Effects of Pioglitazone on Glucose Dependent Insulinotropic Polypeptide Mediated Insulin Secretion and Adipocyte Receptor Expression in Patients with Type 2 Diabetes

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Abstract
Incretin hormone dysregulation contributes to reduced insulin secretion and hyperglycemia in patients with Type 2 Diabetes Mellitus (T2DM). Resistance to glucose dependent insulinotropic polypeptide (GIP) action may occur through desensitization or down-regulation of β-cell GIP receptors (GIP-R). Studies in rodents and cell lines show GIP-R expression can be regulated through peroxisome proliferation-activated receptor-γ (PPARγ) response elements (PPRE). Whether this occurs in humans is unknown. To test this, we conducted a randomized, double-blind, placebo-controlled trial of pioglitazone therapy on GIP-mediated insulin secretion and adipocyte GIP-R expression in subjects with well controlled T2DM. Insulin sensitivity improved, but the insulinotropic effect of infused GIP was unchanged following 12 weeks of pioglitazone treatment. In parallel, we observed increased GIP-R mRNA expression in subcutaneous abdominal adipocytes from subjects treated with pioglitazone. Treatment of cultured human adipocytes with troglitazone increased PPARγ binding to GIP-R PPRE. These results show PPARγ agonists regulate GIP-R expression through PPRE in human adipocytes, but suggests this mechanism is not important for regulation of the insulinotropic effect of GIP in subjects with T2DM. Since GIP has anti-lipolytic and lipogenic effects in adipocytes, the increased GIP-R expression may mediate accretion of fat in patients with T2DM treated with PPARγ agonists.
**Introduction**

Obesity and Type 2 Diabetes Mellitus (T2DM) are growing global public health crises. World-wide, more than 650 million people are obese and more than 422 million are affected by T2DM[1]. The pathophysiology of T2DM is complex, with multiple metabolic abnormalities, including defects in incretin secretion and action, contributing to hyperglycemia in those with established disease. The incretin hormones Glucagon-Like Peptide-1 (GLP-1) and Glucose-Dependent Insulino tropic Polypeptide (GIP) are secreted by enteroendocrine cells following nutrient ingestion and are responsible for stimulating more than 50% of post-prandial insulin secretion[2]. Both GLP-1 and GIP are rapidly degraded by dipeptidyl peptidase-4 (DPP-4) following secretion into the circulation. The secretion and clearance of GLP-1 and GIP are largely normal in those with T2DM, however the insulinotropic effect to these hormones is markedly diminished[3, 4]. Blunted insulinotropic responses to GLP-1 can be overcome by infusions that achieve supraphysiologic levels, however even supraphysiologic GIP levels fail to stimulate insulin secretion in most patients with T2DM[5-7]. As a consequence, the development of incretin-based therapies for the treatment of T2DM has largely focused on the creation of GLP-1 analogues and inhibitors of DPP-4 action[8, 9].

The incretin hormones act through specific G-protein coupled membrane receptors that are widely expressed throughout the body[10]. The GIP receptor (GIP-R) is a GαS-protein coupled receptor found in many tissues including pancreatic islets, adipose tissue, stomach, central nervous system, adrenal cortex, bone, heart, and endothelium[11, 12]. In the presence of glucose, activation of the β-cell GIP-R induces insulin secretion, increases proinsulin synthesis, and promotes proliferative and survival pathways[12-14]. One theory supposes impairment of the insulinotropic effect of GIP in patients with T2DM to be due to decreased GIP-R levels in the pancreatic β-cells[15, 16]. The proposed reduction of GIP-R expression might occur through dysregulation of associated transcription factors and may be reversible with improved glycemic control[17-19]. However, simple down-regulation of the receptor would not explain preservation of early incretin responses with defective late phase responses in obese patients with T2DM[3].
In addition to β-cells, adipocytes are also an important target of GIP signaling. Fat ingestion is a strong stimulator of GIP secretion[20]. Activation of GIP-R in human adipocytes and cell lines in the presence of insulin increases lipoprotein lipase expression, promoting triglyceride storage[21, 22]. Global GIP-R knock-out mice show resistance to weight gain and elevated rates of fat oxidation when fed high-fat diets, effects that can be reversed with rescue of adipose tissue specific GIP-R expression[23-25]. Similarly, adipose specific GIP-R knock-out mice gained less weight on high-fat diets but without changes in fat oxidation[26]. GIP-R expression in human adipose tissue decreases with insulin resistance and with increasing BMI[27-29]. In patients with T2DM, the attenuated insulinotropic effect of GIP appears to be balanced by increased lipogenic activity, suggesting tissue specific alteration of GIP action[30]. Dysregulation of GIP-R signaling in adipocytes may contribute to insulin resistance, altered lipid metabolism, and adipose tissue dysfunction in obesity and T2DM.

