Pioglitazone improves insulin sensitivity through reduction in muscle lipid and redistribution of lipid into adipose tissue

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THE RAPID INCREASE in the prevalence of obesity in developed countries has been accompanied by a parallel increase in the prevalence of type 2 diabetes (T2DM) (8). It is now well established that the development of T2DM results from an interaction of a subject’s genetic makeup and his/her environment. The development of obesity seems to be an important factor portending the development of insulin resistance, which in the presence of β-cell dysfunction results in alterations in glucose tolerance (7, 11).

An important link between obesity and insulin resistance involves lipid deposition in ectopic sites, such as skeletal muscle, islets, and liver (20, 28), with resulting functional impairment that has been called lipotoxicity (9). Because peripheral insulin resistance occurs early in the development of diabetes, and most peripheral glucose disposal occurs in skeletal muscle, intramyocellular lipid (IMCL) accumulation has been the focus of much research into diabetes treatment and prevention. Previous studies have demonstrated a strong correlation between IMCL content and insulin resistance (22, 24), but the factors leading to this accumulation are not clear. In a recent study of young adults with normal body mass index (BMI) and a family history of T2DM, increased IMCL was accompanied by decreased mitochondrial oxidative phosphorylation, suggesting that a defect in some component of muscle lipid oxidation is present (23). Studies of genetically altered rodents have demonstrated a direct relationship between muscle lipid oxidation and insulin sensitivity (15, 19, 25).

Because of the important role of insulin resistance in T2DM, considerable drug development has been focused on insulin sensitizers, which currently include the biguanide, metformin, and the thiazolidinediones pioglitazone and rosiglitazone. Although these drugs are effective in the treatment of diabetes, the mechanisms of action are not well understood. Thiazolidinediones are agonists for the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ), which is expressed at high levels in adipose tissue but at low levels in muscle (29). Paradoxically, the improved insulin sensitivity after thiazolidinedione treatment is primarily a function of increased skeletal muscle glucose transport (9). Hence, the beneficial effects of these drugs implicate either an indirect effect through an adipose tissue-muscle metabolic relationship or a direct effect of thiazolidinediones on the low levels of muscle PPARγ.

We hypothesized that thiazolidinediones, but not metformin, improve insulin sensitivity independently of lowering blood glucose through a diversion of lipid away from ectopic sites and into subcutaneous fat. To test this hypothesis, we examined insulin sensitivity, IMCL, and muscle oxidative capacity in euglycemic subjects with impaired glucose tolerance (IGT) before and after treatment with pioglitazone or metformin.

METHODS

Subjects. All subjects provided written, informed consent under a protocol that was approved by the Institutional Review Board of University of Arkansas for Medical Sciences (UAMS) and was conducted at the UAMS General Clinical Research Center. Subjects in good health were recruited by local advertisement and underwent an initial 75-g oral glucose tolerance test (OGTT). Subjects were included if the fasting
**Table 1. Baseline characteristics of subjects and changes in response to either metformin or pioglitazone**

<table>
<thead>
<tr>
<th></th>
<th>Metformin</th>
<th>Pioglitazone</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>93.5 ± 4.3</td>
<td>94.6 ± 4.1</td>
<td>0.74 ± 0.2</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>33.2 ± 0.9</td>
<td>33.0 ± 0.9</td>
<td>0.36 ± 1.4</td>
</tr>
<tr>
<td>%Fat</td>
<td>37.5 ± 2.0</td>
<td>37.2 ± 1.9</td>
<td>41.5 ± 1.5</td>
</tr>
<tr>
<td>Lean body mass, kg</td>
<td>58.6 ± 3.3</td>
<td>59.5 ± 3.4</td>
<td>52.8 ± 2.2</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>35.3 ± 2.4</td>
<td>35.1 ± 2.1</td>
<td>37.9 ± 2.6</td>
</tr>
<tr>
<td>Fasting glucose, mM</td>
<td>4.9 ± 0.1</td>
<td>4.5 ± 0.1*</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>2-h Glucose mM</td>
<td>9.3 ± 0.4</td>
<td>8.6 ± 0.7</td>
<td>9.6 ± 0.3</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>5.3 ± 0.7</td>
<td>5.2 ± 0.1</td>
<td>5.3 ± 0.1</td>
</tr>
<tr>
<td>Mean glucose-OGTT, mM</td>
<td>8.7 ± 0.4</td>
<td>7.8 ± 0.4</td>
<td>9.0 ± 0.3</td>
</tr>
<tr>
<td>Mean insulin-SI, pM</td>
<td>469.2 ± 72.9</td>
<td>412.2 ± 92.4</td>
<td>67.8 ± 18.2 132.7 ± 115.2*</td>
</tr>
<tr>
<td>HbS, %</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1*</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>Total cholesterol, mM</td>
<td>4.7 ± 0.9</td>
<td>4.7 ± 0.3</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>Triglyceride, mM</td>
<td>1.9 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>LDL, mM</td>
<td>2.5 ± 0.2</td>
<td>2.6 ± 0.3</td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td>HDL, mM</td>
<td>1.3 ± 0.1</td>
<td>1.3 ± 0.2</td>
<td>1.2 ± 0.6</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Tx, treatment; BMI, body mass index; OGTT, oral glucose tolerance test; SI, insulin sensitivity index; LDL and HDL, low- and high-density lipoprotein. There were no significant differences between two groups at baseline characteristics. *P < 0.05 vs. baseline in each group. †P < 0.005 vs. baseline in each group. ‡Baseline SI was slightly higher in metformin group compared with pioglitazone group (P = 0.12). To convert glucose mmol/l to mg/dl, multiply by 18; insulin pM to μU/ml, divide by 6; cholesterol mmol/l to mg/dl, multiply by 38.67; triglyceride mmol/l to mg/dl, multiply by 88.57.

