Pioglitazone decreases fasting and postprandial endogenous glucose production in proportion to decrease in hepatic triglyceride content

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Short running title: Pioglitazone and endogenous glucose production

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Objective: Hepatic triglyceride is closely associated with hepatic insulin resistance and is known to be decreased by thiazolidinediones. We studied the effect of pioglitazone on hepatic triglyceride content and consequent effect on postprandial endogenous glucose production (EGP) in type 2 diabetes.

Research Design And Methods: 10 subjects with type 2 diabetes on sulfonylurea therapy were treated with pioglitazone (30mg daily) for 16 weeks. EGP was measured using a dynamic isotopic methodology after a standard liquid test meal both before and after pioglitazone treatment. Liver and muscle triglyceride levels were measured by $^1$H magnetic resonance spectroscopy and intra-abdominal fat content measured by magnetic resonance imaging.

Results: Pioglitazone treatment reduced mean plasma fasting glucose and mean peak postprandial glucose levels. Fasting EGP decreased after pioglitazone treatment (16.6 ± 1.0 vs. 12.2 ± 0.7 μmol • kg$^{-1}$ • min$^{-1}$, p=0.005). Between 80 – 260 minutes post-prandially, EGP was two-fold lower on pioglitazone (2.58 ± 0.25 vs. 1.26 ± 0.30 μmol • kg$^{-1}$ • min$^{-1}$, p< 0.001). Hepatic triglyceride content decreased by ~ 50% (p = 0.03) and muscle (anterior tibialis) triglyceride content decreased by ~ 55% (p = 0.02). Hepatic triglyceride content was directly correlated with fasting EGP (r = 0.64, p = 0.01) and inversely correlated to percentage suppression of EGP (time 150 min, r = -0.63, p = 0.02). Muscle triglyceride, subcutaneous fat and visceral fat content were not related to EGP.

Conclusions: Reduction in hepatic triglyceride by pioglitazone is very closely related to improvement in fasting and postprandial EGP in type 2 diabetes.
Hepatic triglyceride has been shown to be strongly associated with hepatic insulin resistance in type 2 diabetes [1-3]. The exact mechanism by which hepatic triglyceride induces hepatic insulin resistance is unknown but is thought to relate to accumulation of intracellular fatty acid metabolites and consequent activation of a serine kinase cascade and induction of cellular insulin resistance [4]. Reduction in hepatic triglyceride content by moderate weight reduction normalizes rates of basal endogenous glucose production (EGP) in patients with type 2 diabetes [2]. Hepatic insulin resistance is also associated with impaired postprandial suppression of EGP in type 2 diabetes, but the effect of reduction of hepatic triglyceride content on postprandial suppression of EGP in type 2 diabetes is unknown.

Thiazolidinediones (TZDs) such as pioglitazone, possess insulin sensitizing properties and have been shown to decrease hepatic triglyceride content in type 2 diabetes [5]. As TZDs have been shown to reduce both fasting and postprandial glucose levels [6, 7], we hypothesized that pioglitazone treatment in type 2 diabetes would reduce hepatic triglyceride content and consequently reduce basal and postprandial EGP. In addition, visceral fat has also been implicated in hepatic insulin resistance and pioglitazone has been reported to decrease visceral fat content [8]. The effect of this on EGP requires to be defined.

To test our hypothesis, we used non-invasive $^1$H magnetic resonance spectroscopy (MRS) to assess hepatic triglyceride and intramyocellular triglyceride content and magnetic resonance imaging (MRI) to quantify intra-abdominal fat content in ten type 2 diabetes patients before and after treatment with pioglitazone treatment. EGP was measured using a dynamic isotopic methodology after a standard liquid test meal.