The GIP-R promoter regions contain functional peroxisome proliferation activated receptor-γ (PPARγ) response elements (PPRE) which have been shown to increase GIP-R expression following thiazolidinedione (TZD) treatment in rodent islets and adipocyte cell lines[18, 31]. Patients treated with TZDs manifest increased insulin sensitivity and improved glucose control, but also demonstrate weight gain. The mechanisms underlying these effects are not well understood. We hypothesized that treatment with the TZD pioglitazone (PIO) would improve GIP resistance and increase the insulinotropic effect of GIP on β-cells. We also hypothesized that TZD treatment would increase GIP-R expression in human adipocytes and that this might be an important pathway mediating improved glycemic control, lipid metabolism, and weight gain. To test these hypotheses, we conducted a randomized, double-blind, placebo-controlled trial of PIO in 24 subjects with well-controlled T2DM. We assessed glycemic control, insulin secretory responses, and lipid regulation using oral and intravenous glucose tolerance tests and a GIP infusion, mixed meal tolerance testing, body composition, and adipose tissue gene expression before and after 12 weeks of treatment.

Methods

Subjects
Twenty-four subjects with well controlled type 2 diabetes (HbA1c <7.0%; 55 mmol/mol) treated with diet and exercise (n=9) or metformin (500-1000 mg/day; n=15) were enrolled in a 12-week, randomized, double-blinded, PIO (45mg/day, n=12) or placebo (PBO, n=12) controlled trial (NCT00656864). Subject characteristics are presented in Table 1. Subjects were excluded from the study if they had acute or chronic health conditions, such as heart or kidney failure or cirrhosis, or if they were currently taking or had taken a TZD, sulfonylurea, DPP-4 inhibitor, GLP-1 analog, insulin, or weight loss medication within the past 6 months. This study was approved by the University of Vermont Institutional Review Board. All subjects provided written informed consent prior to participating in any study procedures.

**Clinical Study Design**

Subjects had a screening visit to verify inclusion criteria and provide informed consent. Prior to randomization, all subjects had a baseline oral glucose tolerance test (OGTT), mixed meal test (MMT), frequently sampled intravenous glucose tolerance test (IVGTT), graded glucose infusion and assessment of GIP stimulated insulin secretion (GIP-SIS), measurement of body composition by whole body dual-energy x-ray absorptiometry (DXA), and subcutaneous abdominal adipose tissue biopsies. All procedures were performed after an overnight fast and were conducted with one week between each metabolic test. Following baseline testing, subjects were randomized to receive 45 mg/day PIO or PBO. After 12 weeks of treatment, subjects underwent a second round of metabolic testing, and adipose tissue biopsies. All procedures and visits occurred at the University of Vermont Diabetes Research Center or University of Vermont General Clinical Research Center. HbA1c and plasma lipids were measured at the Fletcher Allen Hospital clinical pathology lab according to standard methods.

**Glucose and Mixed Meal Tolerance Testing**

For the OGTT, 75g of glucose (300 kcal) was delivered as a flavored beverage. The MMT consisted of a 300 kcal beverage containing 50% carbohydrate, 15% protein, and 35% fat (Boost Plus®; Nestle, Vevey, Switzerland). Blood samples were collected at baseline and every 30 min for 3 hours for both tests. The IVGTT consisted of an
intravenous bolus of 0.3g dextrose / kg body weight followed by an insulin bolus (0.06 IU / kg body weight) 20 minutes later[32]. All blood samples were collected on ice and aliquoted for measurement of glucose (K+ Oxalate tubes), insulin and c-peptide (serum-separating tubes), and total incretin hormones (K+ EDTA with aprotinin; Bayer Pharmaceuticals, West Haven, CT). Glucose was measured by the glucose oxidase method (YSI Inc, Yellow Springs, OH), insulin by immunoassay (Alpco, Salem, NH), c-peptide by immunoassay (Meso-Scale Diagnostics, Rockville, MD), non-esterified free fatty acids (FFA) by an enzymatic colorimetric method (WAKO Diagnostics, Mountain View, CA). Incretin hormones were measured using specific C-terminally directed assays detecting total GLP-1 and GIP[33]. Insulin sensitivity was modeled from the OGTT (OGIS) according to the method of Mari et al[34]. Insulin sensitivity (S_i) and the acute insulin response to glucose (AIR_g) were modeled from the IVGTT using the Minimal Model[35]. Insulin secretion rates were calculated from c-peptide levels by population deconvolution[36].