Glucose was under 6.1 mmol/l (110 mg/dl), the 2-h postchallenge glucose was 7.8–11.1 mmol/l (140–199 mg/dl), ages were 35–65 yr, and BMI was 27–38 kg/m². Subjects with a history of coronary artery disease or the concomitant use of fibrates, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers were excluded. All subjects were weight stable for at least 3 mo. Subjects underwent a 2-wk run-in period on a 35% fat, eucaloric diet and were maintained on this diet throughout the study. Total body fat and muscle content were determined by dual-energy X-ray absorptiometry (DEXA) and visceral and subcutaneous fat by abdominal CT scan. All subjects underwent an insulin-modified, frequently sampled intravenous glucose tolerance study (FSIGTT, described below), followed by baseline and muscle and fat biopsies. They were then randomized to metformin or pioglitazone for a 2-wk dose escalation followed by 8 wk at a maximum dose (1,000 mg of metformin twice daily or 45 mg of pioglitazone daily). Compliance and laboratory tests were monitored three times during the follow-up. After 10 wk of treatment, the oral and intravenous glucose tolerance tests, DEXA, CT scan, and biopsies were repeated.

**Insulin sensitivity measurement.** Insulin sensitivity was measured by an insulin-modified intravenous glucose tolerance test using 11.4 g/m² glucose and 0.04 U/kg insulin, as described elsewhere (2). Insulin was measured using an immunoochemiluminescence assay (MLT Assay, Wales, UK) in the GCRC Core Laboratory. The assay has a sensitivity of 0.25 nU/l for insulin, with 1% cross-reactivity with proinsulin and 4–8% coefficient of variation. Plasma glucose was measured in duplicate by a glucose oxidase assay. Insulin sensitivity was calculated from the insulin and glucose data by use of the MinMod program (1).

**Visceral and subcutaneous fat measurement.** Abdominal subcutaneous and visceral adipose tissue was determined from the cross-sectional fat distribution using the Slicomatic program (Tomovision, Montreal, Canada) to analyze the CT scan images at the level of the umbilicus. The ranges of Hounsfield units used to assess the quantity of fat were −250 to −40.

**Muscle biopsy, immunohistochemistry, and measurement of IMCL.** Muscle samples were taken from the vastus lateralis under local anesthesia (1% lidocaine without epinephrine) and immediately processed for fiber typing by immunohistochemistry using monoclonal antibodies, as described previously (3, 12). Muscle lipid content was determined by Oil Red O staining (16), and images of the muscle sections were analyzed using the NIH Image program v. 1.60. The software was set to recognize fat droplets of 0.40 μm² or larger. The total area of fat droplets in a given muscle fiber was divided by the total area of the same muscle fiber, giving a percentage of lipid content in the muscle fiber.

**Muscle lipid oxidative enzymes.** To assess the oxidative capacity of the muscle, we measured the activity of succinate dehydrogenase (SDH) (12) and the expression of genes involved in fatty acid oxidation, including carnitine palmitoyltransferase I (CPT I), PPARα, and PPARδ. Gene expression was measured by real-time PCR using SYBR Green (Applied Biosystems, Foster City, CA) on a Rotor-Gene 3000 Real-Time Amplification System (Corbett Research, Sydney, Australia). Levels of mRNA were expressed relative to 18S RNA.

**Statistical analysis.** All analyses were performed using SAS v. 9.1 (SAS Institute, Cary, NC). Baseline and posttreatment variables within groups were compared by paired t-test. To test the posttreatment differences between the two drug treatment groups, we applied covariance analysis with adjustments for baseline values. Nonnormal data were logarithmically transformed to normality before testing. A P value of 0.05 was considered to be significant. All data are expressed as means ± SE. Mean glucose and insulin during OGTT was calculated from 0-, 30-, 60-, 90-, and 120-min samples.