RESEARCH DESIGN AND METHODS

Subjects. We studied 10 sub-optimally controlled healthy subjects with type 2 diabetes (6M;4F, nine Caucasians, one Asian; \( \geq 2 \) years duration; mean age 52.1 ± 2.8 years (range: 38-64 years); HbA1c > 7.5%; no history of weight loss or ketonuria at diagnosis) on maximum tolerated sulfonylurea treatment, who required additional anti-diabetes medications. Apart from sulfonylurea treatment, no subject was taking any medications that would affect glucose or lipid metabolism and subjects on statins were excluded. Subjects who had previous treatment with metformin, thiazolidinediones, or insulin, and those with history of non-compliance with treatment were excluded. Two subjects were on tolbutamide, three were on gliclazide and five were on gliclazide treatment.

Study design. After a four-week run-in period to ensure metabolic stability, pioglitazone 30mg once daily was given for 16 weeks. Two metabolic study days were undertaken, one before and one after pioglitazone treatment. Localized $^1$H- magnetic resonance (MR) spectra of liver and skeletal muscle and abdominal fat distribution were obtained before and after completion of pioglitazone treatment. The study protocol was approved by the Newcastle and North Tyneside Local Research Ethics Committee. Full verbal and written explanation was given and written consent was obtained before commencement of the studies.

Study protocol. Each subject was studied twice: once before pioglitazone treatment and once after 16-weeks of pioglitazone treatment. Subjects refrained from alcohol or vigorous exercise for 3 days prior to the study day and consumed their habitual weight-maintaining diet. After a 12-hour overnight
fast, all subjects arrived at 0700 and anthropometric measurements were recorded. Bioimpedance was performed using a Holtain BC Analyser (Holtain, Dyfed, UK), and the percentage of body fat was derived. An intravenous cannula for infusion was sited in an antecubital fossa vein, and a second cannula in a distal forearm vein, this hand being placed in a heated box at 50°C to allow sampling of arterialized blood. Baseline blood samples were then taken. After patients were rested for 30 minutes, at time -120 minutes, a fasting plasma glucose adjusted-prime of 6,6-dideuterated glucose was given intravenously [9] and a continuous infusion of 6,6-dideuterated glucose (0.04 mg·ml$^{-1}$·min$^{-1}$) was commenced. A period of 120 min was allowed for equilibrium of 6,6-dideuterated-glucose; the end of this period was taken to be time 0. At this time a standard liquid test meal (100 g carbohydrate, 12.5 g fat, 16 g protein) containing 2g of 2-deuterated (2-D) glucose was given and the subjects consumed this over a 10-minute period. The rate of infusion of 6,6-dideuterated glucose was adjusted in a stepwise fashion to match the anticipated pattern of endogenous glucose release after the meal and was as follows: -120 to 0 min:100% of basal infusion rate; 0–3 min, 100%; 3-8 min, 70%; 8–18 min, 55%; 18–28 min, 30%; 28-45 min, 15%; 45–70 min, 25%; 70-160 min, 35%; 160–270 min, 55%; 270-360 min, 80%. The metabolic study was finished at 360 min. The rate of infusion was determined iteratively from 8 pilot studies. Plasma enrichments of 6,6-dideuterated glucose were analyzed in each pilot study and rate of infusion modified in subsequent studies until the anticipated pattern of EGP was mimicked.

Frequent blood samples were taken for measuring plasma glucose, 2-deuterated glucose, 6,6-dideuterated glucose, triglycerides, free fatty acids (FFA), insulin, C-peptide, and glucagon. Substrate oxidation rates were calculated from indirect calorimetry data derived from constant flow hood calorimeter (Delta Trac 17), using standard formulae [10]. In one subject, the post pioglitazone study was terminated at 260 minutes due to symptoms of hypoglycemia (blood glucose: 3.7 mmol/L). The same subject had reported symptoms of hypoglycemia during the pioglitazone treatment period and sulfonylurea dose had been reduced. The study was stopped at 120 min post-meal ingestion in one other subject on both study days due to vasovagal symptoms.

