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

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Rasouli, Neda, Ulrika Raue, Leslie M. Miles, Tong Lu, Gina B. Di Gregorio, Steven C. Elbein, and Philip A. Kern. Pioglitazone improves insulin sensitivity through reduction in muscle lipid and redistribution of lipid into adipose tissue. Am J Physiol Endocrinol Metab 288:E930–E934, 2005. First published January 4, 2005; doi:10.1152/ajpendo.00522.2004.—Patients with insulin resistance often manifest increased intramyocellular lipid (IMCL) along with increased visceral adipose tissue. This study was designed to determine whether the insulin sensitizer drugs pioglitazone and metformin would improve glucose intolerance and insulin sensitivity by decreasing IMCL. In this study, 23 generally healthy subjects with impaired glucose tolerance were randomized to receive either pioglitazone 45 mg/day or metformin 2,000 mg/day for 10 wk. Before and after treatment, we measured insulin sensitivity and abdominal subcutaneous and visceral adipose tissue with CT scanning. In addition, muscle biopsies were performed for measurement of IMCL and muscle oxidative enzymes. After treatment with pioglitazone, 2-h glucose fell from 9.6 mmol/l (172 mg/dl) to 6.1 mmol/l (119 mg/dl), whereas there was no change in 2-h glucose with metformin. With pioglitazone treatment, there was a 65% increase in insulin sensitivity along with a 34% decrease in IMCL (both P ≤ 0.002). This decrease in IMCL was not due to increased muscle lipid oxidation, as there were no changes in muscle lipid oxidative enzymes. However, pioglitazone resulted in a 2.6-kg weight gain along with a significant decrease in the visceral-to-subcutaneous adipose tissue ratio. In contrast, metformin treatment resulted in no change in insulin sensitivity, IMCL, oxidative enzymes, or adipose tissue volumes. Pioglitazone improved glucose tolerance and insulin sensitivity by reducing IMCL. This reduction in IMCL was not due to an increase in muscle lipid oxidation but to a diversion of lipid from ectopic sites into subcutaneous 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, when 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 plasma glucose was less than 140 mg/dl and hemoglobin A1C level was less than 6%.

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DEXA, CT scan, and biopsies were repeated. 2-wk dose escalation followed by 8 wk at a maximum dose (1,000 mg of 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 ± 0.38 kg (P = 0.014) but with an increase in lean body mass of 1.8 ± 0.47 kg, P = 0.03). 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 pioglitazone gained fat at a higher rate than subjects treated with metformin.

### Table 1. Baseline characteristics of subjects and changes in response to either metformin or pioglitazone

<table>
<thead>
<tr>
<th>Metformin</th>
<th>Pioglitazone</th>
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<tbody>
<tr>
<td><strong>Baseline</strong></td>
<td><strong>Post-Tx</strong></td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>93.9 ± 4.3</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>33.3 ± 0.9</td>
</tr>
<tr>
<td><strong>%Fat</strong></td>
<td>37.5 ± 2.0</td>
</tr>
<tr>
<td><strong>Lean body mass, kg</strong></td>
<td>58.6 ± 3.3</td>
</tr>
<tr>
<td><strong>Fat mass, kg</strong></td>
<td>55.3 ± 2.4</td>
</tr>
<tr>
<td><strong>Fasting glucose, mEq/L</strong></td>
<td>4.9 ± 0.1</td>
</tr>
<tr>
<td><strong>2-h Glucose mM</strong></td>
<td>9.3 ± 0.2</td>
</tr>
<tr>
<td><strong>Hb A1C, %</strong></td>
<td>5.3 ± 0.2</td>
</tr>
<tr>
<td><strong>Mean glucose-OGGT, mM</strong></td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td><strong>Mean insulin-OGGT, pM</strong></td>
<td>469.2 ± 79.8</td>
</tr>
<tr>
<td>*<em>S_0, 10^{-5} <em>min^{-1/2}pM^{-1/2}</em></em></td>
<td>2.7 ± 0.4</td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dL</strong></td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td><strong>Triglyceride, mg/dL</strong></td>
<td>1.9 ± 0.3</td>
</tr>
<tr>
<td><strong>LDL, mg/dL</strong></td>
<td>2.5 ± 0.2</td>
</tr>
<tr>
<td><strong>HDL, mg/dL</strong></td>
<td>1.3 ± 0.1</td>
</tr>
</tbody>
</table>

Data are presented as means ± SE. Tx, treatment; BMI, body mass index; OGGT, oral glucose tolerance test; S_0, 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 S_0 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 pmol/l to μU/ml, divide by 6; cholesterol mmol/l to mg/dl, multiply by 38.67; triglyceride mmol/l to mg/dl, multiply by 22.6.

**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 kg (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 pioglitazone gained fat at a higher rate than subjects treated with metformin.

### 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 OGGT was calculated from 0-, 30-, 60-, 90-, and 120-min samples.
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 vs. 4.6 ± 0.6% after treatment, P = 0.001 vs. baseline), whereas IMCL in type I fiber was unchanged with metformin (6.3 ± 1.2% vs. baseline; P = 0.5 vs. baseline). The decrease in IMCL with pioglitazone treatment was significant for type II fibers but not with metformin (2.6 ± 0.5 to 2.2 ± 0.5%, P = 0.16 vs. baseline; Fig. 3). The changes in IMCL in type II fibers were not significant after pioglitazone (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 muscle SDH (a mitochondrial enzyme in the TCA cycle). Were the decrease in IMCL the result of increased muscle lipid oxidative capacity, SDH activity should have increased with pioglitazone but not metformin treatment. In contrast, SDH activity was unchanged with either metformin or pioglitazone treatment (Table 2); indeed, SDH activity increased slightly with pioglitazone treatment (P = 0.07). Furthermore, mRNA levels of genes involved in muscle lipid oxidation including CPT I, PPARα, and PPARδ 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.

### Table 2. SDH activity and expression of PPARα, PPARδ, and CPT I

<table>
<thead>
<tr>
<th>Gene</th>
<th>Metformin Baseline</th>
<th>Metformin Post-Tx</th>
<th>Pioglitazone Baseline</th>
<th>Pioglitazone Post-Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDH activity</td>
<td>130.7±15.5</td>
<td>121.3±12.2</td>
<td>119.8±21.1</td>
<td>98.1±18.2</td>
</tr>
<tr>
<td>PPARα</td>
<td>1.50±0.2</td>
<td>1.5±0.2</td>
<td>1.9±0.3</td>
<td>1.6±0.3</td>
</tr>
<tr>
<td>PPARδ</td>
<td>1.2±0.1</td>
<td>1.4±0.1</td>
<td>1.2±0.1</td>
<td>1.3±0.1</td>
</tr>
<tr>
<td>CPT I</td>
<td>1.1±0.1</td>
<td>0.9±0.1</td>
<td>0.8±0.1</td>
<td>1.0±0.2</td>
</tr>
</tbody>
</table>

SDH, succinate dehydrogenase; PPAR, peroxisome proliferator-activated receptor; CPT I, carnitine palmitoyltransferase I. There was no significant change in SDH activity (expressed as optical density units/min × 10⁻⁴) in response to either metformin or pioglitazone. Expression of PPARα, PPARδ, and CPT I measured by real-time PCR genes (cycle no. relative to 18S RNA) did not change significantly after medications.
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 $S_I$ and also resulted in a significant decrease in type I fiber IMCL. The $S_I$ 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 $S_I$, 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 $S_I$.

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 1H 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 TRIOPOD 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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