**Graded Glucose Infusion and GIP Stimulated Insulin Secretion**
Following each IVGTT, subjects underwent graded glucose infusion followed by a GIP infusion. After the 3-hour IVGTT, a 20% dextrose was administered at 300mL/hr for 30 min, 450mL/hr for 30 min, then the dextrose infusion was continued while GIP (2 pmol/kg/min) was co-infused for another 30 minutes. Glucose stimulated insulin secretion (GSIS) was calculated as a linear insulin to glucose ratio during the first portion of the graded glucose infusion. GIP-SIS was calculated as the incremental difference between the insulin concentration for a given glucose along the GSIS line and the plasma insulin level during the GIP infusion. Glucose stimulated insulin secretion rates (GS-ISR) and GIP stimulated insulin secretion rates (GIP-SISR) were calculated by the same method using insulin secretion rates derived from c-peptide data.

**Adipose Tissue Biopsy and Fractionation**
Subcutaneous peri-umbilical abdominal adipose tissue was obtained by percutaneous needle biopsy under local anesthesia. Tissue was aspirated into sterile phosphate buffered saline and washed twice to remove any blood. Whole tissue samples were then frozen in
liquid nitrogen and stored at -80°C. Adipose tissue from needle biopsies were also incubated in saline with collagenase (Worthington Biochemical Corp, Lakewood, NJ) at 37°C for 80 minutes, passed through a 200 µm pore filter, and centrifuged to pellet stromal vascular cells (SVC). Following centrifugation, tubes were left for 5 minutes allowing adipocytes to separate by floatation. Isolated adipocytes and SVCs were then snap frozen in liquid nitrogen, and stored at -80°C.

**Human Adipocyte Culture**

Preadipocytes were cultured and differentiated into adipocytes using previously established protocols with slight modifications[37]. Preadipocytes were expanded from stromal vascular fractions in growth media (αMEM with nucleosides; Gibco ThermoFisherScientific, Waltham, MA), 2.5% fetal bovine serum (Gibco), 10 ng/mL human epidermal growth factor (Gibco), 1 ng/mL human fibroblast growth factor-2 (Gibco), 5.5 mM glucose, and 10 nM human insulin (Humulin R; Eli Lilly and Co, Indianapolis, IN) at 37°C with 5% CO₂ for no more than two passages. Preadipocytes were sub-cultured at 4.5 X 10⁴ per well in 6-well plates for 10 days. Media were changed after the first 24 hours, then every 72 hours. Preadipocyte plates were triggered to differentiate using αMEM/F12 with 17.5 mM glucose (Gibco), 15 mM HEPES (Gibco), 2 nM triiodothyronine (T3; Sigma-Aldrich, St. Louis, MO), 10 mg/L transferrin (Sigma), 17 µM pantothenate (Sigma), 33 µM biotin (Sigma), 100 nM dexamethasone (Sigma), 1 µM troglitazone (Sigma), 0.5 mM 3-isobutyl-1-methylxanthine (Sigma), and 100 nM human insulin. Cells were incubated in the trigger medium for 4 days, then changed to maintenance media (DMEM/F12 with 5.5 mM glucose, 10 nM insulin, 15 mM HEPES, 1 nM T3, 10 mg/L transferrin, 17 µM pantothenate, 33 µM biotin, 10 nM dexamethasone). Adipocytes were allowed to mature for 12 days and media were changed every 72 hours. For the final 72 hours, paired cultures were treated with 10 µM troglitazone or vehicle to activate PPARγ for chromatin immunoprecipitation assays.

**Gene Expression Analysis**

Adipose tissue and adipocyte RNA were extracted using RNeasy Lipid Tissue Mini Kits (Qiagen; Valencia, CA) with RNase-Free DNase treatment (Qiagen). A cDNA library
was made using Advantage-RT-for-PCR kits (Clontech; Mountain View, CA) using 1 ug RNA template and oligo-dT primers. Gene expression was measured by real-time PCR on an Applied Biosystems 7300 (Life Technologies Inc.; Carlsbad, CA) using TaqMan primers for PPARγ (Hs01115510_m1) and GIP-R (Hs00609210_m1). All expression data were normalized to GAPDH (4326317E). Relative expression levels were calculated using the -ΔΔCt method.