**Metabolite and hormone assays.** Plasma glucose was measured on a Yellow Springs glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH, USA). FFA was measured on a Roche Cobas centrifugal analyzer by using a Wako kit (Wako Chemicals, Neuss, Germany). Plasma triglycerides were measured on a Roche Cobas centrifugal analyzer, using a colorimetric assay (ABX Diagnostics, Montpellier, France). Serum insulin and C-peptide assays were both measured using Dako ELISA kits (Dako; Ely, Cambridge, UK). Plasma glucagon was measured using the glucagon RIA kit (Linco Research, Missouri, USA) and tubes counted using a Packard Cobra gamma counter. $^2$H$_2$ percent enrichment in plasma glucose was determined by gas chromatography-mass spectroscopy (GC-MS), using a Thermo Voyager single quadrupole mass spectrometer interfaced to a Thermo Trace GC, with automated injection via a Thermo AS2000 autosampler (Thermo Scientific, Waltham, MA, USA). The coefficient of variation (CV) for the precision of plasma $^2$H$_2$ APE measurement was 4.1%.

**Calculations of EGP.** The profile of exogenous glucose concentration, i.e., the component of total glucose concentration due to exogenous glucose ingestion, was initially calculated from 2-D glucose [11]. We then calculated the time course of the endogenous glucose concentration, i.e., the component of
total glucose due to EGP only, by subtracting
the calculated exogenous component and the
6,6-dideuterated glucose concentration from
the measured total glucose concentration. The
steady-state values of plasma clearance rate
(PCR) and basal EGP (basal EGP = PCR x
basal glucose concentration) were estimated
from the 6,6-dideuterated glucose decay curve
after the prime dose of 6,6-dideuterated
-glucose given 2 h before the meal [12]. Since
6,6-2H2-glucose had been infused mimicking
the expected behaviour of EGP, the ratio of
6,6-2H2-glucose to endogenously produced
-glucose was almost constant (tracer-to-tracee
clamp technique), allowing reliable estimation
of EGP [13]. EGP was calculated using both
the model of Steele et al [14,15] and two-
compartment model of Radziuk et al [16],
with the tracer-to-tracee ratio derivative
calculated after the signal was smoothed
using an algorithm based on stochastic
nonparametric deconvolution [17]. Calculated
EGP was similar in both models and the
results from the Steele model are presented.
In three subjects, the EGP profiles could not
be assessed due to modelling factors in the
postprandial period and their data were
excluded from the EGP analysis. Relationships
between EGP and its
determinants were determined in all subjects
with paired data available (seven subjects).

\[ ^1H \text{ magnetic resonance spectroscopy.} \]
Localized \(^1H\)-nuclear MR spectra of liver and
muscle were obtained in a 1.5 Tesla MR
scanner with a \(^1H\) transmitter/receiver coil
placed over the relevant tissue. Liver spectra
were acquired by applying the breath hold-
triggered stimulation echo acquisition
sequence. Spectra were collected without
water suppression, using a point-resolved
spectroscopy sequence [18], with a 2 x 2 x 2-
cm voxel in the soleus muscle and anterior
tibialis muscle and a 3 x 3 x 3-cm voxel in the
-liver, using an echo time of 25 ms and
repetition time of 5,000 ms with 32
acquisitions and a spectral resolution of 1 Hz.

Spectra were analyzed using an MR user
interface [19]. Automatic phase correction
was performed using the water peak in the
spectra, and the water peak was assigned to
4.68 ppm. Lipid spectral peaks were assigned
as described in Boesch et al. [20] and spectra
peak amplitudes estimated by fitting
Lorentzian curves to the spectra. To calculate
molar density of triglycerides from the
amplitudes of water and intramyocellular lipid
estimated in the spectral fitting, we used the
formulae described by Szczepaniak et al [21].

\[ \text{Abdominal fat distribution.} \]
The volume of
total and visceral fat compartments were
determined as described previously [22]. To
determine the visceral fat content, a region
encompassing the viscera was drawn
manually by an operator using a computer
mouse. The visceral fat volume was
determined by multiplying the number of
pixels brighter than the threshold in this
region by the pixel size and slice thickness.
Regions were also drawn manually around
brightly appearing structures such as bone
marrow in order to subtract their contribution
from the total fat volume. The subcutaneous
fat volume was quantified by subtracting the
visceral fat volume from the total fat volume.
The intra-subject co-efficient of variation in
measurement of subcutaneous and visceral fat
were 4.9 % and 3.8 % respectively.

