 720
% HbA _{1c}	2.6 \pm 0.3	4.5 \pm 1.2	4.8 \pm 1.6	2.8 \pm 0.2	5.4 \pm 1.8	3.6 \pm 1.6*
HDL (mM)	1.9 \pm 0.3	3.1 \pm 0.5	3.0 \pm 0.5	1.8 \pm 0.4	2.6 \pm 0.8	3.4 \pm 0.8***
TG (mM)	1.2 \pm 0.4	1.5 \pm 0.3	1.2 \pm 0.3	1.0 \pm 0.3	1.1 \pm 0.3	0.8 \pm 0.2*

Basal blood samples were taken from the retrobulbar, intraorbital, capillary plexus in anesthetized mice. The results are expressed as mean \pm SEM. * P <0.05, ** P <0.01, *** P <0.001. HDL- high density lipoprotein, TG- triglyceride.

Figure legends

Fig. 1 A) Body weight in db/+ and db/db mice during the 8-week PPAR δ agonist (NNC 61-5920) treatment period. B) Average 24-h food intake in db/+ and db/db mice during the first 6 weeks of the study. Results are presented as mean \pm SEM.

Fig. 2 Oral glucose tolerance test (75 mg glucose/mouse) performed in over night fasted db/+ and db/db mice before and after 58 days of treatment with the PPAR δ agonist NCC 61-5920. Plasma levels of glucose at A) day 0 and at B) 58 days treatment and the corresponding insulin levels at C) day 0 and D) at day 58. Data are presented as mean \pm SEM, with n=6 in the different treatment groups.

Intravenous glucose tolerance test (1 g/kg glucose) was performed in 4-h fasted, anaesthetized mice after 60 days of treatment with the PPAR δ agonist NNC 61-5920 and in control mice db/+ and db/db administered with vehicle only. Plasma levels of E) glucose and F) insulin were measured at the indicated time points. Data are presented as mean \pm SEM, with n=7-9 in the different treatment groups.

Fig. 3 Diazoxide supplemented glucose-insulin test (DSGIT) was performed by injection of diazoxide ten minutes prior to an intravenous glucose bolus (1 g/kg) in 4-h fasted, anaesthetized mice after 60 days of treatment with the PPAR δ agonist NNC 61-5920. Plasma levels of glucose in mice treated with A) vehicle or B) NNC 61-5920 with corresponding insulin levels, C) and D), respectively. E) Mean glucose tolerance as KG5-20 min in relation to mean 1-5 min insulin peak in control db/db mice (filled square) and in PPAR δ -agonist treated mice. Data are presented as mean \pm SEM, with n=7-9 in the different treatment groups.

Fig. 4A) Insulin secretion from isolated islets from vehicle and NNC 61-5920 treated mice. Islets were isolated after 61 days of treatment with the PPAR δ agonist. Data are presented from two independent experiments where islets from two mice were pooled in each treatment group. Batches of three islets in eight replicates were analysed for insulin secretion. Data are presented as mean \pm SEM.

* $P < 0.05$, ** $P < 0.01$ B) Palmitate oxidation in isolated islets measured from vehicle or NCC 61-5920 treated db/db mice. Data are presented from two independent experiments where islets from two mice were pooled in each treatment group. Batches of 25 islets in quadruplicates were analysed for ^{14}C -palmitate oxidation. Data are presented as mean \pm SEM. ** $P < 0.01$. Islet morphology in C) db/+, db/db vehicle and db/db NNC 61-5920 treated mice (61 days). D) Beta and alpha cell mass were calculated in the three different groups. Data are presented as mean \pm SEM, with $n=4-6$ in the different treatment groups.