Chromatin Immunoprecipitation (ChiP) Assay
Chromatin immunoprecipitation assay was performed on cultured human adipocytes treated with 10 uM troglitazone or vehicle (DMSO) for 72 hr using ChIP-IT kit (Active Motif; Carlsbad, CA). Cells were fixed using formaldehyde solution and chromatin sheared by enzymatic digestion into DNA fragments averaging 300-500 bp. Rabbit polyclonal antibody against PPARγ (PA3-821A; Thermo-Fisher) was incubated overnight with precleared chromatin, and parallel samples were incubated with negative-control IgG provided with the kit. Protein G-agarose beads were then added and incubated 1.5 h at 4°C. After reversing the cross-links, DNA was isolated and real-time PCR (ABI 7300) was performed with target validated SYBR Green primer pairs (Qiagen, EpiTect Human GIP-R ChIP assay, Cat No. GPH100697(-) 04kb) for the flanking regions of human GIP-R PPRE promoter (Gene Bank: NM_000164.2, Chr19:46167667-46167684). PCR conditions were 1 cycle of 95°C for 10 min, 40 cycles of 95°C for 15 s, 60°C for 1 min. The ChiP qPCR DNA fractions for PPARγ and NIS (non-immune serum) were normalized with input DNA to get ΔCt value and ΔΔCt was obtained by normalizing NIS background. The fold enrichment of PPARγ on GIP-R promoter was calculated by $2^{-\Delta\Delta C_t[PPAR/NIS]}$. The ChiP was performed on three independent human adipocyte cultures for vehicle (DMSO) or troglitazone treated samples.

Statistical Analysis
Study data were collected using REDCap electronic data capture tools hosted at the University of Vermont (REDCap Consortium; Vanderbilt, TN). All data were loaded into STATA v11.2 (StataCorp LP; College Station, TX). Demographic, metabolic, and ChiP data were normally distributed. Gene expression data underwent natural logarithm
transformation prior to analysis. Categorical data were analyzed by Fisher’s exact test. Continuous data were analyzed by t-test, paired where appropriate. Pre-specified primary outcomes were change in GIP-SIS and GIP-R expression. *A priori* power calculation indicated 80% power to detect 120% change in either parameter with 95% confidence. The study was not powered for multiple covariate analysis, but exploratory analysis with simple linear regression modeling was conducted using pre-study values as a covariate. All data are presented as mean±SD. A p-value <0.05 was considered significant.

**Results**

*Changes in Weight and Glycemic Control after Pioglitazone Treatment*

At baseline, the age, weight, body composition, glycemic control, use of metformin and statins and all measured parameters were similar between subjects that were randomized to the PIO and PBO group ([Table 1](#)). After 12 weeks of treatment, subjects on PIO gained more weight than those on PBO (p<0.001), increased their BMI (p<0.001), and increased DXA fat mass (p=0.008) ([Supplementary Table 1](#)). Overall glycemic control, measured by HbA1c was significantly better in subjects on PIO compared to PBO (p=0.04). Treatment with PIO reduced fasting glucose levels (p=0.008) and fasting insulin concentrations trended lower compared to PBO (p=0.09). The homeostatic model of insulin resistance (HOMA-IR) was reduced in subjects treated with PIO (p=0.03).

*Pioglitazone Increased Insulin Sensitivity without Altering Incretin Release*

Insulin sensitivity (S_i) derived from IVGTT was increased by treatment with PIO (p=0.03) while first phase insulin secretion (AIR_g) was unchanged compared to PBO (p=0.7) ([Figure 1](#)). Areas under the curve (AUC) for glucose and insulin during the OGTT were significantly lower with PIO treatment compared to PBO (p=0.002 and 0.003, respectively) ([Figure 2](#)). Insulin sensitivity derived from the OGTT (OGIS) was significantly improved by treatment with PIO (p=0.002). GLP-1 and GIP release following OGTT was not changed by treatment with PIO (p=0.15 and p=0.97, respectively) ([Supplementary Figure 1](#)). Subjects treated with placebo did have a higher GLP-1 AUC at the end of the study compared to baseline (p=0.008), but the post-study levels did not differ between PBO and PIO treatment (p=0.7). Subjects treated with
PIO had decreased glucose AUC during the MMT (p=0.006) compared to placebo (Figure 3). Insulin AUC trended lower with PIO treatment, but did not reach statistical significance (p=0.09). GLP-1 and GIP levels in response to the MMT were not changed by treatment with PIO (p=0.75 and p=0.40, respectively) (Supplementary Figure 2).

**GIP Stimulated Insulin Secretion was not changed by Pioglitazone**

Insulin levels increased linearly during the glucose infusion with the slope of the line defining GSIS (Figure 4). Treatment with PIO reduced GSIS, but this change was not statistically significant (p=0.07). The increase in circulating insulin above GSIS after GIP infusion (GIP-SIS) was reduced following treatment with PIO (p=0.03) and unchanged in those receiving PBO (p=0.9). Due to potential changes in hepatic insulin extraction, we also calculated insulin secretion rates (ISR) from c-peptide levels (Figure 5). The GS-ISR was reduced ~50% in subjects treated with PIO (p<0.001), however, the GIP-SISR was not changed by treatment with PIO (p=0.5).