\[ \text{Statistical analysis.} \]
All data are expressed as
means ± SE. Statistical analyses were
performed using MINITAB software
(Minitab, State College, PA). Comparisons
were carried out using Student's t-test (two
tailed) where appropriate and Wilcoxon
signed rank test used to compare EGP.
Relationships were tested using the linear
correlation analysis. Stepwise regression
analysis was used for determining the
determinants of EGP. A P-value of <0.05 was
considered to indicate statistical significance.

\[ \text{RESULTS} \]
Plasma glucose, HbA1c and body weight. Fasting mean plasma glucose (10.1 ± 0.5 vs. 7.8 ± 0.5 mmol/L, p= 0.02) and HbA1c (8.6 ± 0.3 vs. 6.7 ± 0.38 % p< 0.001) were significantly lower after pioglitazone treatment [Table 1]. After 16 weeks of pioglitazone treatment, both body weight and body mass index increased significantly and increases in both fat mass and fat-free mass contributed to the overall weight gain [Table 1].

After meal ingestion, plasma glucose rose on both study days, but mean peak plasma glucose was significantly lower (18.7 ± 2.3 vs. 15.6 ± 3.8 mmol/L, p< 0.05), and delayed (100 min vs. 150 min) after pioglitazone treatment. The postprandial plasma glucose remained significantly lower until the end of the study (360 min: 9.4 ± 1.0 vs. 6.5 ± 0.8, p= 0.04) [Fig. 1A].

Plasma insulin and C-peptide. Fasting insulin levels (102.2 ± 14.8 vs. 81.1 ± 14.8 pmol/L, p = NS) and post-meal mean peak insulin levels (506.2 ± 62.5 vs. 464.5 ± 87.5 pmol/L, p =NS) did not change significantly after pioglitazone treatment [Fig. 1B]. However, fasting C-peptide levels were significantly reduced after pioglitazone treatment (1.3 ± 0.1 vs. 1.0 ± 0.1 nmol/L, p =0.005) and remained significantly lower until 80 minutes postprandially [Fig. 1C].

Insulin secretion rates and hepatic insulin extraction. Using C-peptide and plasma insulin levels, insulin secretion rate and hepatic insulin extraction were derived. Insulin secretion rates were similar in the postprandial period both at baseline and after pioglitazone treatment (peak insulin secretion rate 1197 ± 125 vs. 1205 ± 165 pmol/min; p = NS). Similarly, mean postprandial hepatic insulin extraction was unchanged after pioglitazone treatment (39.3 ± 0.01 % vs. 40.2 ± 0.01 %, p = NS).

Glucagon. Fasting glucagon levels were significantly decreased on pioglitazone treatment (150.9 ± 24.2 vs. 112.4 ± 17.2 pg/ml, p= 0.005) and there was a lower and delayed postprandial peak (207.4 ± 24.1 pg/ml at 20 min vs. 150.1 ± 23.9 pg/ml at 40 min, p < 0.01). Postprandial glucagon levels reached a nadir at 210 minutes on both study days and then rose slightly on both study days during the postabsorptive period [Fig. 1D].

FFA and Triglyceride. Fasting plasma FFA levels were slightly but not statistically significantly lower (0.73 ± 0.08 vs. 0.66 ± 0.05 μmol/L, p= NS). Postprandially, FFA suppressed on both study days, more so following pioglitazone treatment (0.20 ± 0.04 vs. 0.08 ± 0.02 μmol/L at 150 min [nadir], p= 0.018). Levels remained significantly lower until 210 min (0.22 ± 0.04 vs. 0.10 ± 0.02 μmol/L, p= 0.03) [Fig. 2A]. Fasting plasma triglyceride levels were slightly lower after pioglitazone treatment (2.3 ± 0.4 vs. 1.9 ± 0.6 mmol/L, p= NS) and remained lower throughout the postprandial period [Fig. 2B].