Fig. 5 Muscle palmitate oxidation in A) EDL and in B) soleus muscle and glucose oxidation in C) EDL and D) soleus muscle. Muscle insulin sensitivity was estimated by measurement of E) insulin-stimulated glycogen synthesis. The experiments were performed in db/+, db/db vehicle treated and in 61 days NCC 61-5920 treated mice. Data are presented as mean \pm SEM. * $P < 0.05$

Fig 1

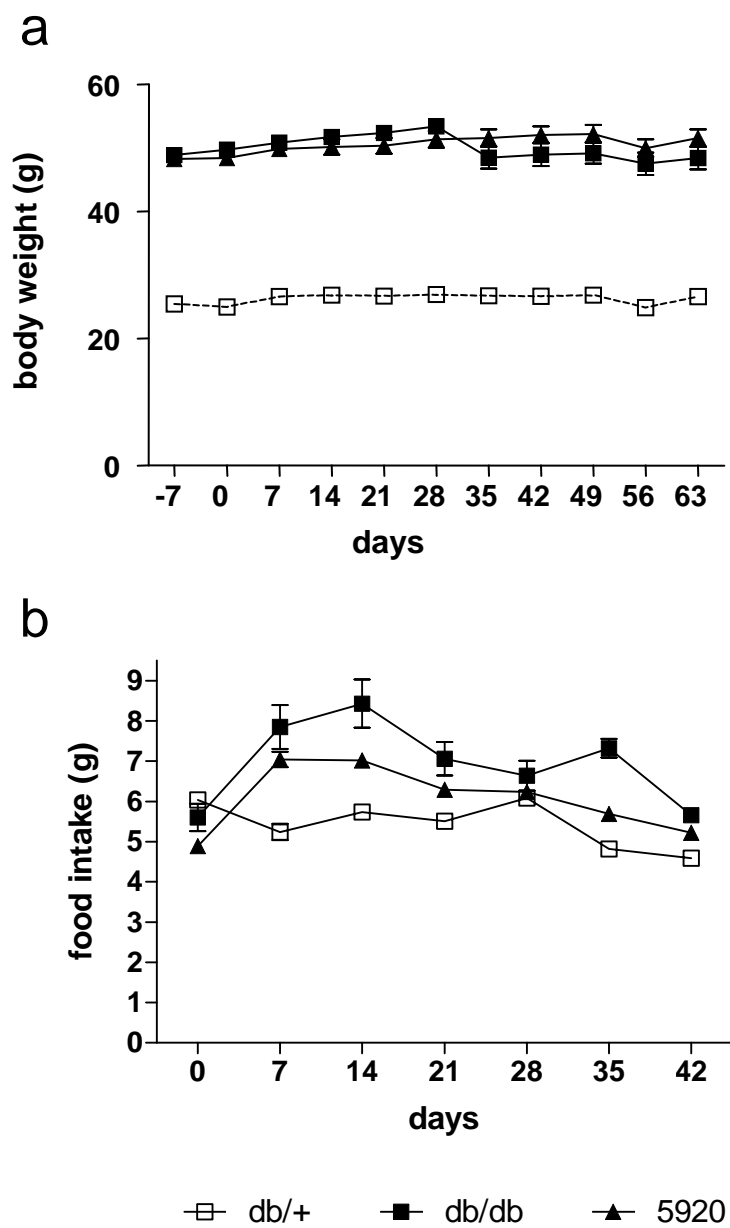
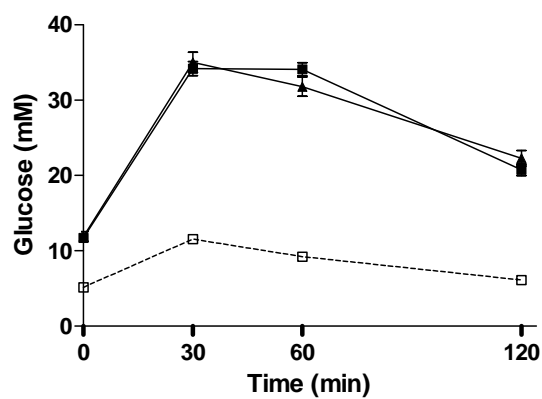
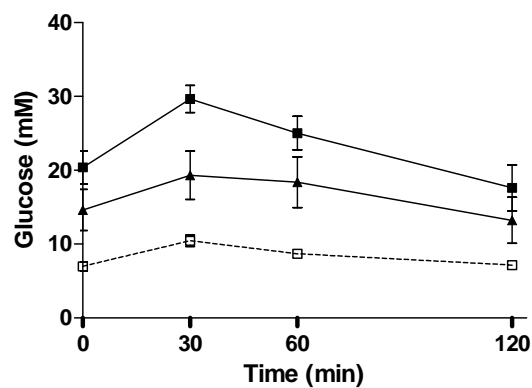