**Pioglitazone improves Lipid Profile and Free Fatty Acid Suppression**

Circulating triglycerides and cholesterol / HDL ratios were significantly reduced by treatment with PIO (p=0.04 and 0.03, respectively), while total cholesterol, HDL, and LDL were not (p>0.3). Free fatty acid suppression during IVGTT was increased from 68% to 85% following treatment with PIO (p=0.007), and increased from 57% to 72% during MMT (p=0.04) (Figure 6).

**Adipose Gene Expression Data**

Expression of PPARγ in adipocytes was not different between subjects randomized to PIO or PBO at baseline or following the 12 weeks of treatment (Figure 7a). Subjects treated with PIO had increased adipocyte GIP-R expression (p=0.015) while those treated with PBO did not (p=0.15; Figure 7b). Post-study GIP-R expression was associated with increased FFA suppression during IVGTT (p=0.02) and reductions in cholesterol / HDL ratio (p=0.002) by simple linear regression, but had no association with alterations in glucose and insulin homeostasis, weight, or changes in body composition after controlling for pre-study gene expression. Treatment with PIO and metformin was
associated with an attenuated increase in GIP-R expression compared to PIO alone (p=0.009 for the interaction).

**PPARγ directly regulates GIP-R expression in Human Adipocytes**

Since adipose GIP-R gene expression was increased in subjects treated with PIO, we asked if this was a direct effect of PPARγ binding. We treated differentiated human adipocyte cultures with 10 uM troglitazone or vehicle in parallel (n=3 paired cultures). PPARγ was bound to the GIP-R promoter in cultures treated with vehicle and treatment with troglitazone increased the binding by more than twofold (p=0.03; Figure 7c). These data show that PPARγ binds to the human GIP-R promoter and that treatment with a TZD increases PPARγ occupancy of the GIP-R PPRE.

**Discussion**

Impairment of incretin function is a hallmark of type 2 diabetes mellitus but mechanisms involved in the dysregulation of the incretin system are not fully delineated. PPARγ (NR1C3 gene) is a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors. As a transcriptional regulator, PPARγ must be ligand activated, undergo heterodimerization with retinoid X receptors (RXR: NR2B), recruit cofactors, and recognize a response element (PPRE) in the promoter region of target genes[38-40]. Transcriptional activation of PPARγ induces diverse biological actions, including lipid metabolism, cellular growth and differentiation. TZDs are orally active agents that are high-affinity activators of PPAR-γ[41]. Clinical trials of TZDs demonstrate improved glucose and lipid control, enhanced peripheral insulin sensitivity, and preservation of β-cell function in prediabetes and early T2DM compared to other therapies[42, 43].

Contrary to our hypothesis, we found that treatment with PIO did not increase the insulinotropic effect of GIP in subjects with well-controlled T2DM. The lack of effect likely reflects the relatively good glycemic control of these study subjects. It is possible that β-cell expression of GIP-R was not down-regulated and therefore could not be enhanced with PIO. This notion is supported by robust pre-study insulin secretion in
response to GIP infusion. These subjects had well-controlled T2DM and, possibly, a preserved insulinotropic incretin response. Conversely, the effects of PIO treatment along other pathways or in other tissues may predominate, resulting in a lack of change in response to incretins. Subjects treated with PIO did have significant improvement of insulin sensitivity with increased $S_i$ and OGIS in concert with reduced GS-IS. In addition to the lack of improvement in GIP-SISR, we did not observe any alteration in first phase insulin secretion or GSIS by PIO treatment. These results are consistent with an overall improvement in peripheral glucose disposal without an increase in $\beta$-cell function, suggesting islet incretin function was not impaired at baseline.

PIO treatment caused increases in fat mass, reduced circulating triglycerides and the cholesterol / HDL ratio, and increased FFA suppression during IVGTT and MMT, effects consistent with amplified lipid storage. While we do not have direct evidence of a functional effect of increased adipocyte GIP-R expression, the change in adipose GIP-R could contribute to the well-described weight gain and increases in subcutaneous adipose tissue that occur with TZD treatment[44]. Acute GIP infusions in obese subjects with untreated type 2 diabetes resulted in more FFA suppression and subcutaneous adipose triglyceride storage during a hyperglycemic clamp compared to placebo[30]. This lipogenic effect was not seen in metabolically normal lean or obese subjects, or in obese subjects with impaired glucose tolerance, causing the authors to conclude that there is a dissociation of the lipogenic and insulinotropic actions of GIP in type 2 diabetes which may increase obesity and insulin resistance. We found associations between increased adipocyte GIP-R expression and improved lipid metabolism, but whether elevated adipocyte GIP-R contributes to fat accumulation or improved lipid metabolism during PPAR$\gamma$ agonist treatment requires further investigation.