EGP. At the end of the first 120 minute basal period, mean plasma APE of 6,6-2H2-glucose were 1.48 ± 0.05 and 1.61 ± 0.09 before and after pioglitazone treatment respectively. The rate of fall of plasma 6,6-2H2-glucose APE consequent upon the change in infusion protocol was similar during the postprandial period on both study days (31% and 38% at 30 min and 47% and 51 % at 60 minutes). At nadir, between 120 and 180 min, plasma APE were 0.6 ± 0.01 and 0.7 ± 0.01 before and after pioglitazone treatment respectively. Fasting EGP was significantly reduced after pioglitazone treatment (16.6 ± 1.0 vs. 12.2 ± 0.7 μmol • kg⁻¹•min⁻¹, p=0.005) [Fig 3]. Although postprandial suppression of EGP was rapid on both study days (at 40 min: 96% suppression [baseline study] and 93% suppression [pioglitazone study]), postprandial EGP was significantly lower at 150 min after pioglitazone treatment (2.50 ± 0.61 vs. 0.82 ± 0.25 μmol • kg⁻¹•min⁻¹, p= 0.05). Between 80 – 260 minutes, mean EGP was two-fold lower following pioglitazone treatment (2.58 ± 0.25 vs. 1.26 ± 0.30 μmol •
kg\(^{-1}\)•min\(^{-1}\), p< 0.001) [Fig. 3]. When expressed as percentage suppression from baseline, postprandial suppression was still greater after pioglitazone treatment (82% vs. 90%, between 180 – 210 min, p = 0.02). Fasting EGP was directly correlated with hepatic triglyceride content before and after pioglitazone treatment (r = 0.64, p = 0.01) [Fig. 4A]. Likewise, hepatic triglyceride content correlated inversely with percentage suppression of EGP at 150 minutes (r = -0.63, p = 0.02) [Fig. 4B]. There was a strong negative correlation between FFA levels at nadir (150 minutes) and percentage suppression of EGP at 210 minutes (r = -0.87, p < 0.001) [Fig. 4C]. A stepwise regression analysis, with EGP (fasting and postprandial) as the dependent variable and hepatic triglyceride, muscle triglyceride, plasma insulin, plasma glucagon, visceral fat, plasma FFA, age and BMI as independent variables showed that for fasting EGP, hepatic triglyceride was the most significant and independent variable (step 1, adj R\(^2\) = 53, p < 0.001) followed by fasting plasma glucagon (step 2, adj R\(^2\) = 84; p = 0.005). For postprandial percentage suppression of EGP at 150 minutes, hepatic triglyceride was the only significant and independent variable (step 1; adj R\(^2\) = 36; p = 0.03). There was no independent relationship between changes in plasma glucagon or molar insulin-glucagon ratio with EGP.

\(^{1}\)H-MR spectroscopy. Despite increase in body weight, hepatic triglyceride content decreased by ∼50% following pioglitazone treatment (140.1 ± 28.1 vs. 67.0 ± 10.3 \(\mu\)mol/g, p= 0.03) [Fig. 5A] and tibialis anterior muscle triglyceride content decreased by ∼55% (8.37 ± 1.6 vs. 3.65 ± 1.14 \(\mu\)mol/g, p= 0.02) [Fig. 5B]. Soleus triglyceride content was slightly lower after pioglitazone treatment (23.8 ± 3.3 vs. 20.8 ± 3.1 \(\mu\)mol/g, p= NS). Intramyocellular triglyceride content did not correlate with fasting (r = 0.27, p = 0.37) or mean postprandial EGP [80-260 min] (r = 0.19, p = 0.52).

Abdominal fat content. Pioglitazone treatment was associated with a significant increase in subcutaneous fat content (10.8 ± 1.4 vs. 12.5 ± 1.7 L, p= 0.003) [Fig 5D] and decreased visceral fat to subcutaneous fat ratio (0.68 ± 0.1 vs. 0.57 ± 0.1, p= 0.02). Total visceral fat content was unchanged (6.2 ± 0.3 vs. 6.2 ± 0.5 L, p= NS) [Fig 5C]. Visceral fat content did not correlate with fasting (r = -0.05, p = 0.88) or mean postprandial EGP [80-260 min] (r = 0.01, p = 0.76).