Fig 2

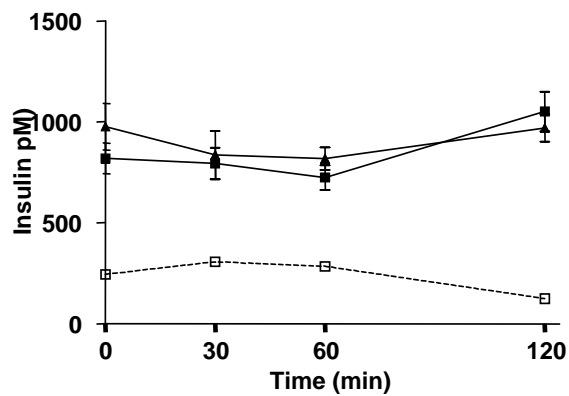
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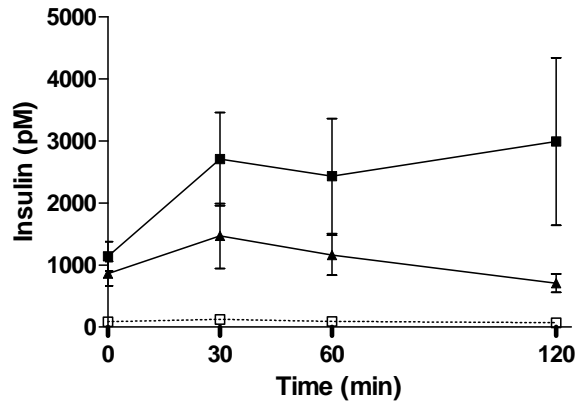
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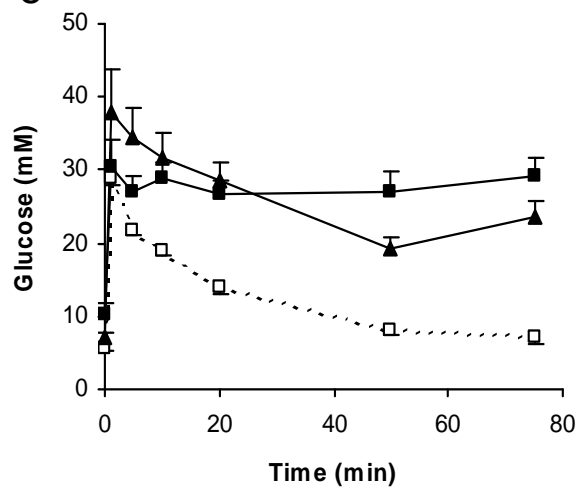
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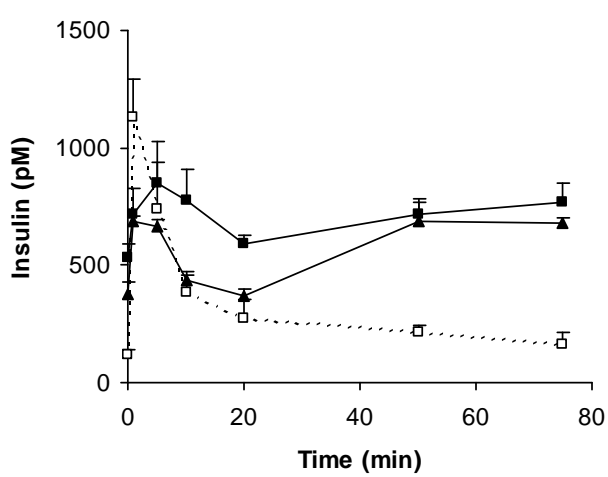
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---□--- db/+ —■— db/db —▲— 5920