The PPAR family has a wide cistrome driving a range of gene promoters, thus, our observed effects may represent contributions from other pathways promoted by PPAR binding[38]. Our results suggest dysregulated adipocyte GIP-R may be a key feature of the altered incretin response in type 2 diabetes, but the functional role of adipose GIP-R in type 2 diabetes is not fully understood[45]. Diminished GIP or discordant action in clinical and experimental models of T2DM may be directly related to tissue specific down-regulation of GIP-R or reduced recycling of the receptor to the cell
surface[10, 30, 45]. Obesity may be associated with down regulation of GIP-R in adipose tissue and adipocytes which could contribute to insulin resistance[27-29]. We speculate that reduced adipose GIP-R expression could result from attenuated adipose PPARγ signaling and may be one mechanism causing incretin dysfunction in T2DM. A clearer understanding of the functional changes of GIP-R signaling induced by type 2 diabetes is especially important as dual incretin agonists have a more profound effect on weight loss than GLP-1-R agonists[46]. Emerging therapies utilizing direct GLP-1 and GIP agonists will benefit greatly from a more thorough understanding of extra-pancreatic incretin biology. Our results suggest the potential for synergy of TZD and incretin agonist therapy[47].

**Acknowledgements**

W.G.T assisted with conduct of the clinical study, assisted with ChIP experiment design, performed adipose culture, analyzed the data, and wrote the manuscript. D.G. designed and performed the ChIP experiments, assisted with adipose culture, and edited the manuscript. O.S. processed adipose tissue biopsies and conducted gene expression analyses. C.F.D. and J.J.H. performed and interpreted incretin hormone measurements and edited the manuscript. D.E. provided the GIP hormone, assisted with study analysis and interpretation, and edited the manuscript. R.E.P. designed and conducted the clinical study, oversaw data analyses, and edited the manuscript. W.G.T and R.E.P are the guarantors of this work, had full access to all the data, and take responsibility for the accuracy and integrity of the data and analyses.

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Reference


30. Thondam, S.K., et al., *Glucose-dependent insulinogetic polypeptide promotes lipid deposition in subcutaneous adipocytes in obese type 2 diabetes patients:*


Table 1 – Baseline Clinical Data

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Data are presented as counts or mean ± standard deviation

No differences between groups by t-test of Fisher’s exact test
Figure Legends

Figure 1 – Intravenous Glucose Tolerance Test. Plasma glucose (solid lines; black square – before; white circle – after) and insulin levels (dashed lines; black triangle – before; white diamond – after) during intravenous glucose tolerance test before and after treatment with placebo (a) or pioglitazone (b).

Figure 2 – Oral Glucose Tolerance Test. Plasma glucose levels during oral glucose tolerance test before (black square) and after (grey circle) intervention in subjects treated with pioglitazone (PIO) (b) or placebo (PBO) (a). Plasma insulin levels for subjects treated with pioglitazone (d) or placebo (c). Total glucose excursion (e) and insulin secretion (f) measured by the area under the curve (AUC) presented as box and whisker plots (dark grey – before; light grey – after). * p < 0.01 by Students t-test; n.s. – non-significant.

Figure 3 – Mixed Meal Test. Plasma glucose levels during mixed meal test before (black square) and after (grey circle) intervention in subjects treated with pioglitazone (PIO) (b) or placebo (PBO) (a). Plasma insulin levels for subjects treated with pioglitazone (d) or placebo (c). Total glucose excursion (e) and insulin secretion (f) measured by the area under the curve (AUC) presented as box and whisker plots (dark grey – before; light grey – after). * p < 0.01 by Students t-test; n.s. – non-significant.

Figure 4 – GIP Stimulated Insulin Secretion. Plasma glucose (solid lines; black square – before; white circle – after) and insulin levels (dashed lines; black triangle – before; white diamond – after) during intravenous glucose and GIP infusion before and after treatment with placebo (a) or pioglitazone (b). Scale for glucose is on the left; insulin on the right. The arrows mark the beginning of the glucose and GIP infusions. Insulin secretion analyses before and after treatment with placebo (c) or pioglitazone (d). Dashed lines represent glucose-stimulated insulin secretion (GSIS) and the increment above GSIS following GIP infusion defines the GIP-SIS.