**RESULTS**

**Fat distribution and insulin sensitivity in response to treatment.** The baseline characteristics of the 23 subjects (12 metformin and 11 pioglitazone) who completed the study did not differ by treatment group, as shown in Table 1. After 10 wk of treatment with pioglitazone or metformin, there were no significant changes in fasting lipid profiles in either group (Table 1). Subjects in the metformin group remained weight stable during the drug treatment phase. However, subjects in the pioglitazone group gained 2.63 ± 0.65 kg (P = 0.004) without any significant change in total body adipose tissue (0.83 ± 0.51 kg, P = 0.16) but with an increase in lean body mass of 1.8 ± 0.42 (P = 0.003). Abdominal subcutaneous and visceral adipose tissue volumes were measured by CT scanning. As shown in Fig. 1, there was no change from baseline in adipose tissue volumes with metformin. In contrast, subjects treated with

![Fig. 1. Areas of subcutaneous (SQ) and visceral (V) fat were measured by CT scan of the abdomen at umbilical level. After treatment with metformin (MET), SQ or V fat area did not change; however, SQ fat increased and V fat decreased slightly and the ratio of V to SQ fat (V/SQ) decreased significantly after pioglitazone (PIO; *P = 0.01).](http://ajpendo.physiology.org/doi/10.1152/ajpendo.00439.2004)
pioglitazone showed a small increase in abdominal subcutaneous fat (494 ± 42 to 515 ± 42 cm², P = 0.18) and a slight decrease in visceral fat (166 ± 19 to 162 ± 21 cm², P = 0.49), resulting in a statistically significant decrease in the ratio of visceral to subcutaneous fat (0.35 ± 0.04 to 0.32 ± 0.04, P = 0.015; Fig. 1). When the posttreatment visceral-to-subcutaneous fat ratios were compared between treatment groups, the ratios were lower in the pioglitazone group compared with the metformin group (P = 0.05).

The insulin sensitivity index (S₁) increased 65% with pioglitazone treatment (1.84 to 3.02 × 10⁻⁵ min⁻¹/pM, pre- vs. posttreatment, P = 0.002) but did not improve with metformin (2.66 ± 0.39 to 2.50 ± 0.30 × 10⁻⁵ min⁻¹/pM, P = 0.90; Fig. 2). Consistent with this observation, oral glucose tolerance improved with pioglitazone but not with metformin (Table 1). After treatment with pioglitazone, 2-h blood glucose decreased from 9.6 ± 0.3 (172 ± 5.7 mg/dl) to 6.6 ± 0.5 mmol/l (119 ± 8.8 mg/dl, P < 0.0006), whereas there was no significant change in 2-h glucose with metformin.

IMCL content and fiber type and size did not differ between groups at baseline and did not change with either therapy. In contrast, pioglitazone decreased IMCL in type I fibers by 34% (7.0 ± 0.6% at baseline to 4.6 ± 0.6% after treatment, P = 0.001 vs. baseline), whereas IMCL in type II fiber was unchanged with metformin (6.3 ± 1.2% at baseline to 5.9 ± 0.7% after treatment, P = 0.6 vs. baseline; Fig. 3). The changes in IMCL in type II fibers were not significant after pioglitazone (2.6 ± 0.5 to 2.2 ± 0.5%, P = 0.16 vs. baseline) or metformin (2.6 ± 0.7 to 2.9 ± 0.7%, P = 0.36 vs. baseline).

**Fatty acid oxidation.** The decrease in IMCL with pioglitazone might result from decreased lipid uptake, increased muscle lipid oxidation, or a combination of the two. To assess the oxidative capacity of the muscle, we measured the activity of SDH (a mitochondrial enzyme in the TCA cycle). Were the decrease in IMCL the result of increased muscle oxidative capacity, SDH activity should have increased with pioglitazone but not metformin treatment. In contrast, SDH activity was unchanged with either metformin or pioglitazone therapy (Table 2); indeed, SDH activity decreased slightly with pioglitazone treatment (P = 0.07). Furthermore, mRNA levels of genes involved in muscle lipid oxidation including CPT I, PPARα, and PPARβ were unchanged with either pioglitazone or metformin therapy (Table 2). Thus pioglitazone decreased IMCL without any detectable increase in enzymes associated with muscle lipid oxidation.