Substrate oxidation. Fasting glucose oxidation was significantly lower (2.2 ± 0.2 vs. 1.5 ± 0.2 mg/kg/min, p < 0.01) after pioglitazone treatment and accounted for 18% and 12% of fasting glucose disposal before and after pioglitazone treatment respectively. Postprandial glucose oxidation remained lower after pioglitazone treatment until 120 minutes after the meal. Fasting (0.4 ± 0.1 vs. 0.2 ± 0.1 mg/kg/min, p =NS) and postprandial lipid oxidation (0.2 ± 0.1 vs. 0.1 ± 0.1 mg/kg/min, P =NS) were slightly but not significantly lower after pioglitazone treatment. Resting and postprandial energy expenditure was similar on both study days.

**DISCUSSION**

In keeping with our hypothesis, pioglitazone treatment in type 2 diabetes resulted in almost 50% decrease in hepatic triglyceride and 55% reduction in muscle triglyceride, despite significant weight gain (~6 kg). This was associated with a 26% decrease in fasting EGP and a two-fold decrease in postprandial EGP. Hepatic triglyceride content was directly correlated with fasting EGP (r = 0.64, p = 0.01) and inversely correlated with percentage suppression of EGP (time 150 min, r = - 0.63, p = 0.02) before and after pioglitazone treatment. By stepwise regression hepatic triglyceride was found to be a significant independent predictor of
fasting EGP, accounting for 53% of its variation (p < 0.001). Similarly, hepatic triglyceride content accounted for 36% of the variation seen in the percentage suppression of postprandial EGP observed at 150 minutes (p = 0.03). In contrast, although muscle triglyceride content decreased with pioglitazone treatment, it did not correlate with EGP. There was no relationship between visceral fat content with EGP.

In type 2 diabetes, hepatic insulin resistance is believed to underlie the elevated basal rates of EGP and subnormal postprandial suppression of EGP [23, 24]. In a recent study, elevated EGP was found to be a primary mechanism for postprandial hyperglycemia in type 2 diabetes [25]. Hepatic triglyceride content is characteristically increased in type 2 diabetes and is an important predictor of hepatic insulin resistance [1, 26] and this is true even independent of obesity [27]. In addition, hepatic triglyceride content correlated negatively with postprandial net hepatic glycogen synthesis in type 2 diabetes [3]. Although the exact cause of the excess accumulation of hepatic triglyceride in type 2 diabetes is unclear, we have previously shown this is likely to be, at least partly secondary to increased postprandial uptake of dietary fatty acids by the liver [28]. Other plausible mechanisms include increased FFA delivery from visceral depots and reduced intra-hepatic FFA oxidation [29].

Hepatic steatosis induced by three-day high-fat feeding, resulted in reduced insulin activation of IRS-1 and IRS-2–associated phosphatidylinositol 3-kinase activity, increased gluconeogenesis and liver specific insulin resistance [30]. By reversal of this principle, a very low-fat diet induced decrease in hepatic triglyceride in patients with type 2 diabetes was shown to markedly improve both EGP suppression during insulin infusion and hepatic insulin sensitivity [2]. Similarly, we observed an almost 50% decrease in hepatic triglyceride content following pioglitazone treatment [Fig. 5A] and this was associated with significant improvement in fasting and postprandial EGP [Fig. 3].