Figure 5 – GIP Stimulated Insulin Secretion Rate. Plasma glucose (solid lines; black square – before; white circle – after) and c-peptide concentration (dashed lines; black triangle – before; white diamond – after) during intravenous glucose and GIP infusion before and after treatment with placebo (a) or pioglitazone (b). Plasma glucose and calculated insulin secretion rate (ISR) before and after treatment with placebo (c) or pioglitazone (d). Scale for glucose is on the left; c-peptide or insulin secretion rate on the right. The arrows mark the beginning of the glucose and GIP infusions. Insulin secretion rate analyses before and after treatment with placebo (e) or pioglitazone (f). Dashed lines represent glucose-stimulated insulin secretion rate (GSISR) and the increment above GSISR following GIP infusion defines the GIP-SISR.

Figure 6 – Free Fatty Acid Suppression. Plasma non-esterified free fatty acids (FFA) before (solid lines; black squares) and after (dashed lines; white circles) treatment with placebo (PBO) or pioglitazone (PIO) during intravenous glucose tolerance testing (IVGTT) (a, b) or mixed meal testing (MMT) (c, d). Percent suppression of circulating
FFA during intravenous glucose tolerance test (e) and mixed meal testing (f) presented as box and whisker plots (dark grey – before; light grey – after). Note the inverted scale. * p < 0.01 by Students t-test; n.s. – non-significant.

**Figure 7 – Adipocyte GIP-R Expression is Directly Increased by PPARγ Activation.**

PPARγ (a) and GIP-R (b) expression in isolated subcutaneous adipocytes from subjects before (dark grey) and after (light grey) treatment with pioglitazone (PIO; n=11) or placebo (PBO; n=9). Cultured human adipocytes treated with 10 uM troglitazone (TZD; n=3) for 72 hours had more PPARγ bound copies of the GIP-R PPRE compared to DMSO vehicle (PBO; n=3) (c). * p < 0.05 by Students t-test; n.s. – non-significant.
Figure 1 – Intravenous Glucose Tolerance Test. Plasma glucose (solid lines; black square – before; white circle – after) and insulin levels (dashed lines; black triangle – before; white diamond – after) during intravenous glucose tolerance test before and after treatment with placebo (a) or pioglitazone (b).
Figure 2 – Oral Glucose Tolerance Test. Plasma glucose levels during oral glucose tolerance test before (black square) and after (grey circle) intervention in subjects treated with pioglitazone (PIO) (b) or placebo (PBO) (a). Plasma insulin levels for subjects treated with pioglitazone (d) or placebo (c). Total glucose excursion (e) and insulin secretion (f) measured by the area under the curve (AUC) presented as box and whisker plots (dark grey – before; light grey – after). * p < 0.01 by Students t-test; n.s. – non-significant.
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Supplementary Table 1 – Follow-Up Clinical Data