![Fig. 3. Oil Red O staining of neutral lipid within skeletal muscle at x40 magnification. A: Intramyocellular lipid (IMCL; lipid droplets are viewed as distinct spots of stain) decreased significantly after pioglitazone. B: IMCL content in type I fiber decreased from 7.0 ± 1.8 to 4.6 ± 1.8% (*P = 0.001 vs. baseline) after pioglitazone treatment but did not change in metformin group. Posttreatment, IMCL was significantly lower in pioglitazone compared with metformin group (P = 0.04).](image-url)
DISCUSSION

In this study, we treated IGT subjects with either metformin or pioglitazone for 10 wk. Because both metformin and pioglitazone lower blood glucose in diabetic subjects, we selected IGT subjects for these studies to avoid glucotoxicity as a confounding factor. In addition to the improvement in blood glucose following the OGTT, pioglitazone significantly increased the $S_t$ and also resulted in a significant decrease in type I fiber IMCL. The $S_t$ is a composite measure that reflects predominantly peripheral glucose disposal in skeletal muscle. This decrease in IMCL was not accompanied by any increase in SDH, CPT I, PPARα, or PPARδ, suggesting that the decrease in IMCL was not due to increased muscle lipid oxidation. Meanwhile, these pioglitazone-treated subjects demonstrated a decrease in the visceral-to-subcutaneous fat ratio in the abdomen, despite a small weight gain.

Thiazolidinediones are agonists for PPARγ, which is present predominantly in adipose tissue, yet these drugs improve peripheral insulin sensitivity, which involves glucose transport mostly into skeletal muscle. Based on the changes observed in adipose tissue distribution and muscle lipid and the improvement in $S_t$, these data strongly suggest that pioglitazone improved insulin sensitivity by diverting lipid from ectopic sites, such as skeletal muscle and visceral fat, into subcutaneous adipose tissue. It is possible that pioglitazone may have direct effects on skeletal muscle, where PPARγ is expressed at a low level; however, these effects in muscle are likely subtle and not detectable using the methods described in these subjects. We did not detect any changes in peripheral insulin sensitivity or IMCL in IGT subjects treated with metformin, suggesting either that metformin is relatively ineffective in IGT subjects or that the predominant effects are to decrease hepatic glucose production, which is not well represented in the $S_t$.

This reversal of lipotoxicity through diverting lipids into adipose tissue has been demonstrated previously in both mice and humans with lipodystrophy. In a mouse model of lipodystrophy, peripheral lipotoxicity was improved either with an adipose tissue transplant or by treatment with a combination of leptin and adiponectin (6, 31). In humans with congenital lipodystrophy, leptin treatment greatly reduced intrahepatocellular lipid content (21). In addition, previous studies of thiazolidinediones in rodents demonstrated reduced IMCL content and restored insulin sensitivity (32), even in the absence of adipose tissue (5). However, human studies of diabetic subjects treated with thiazolidinediones have been conflicting. One study showed no change in total muscle fat using $^1$H nuclear magnetic resonance spectroscopy (18), whereas another study demonstrated reduced muscle lipid content with both troglitazone and metformin (17). It is noteworthy that both studies examined subjects with diabetes, where the direct effects of the drug are hard to distinguish from the reduction in glucotoxicity from the significant lowering of blood glucose. In this study, subjects did not have diabetes but had IGT. Although subjects treated with pioglitazone demonstrated a significant improvement in glucose tolerance, $Hb A_{1C}$ was normal, and there was no change in $Hb A_{1C}$ in either group. Therefore, it is possible that relief of glucotoxicity played a role in the response to pioglitazone; however, this effect was likely very small.

Although numerous studies have demonstrated the effectiveness of metformin and the thiazolidinediones in the treatment of diabetes, only limited data are available to suggest that these drugs are effective in subjects with IGT (4, 14). Recent controlled trials on diabetes prevention, such as the Diabetes Prevention Program (DPP) and Finnish Diabetes Prevention Study (14, 27), have shown that lifestyle modification, including dietary changes, weight loss, and regular exercise, reduced the progression from impaired glucose metabolism to T2DM. Although metformin treatment also reduced the onset of diabetes in the DPP, lifestyle measures were superior to metformin treatment (14). The DPP initially included a troglitazone treatment arm, which was stopped when the drug was withdrawn from the market. However, preliminary results suggested a considerable short-term benefit from troglitazone on diabetes prevention (26), and the TRIPOD study, involving women with previous gestational diabetes who were treated with troglitazone, demonstrated a reduced incidence of diabetes (4). Other studies have shown that exercise and weight loss reduce IMCL (10, 30), and our data in this study demonstrated no effects of metformin on IMCL. Therefore, it is likely that measures directed at reduction of IMCL, such as lifestyle changes and lipid diversion with thiazolidinediones, are more effective at reducing lipotoxicity and therefore more valuable in the treatment of IGT and the prevention of T2DM.

In summary, we showed that pioglitazone, but not metformin, was able to improve insulin sensitivity in IGT subjects through a reduction in muscle lipid along with its redistribution into subcutaneous adipose tissue. This study demonstrates the utility of this mechanism for the treatment of insulin resistance and the metabolic syndrome and perhaps for the prevention of diabetes in subjects who are at high risk of developing T2DM.

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