Increased EGP in type 2 diabetes is contributed to by both increased gluconeogenesis and glycogenolysis [25], but the effect of gluconeogenesis may predominate [31, 32]. The results of this study suggest that pioglitazone induced reduction in EGP is likely to reflect changes in either gluconeogenesis and/or glycogenolysis. In a recent study, pioglitazone treatment improved fasting and postprandial glycemia, principally via inhibition of gluconeogenesis [33]. The effect of TZD’s on glycogenolysis is yet to be determined. Pioglitazone has been shown to decrease gluconeogenesis [34] and inhibit expression of key genes involved in gluconeogenesis [35]. In addition, FFA are potent stimulators of gluconeogenesis and TZD’s decrease FFA levels [36, 37]. Pioglitazone also markedly increase adiponectin levels [38, 39] and adiponectin has been shown to decrease gluconeogenesis and EGP [40, 41]. Hyperglucagonemia is associated with increased basal EGP [42] and we observed major decrease in fasting glucagon levels after pioglitazone treatment. As fasting insulin levels were relatively unchanged after pioglitazone treatment, the effect of pioglitazone on fasting EGP is likely to be secondary to its effect, either directly or indirectly on the liver.

We observed that the initial postprandial suppression of EGP both before and after pioglitazone was relatively normal. Using a similar dynamic tracer methodology, we have previously reported similar initial postprandial suppression of EGP in subjects with type 2 diabetes compared with matched controls [43]. The conventional dual tracer approach with fixed priming and fixed-rate tracer infusion has been extensively used to measure EGP and documents subnormal suppression of EGP in type 2 diabetes [44,
However, by infusing the iv tracer to mimic the expected pattern of EGP, tracer-to-tracee ratio is maintained approximately constant and the EGP estimate is model-independent and is not overestimated [43]. This has recently been quantitatively assessed [13]. In contrast to our observations, a recent study reported a much slower rate of suppression of EGP following pioglitazone [33] and this is likely to be a consequence of using a fixed-rate tracer infusion for a dynamic meal study.

The rapid early suppression of EGP needs to be considered. Hepatic insulin action is relatively unimpaired at high insulin levels [46] and glucose effectiveness is also preserved in type 2 diabetes [47]. In the immediate postprandial period, the change in plasma FFA is fairly subtle and glucagon levels actually rise. It has been suggested that FFA concentrations signal the suppression of EGP (portal hypothesis) [48]. However, our data does not support this, as there is robust suppression (~75%) of EGP despite only a ~15% decrease in FFA by 30 min postprandially. It is hence most likely that the direct effect of rising hepatic sinusoidal insulin is the primary determinant of EGP in this period.

Impaired postprandial suppression of FFA and glucagon is a characteristic feature of type 2 diabetes [44, 49] and both FFA and glucagon [25, 50, 51] have been implicated in the abnormal regulation of EGP. Recently, Woerle et al [25] have demonstrated that postprandial EGP was significantly correlated with insulin-to-glucagon molar ratio, postprandial decrements in both plasma glucagon and plasma FFA. In the current study, between 80-260 minutes after the meal, pioglitazone treatment resulted in a two-fold greater suppression of EGP compared to pre-treatment. Unlike the immediate postprandial period, insulin levels fall during this period and FFA and glucagon may exert a more pronounced effect on EGP. Insulin levels and insulin secretion rates were relatively unchanged between the two study days, but postprandial FFA and glucagon levels were significantly lower following pioglitazone treatment. We observed a strong correlation between the nadir FFA levels and percentage suppression of EGP at 210 minutes [Fig. 4C]. Hence, it is likely that pioglitazone induced interruption of intra-hepatic fatty acid supply may underlie the beneficial effects of EGP during this time.

Although, pioglitazone treatment decreased muscle triglyceride content as seen in previous studies [52, 53], we observed no correlation between muscle triglyceride and EGP. The mechanism of reduction of muscle triglyceride by pioglitazone is unclear. Pioglitazone is postulated to increase tissue fatty acid oxidation through its effect on increasing adiponectin and consequent activation of adenosine monophosphate – activated protein kinase [53], but muscle fatty acid oxidative enzyme levels were not observed to change following pioglitazone treatment in a recent study [52]. In contrast to previous studies, we did not observe any significant change in visceral fat content following pioglitazone treatment. The fact that EGP improved independent of change in visceral fat content implies that visceral fat is unlikely to be a critical determinant of EGP and this clearly refutes the portal hypothesis in the control of EGP [48].