<table>
<thead>
<tr>
<th>Change from Baseline</th>
<th>Placebo</th>
<th>Pioglitazone</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (Δ kg)</td>
<td>−0.5 ± 2.8</td>
<td>2.6 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (Δ kg/m²)</td>
<td>−0.2 ± 1.0</td>
<td>1.0 ± 0.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DXA Fat Free Mass (Δ kg)</td>
<td>−0.6 ± 2.6</td>
<td>0.2 ± 2.0</td>
<td>0.8</td>
</tr>
<tr>
<td>DXA Fat Mass (Δ kg)</td>
<td>−0.4 ± 2.1</td>
<td>1.6 ± 1.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Hemoglobin A1c Δ %</td>
<td>0.15 ± 0.14</td>
<td>−0.27 ± 0.39</td>
<td>0.04</td>
</tr>
<tr>
<td>(Δ mmol/mol)</td>
<td>(1.5 ± 1.4)</td>
<td>(−2.8 ± 4.4)</td>
<td></td>
</tr>
<tr>
<td>Fasting Glucose (Δ mM)</td>
<td>0.3 ± 0.7</td>
<td>−0.9 ± 1.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Fasting Insulin (Δ pM)</td>
<td>2.1 ± 31.7</td>
<td>−37.3 ± 67.2</td>
<td>0.09</td>
</tr>
<tr>
<td>HOMA-IR (Δ)</td>
<td>0.3 ± 1.7</td>
<td>−1.8 ± 2.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Sₗ (Δ pM*min⁻¹/10⁻⁵)</td>
<td>−0.2 ± 0.6</td>
<td>0.3 ± 0.4</td>
<td>0.03</td>
</tr>
<tr>
<td>AIR-g (Δ pM*min⁻¹/10⁻²)</td>
<td>1.7 ± 7.4</td>
<td>−1.1 ± 10.2</td>
<td>0.7</td>
</tr>
<tr>
<td>OGIS (Δ mL<em>min⁻¹</em>m⁻²)</td>
<td>10.5 ± 45.0</td>
<td>49.2 ± 39.5</td>
<td>0.002</td>
</tr>
<tr>
<td>GSIS (Δ pM / mM)</td>
<td>−0.03 ± 0.08</td>
<td>−0.03 ± 0.05</td>
<td>0.07</td>
</tr>
<tr>
<td>GIP-SIS (Δ pM)</td>
<td>−4.1 ± 111.5</td>
<td>−94.5 ± 126.8</td>
<td>0.03</td>
</tr>
<tr>
<td>GSISR (Δ pM<em>kg⁻¹</em>min⁻¹ / mM)</td>
<td>0.06 ± 0.09</td>
<td>−0.16 ± 0.21</td>
<td>0.02</td>
</tr>
<tr>
<td>GIP-SISR (Δ pM<em>kg⁻¹</em>min⁻¹)</td>
<td>−0.4 ± 1.1</td>
<td>−0.9 ± 1.2</td>
<td>0.5</td>
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<tr>
<td>Triglycerides (Δ mg/dL)</td>
<td>−7.8 ± 23.8</td>
<td>−28.6 ± 41.3</td>
<td>0.04</td>
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<tr>
<td>Total Cholesterol (Δ mg/dL)</td>
<td>−8.5 ± 32.1</td>
<td>−7.7 ± 20.6</td>
<td>0.2</td>
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<tr>
<td>LDL (Δ mg/dL)</td>
<td>0.6 ± 19.6</td>
<td>−4.8 ± 12.3</td>
<td>0.2</td>
</tr>
<tr>
<td>HDL (Δ mg/dL)</td>
<td>−0.2 ± 3.7</td>
<td>2.4 ± 7.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Cholesterol / HDL Ratio (Δ)</td>
<td>−0.1 ± 0.9</td>
<td>−0.4 ± 0.5</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation
P-values from paired Student’s t-test of pre and post study data values

**Supplementary Figure 1** – Incretin Secretion During OGTT. Plasma glucagon like peptide-1 (GLP-1) levels during oral glucose tolerance test before (black square) and
after (grey circle) intervention in subjects treated with pioglitazone (PIO) (b) or placebo (PBO) (a). Plasma glucose dependent insulinotropic polypeptide (GIP) levels for subjects treated with pioglitazone (d) or placebo (c). Total GLP-1 excursion (e) and GIP secretion (f) measured by the area under the curve (AUC) presented as box and whisker plots (dark grey – before; light grey – after). * p < 0.01 by Students t-test; n.s. – non-significant.

**Supplementary Figure 2** – Incretin Secretion During MMT. Plasma glucagon like peptide-1 (GLP-1) levels during mixed meal test before (black square) and after (grey circle) intervention in subjects treated with pioglitazone (PIO) (b) or placebo (PBO) (a). Plasma glucose dependent insulinotropic polypeptide (GIP) levels for subjects treated with pioglitazone (d) or placebo (c). Total GLP-1 excursion (e) and GIP secretion (f) measured by the area under the curve (AUC) presented as box and whisker plots (dark grey – before; light grey – after). n.s. – non-significant by Student’s t-test.

**Supplementary Figure 3** – GIP-R Peroxisome Proliferator Response Element Schematic. Comparative sequences of rodent and human GIP-R PPRE over schematic of the PPARγ binding site upstream of the GIP-R transcription site and the location targeted by chromatin immunoprecipitation PCR (ChIP-qPCR).
Supplementary Figure 1 – Incretin Secretion During OGTT

(a) Placebo

(b) Pioglitazone

(c) Placebo

(d) Pioglitazone

(e) AUC GLP-1 (pM*180min^-1)

(f) AUC GIP (pM*180min^-1)
Supplementary Figure 2 – Incretin Secretion During MMT

(a) Placebo

(b) Pioglitazone

(c) Placebo

(d) Pioglitazone

(e) AUC GLP-1 (pmol/min)

(f) AUC GIP (pmol/min)

PBO  PIO

n.s. n.s. n.s. n.s.
Supplementary Figure 3 – GIP-R Peroxisome Proliferator Response Element

Mouse GIPR PPRE : CCCATG-G-AGGTCA
Rat GIPR PPRE   : CCCATG-G-AGGTCA
Human GIPR PPRE : GGGAGA-C-AGGGTA