Fig 3

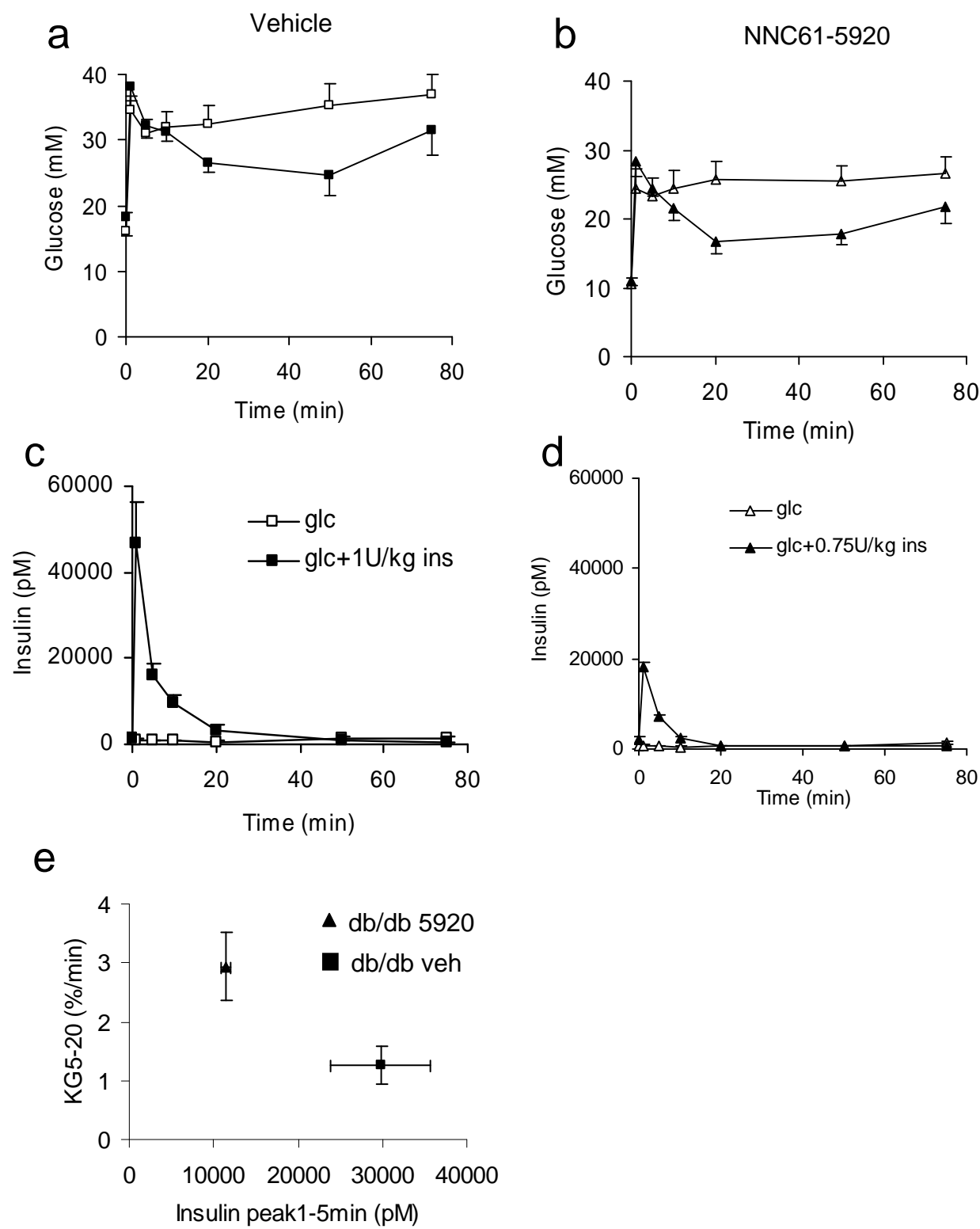