Potential shortcomings of the study must be considered. The small study sample size is a potential weakness of the study, but the subject characteristics are typical for type 2 diabetes and the main findings are clear cut. The small sample size is also likely to explain the lack of statistical significance of lipid oxidation between the study days. Secondly, the study was a within-subject comparative study by design and did not have a placebo arm. Thirdly, gluconeogenesis and glycogenolysis were not separately quantified and hence the precise mechanism of reduction
in EGP with pioglitazone could not been
determined. Nonetheless, the study defines
the precise effects of pioglitazone on
postprandial glucose metabolism and the
determinants of EGP in type 2 diabetes.

In summary, we have demonstrated that
16 week treatment with pioglitazone improves
fasting and postprandial EGP suppression and
decreases liver and muscle triglyceride
content despite overall increase in body
weight. Increased hepatic triglyceride content
is associated with subnormal postprandial
suppression of EGP and is an independent
determinant of fasting and postprandial EGP.
This study provides further insight into the
mechanism of action of pioglitazone and
suggests that hepatic triglyceride is a useful
therapeutic target for management of type 2

diabetes.

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**TABLE 1.** Anthropometric and laboratory measurements before and after pioglitazone treatment for 16 weeks.

<table>
<thead>
<tr>
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<th>Before pioglitazone</th>
<th>After pioglitazone</th>
<th>P value</th>
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<tr>
<td>Body weight (kg)</td>
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<td>BMI (kg/m²)</td>
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<td>Fat mass (kg)</td>
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<td>FPG (mmol/L)</td>
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</tr>
<tr>
<td>Peak plasma glucose (mmol/L)</td>
<td>18.7 ± 2.3</td>
<td>15.6 ± 3.8</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Fasting plasma insulin (pmol/L)</td>
<td>102.2 ± 14.8</td>
<td>81.1 ± 14.8</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting plasma C-peptide (nmol/L)</td>
<td>1.3 ± 0.1</td>
<td>1.0 ± 0.1</td>
<td>0.005</td>
</tr>
<tr>
<td>Fasting plasma TG (mmol/L)</td>
<td>2.3 ± 0.4</td>
<td>1.9 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Fasting plasma FFA (μmol/L)</td>
<td>0.73 ± 0.08</td>
<td>0.66 ± 0.05</td>
<td>NS</td>
</tr>
<tr>
<td>Baseline glucagon (pg/ml)</td>
<td>150.9 ± 24.2</td>
<td>112.4 ± 17.2</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Date are values ± SE; BMI, body mass index; FFM, fat-free mass; FPG, fasting plasma glucose; TG, triglyceride; FFA, free fatty acids; NS, not significant.
Figure 1

Figure 1. Change in plasma glucose (A), plasma insulin (B), plasma C-peptide (C) and plasma glucagon (D) before and after the meal, before pioglitazone (white circles) and after pioglitazone (black circles) treatment. Data are shown as means ± SEM.
Figure 2. Change in plasma triglyceride (A) and plasma FFA (B) before and after the meal, before pioglitazone (white circles) and after pioglitazone (black circles) treatment. Data are shown as means ± SEM.
Figure 3. Change in endogenous glucose production (EGP) during the study period, before pioglitazone (white circles) and after pioglitazone (black circles) treatment. Data are shown as means ± SEM.
Figure 4. Correlation between fasting EGP and liver fat content ($r = 0.64$, $p = 0.01$) (A), postprandial EGP [percentage suppression at 150 min] and liver fat content ($r = -0.63$, $p = 0.02$) (B) and postprandial EGP [percentage suppression at 210 min] and FFA concentration ($r = -0.87$, $p < 0.001$) (C), before pioglitazone (white circles) and after pioglitazone (black circles) treatment.
Figure 5. Liver triglyceride (A) and muscle triglyceride (B) visceral fat (C) and subcutaneous fat (D) content at baseline and after pioglitazone treatment. Data are shown as means ± SEM. * P < 0.04; ** P < 0.001.