Fig 4

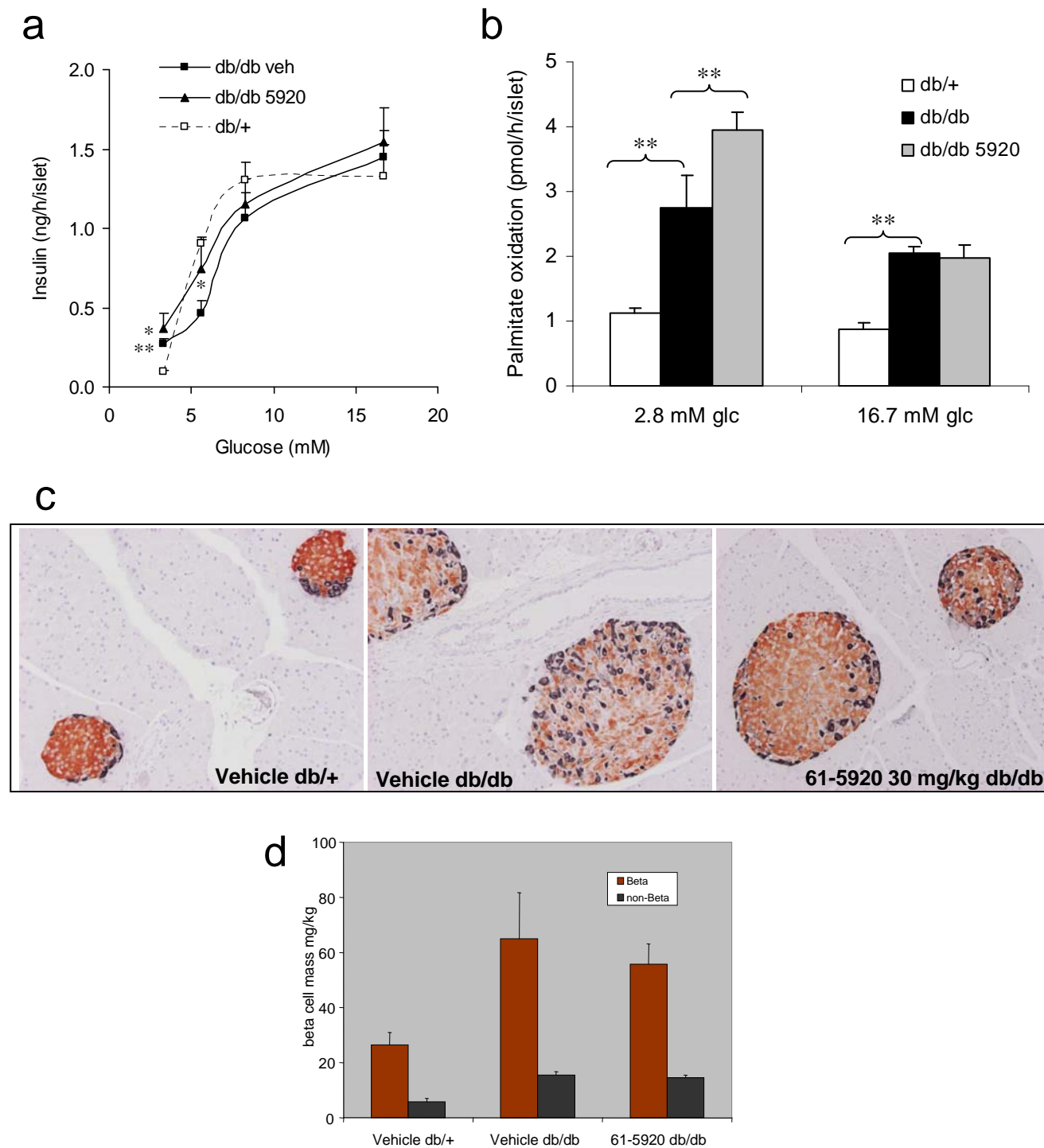


Fig 5

