Pioglitazone-Induced Increase in the Stearoyl-CoA Desaturation Index and Fat Accumulation in Rat Muscles Are Not Related to Lipoprotein Lipase Activity

Masaru Ochiai* and Tatsuhiro Matsuo

Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki, Kita, Kagawa, 761-0795 Japan

Abstract: Muscular insulin resistance is a characteristic of obesity and type 2 diabetes, but little is known about fatty acid (FA) metabolism in insulin-resistant skeletal muscle. In this study, we investigated the effects of the repeated administration of the PPAR-γ agonist pioglitazone on fat accumulation, FA composition, and stearoyl-CoA desaturase (SCD) index in rat tissues. Seventeen 4-week-old male Wistar rats were divided into control (C, n = 9) and pioglitazone treatment (P, n = 8) groups, and all the rats were fed a high-fat and high-sucrose diet for 8 weeks. Vehicle or pioglitazone (3 mg/kg) was orally administered daily to rats in the C group and P group, respectively. In the eighth week of the test period, an oral glucose tolerance test (OGTT) was performed after 12 h fasting. At the end of the test period, serum, liver, perirenal adipose tissue, and skeletal muscles were removed after 12 h fasting. The fasting serum and plasma glucose concentrations and OGTT glucose and insulin levels were significantly lower, while the serum adiponectin concentration was significantly higher in the P group than in the C group. Pioglitazone administration increased fat accumulation in the various muscle types examined, perirenal adipose tissue, and brown adipose tissue (BAT), but decreased fat accumulation in the liver. Pioglitazone administration increased the SCD indices for the muscles, perirenal adipose tissue, and liver, but not those of BAT. The lipoprotein lipase (LPL) activity of the BAT and perirenal adipose tissue, but not the muscles, was higher in the P group than in the C group. These results indicate that pioglitazone administration improved glucose tolerance and increased fat accumulation and SCD indices in the muscles and adipose tissues of rats. The increased fat accumulation was closely correlated with LPL activity in both adipose tissues, but not in the muscles.

Key words: pioglitazone, fat accumulation, fatty acid composition, stearoyl CoA desaturation, lipoprotein lipase

1 INTRODUCTION
Muscular insulin resistance (IR) is a characteristic of obesity and leads to the development of type 2 diabetes. Many studies have suggested that intramuscular lipid accumulation contributes to the development of IR[1,2,3]. However, well-trained athletes also have high intramuscular triacylglycerol (IMTG) concentrations, but preserve insulin sensitivity[4,5]. The molecular mechanisms linking IMTG accumulation and impaired IR have not yet been fully elucidated, although this metabolic paradox indicates that the total amount of IMTGs might not directly impair insulin action. Various studies have demonstrated that saturated fatty acids (SAs) in skeletal muscle are linked to the insulin signaling cascade[6,7]. In accordance with this, increased amounts of saturated FAs in skeletal muscles have been found to be associated with IR, whereas polyunsaturated FAs were associated with improved IR[8]. Therefore, the relationship between saturated and unsaturated FAs in skeletal muscles may contribute substantially to the development of IR[9,10].

Stearoyl-CoA desaturase (SCD) is a rate-limiting enzyme responsible for the conversion of saturated FAs, e.g., palmitic acid (16:0) and stearic acid (18:0), to their respective monounsaturated forms. This enzymatic process is essential for the normal functioning of the cell and is involved in the regulation of lipid metabolism. However, the role of SCD in the development of IR in skeletal muscle is not fully understood. This study aimed to investigate the effects of pioglitazone, a PPAR-γ agonist, on fatty acid metabolism in skeletal muscle and to determine its potential role in the development of IR.

Abbreviations: IR, insulin resistance; IMTG, intramuscular triacylglycerol; FA, fatty acid; SCD, stearoyl-coenzyme A desaturase; TG, triacylglycerol; TZDs, Thiazolidinediones; PPAR, peroxisome proliferator receptor; LPL, lipoprotein lipase; CMC-Na, carboxymethylcellulose sodium salt; OGTT, oral glucose tolerance test; BAT, brown adipose tissue; EDL, extensor digitorum longus; NEFA, non-esterified fatty acid; ELISA, enzyme-linked immunosorbent assay; DG, diacylglycerol
tive unsaturated FAs, i.e., palmitoleic acid (C16:1) and oleic acid (C18:1). The upregulation of SCD1 could result in a significant increase in the rate of triacylglycerol (TG) synthesis and protect against FA-induced IR \(^\text{3, 10}\). Endurance exercise reportedly increases muscular TG synthesis and improves IR by stimulating SCD1 activity in skeletal muscles \(^\text{11}\). Therefore, increasing the proportion of unsaturated FAs in skeletal muscles is thought to be important in improving IR.

Thiazolidinediones (TZDs), which stimulate peroxisome proliferator receptor gamma (PPAR-\(\gamma\)), are known to facilitate the differentiation of adipocytes and generate small adipocytes, resulting in improved muscular IR \(^\text{12}\). PPAR-\(\gamma\) is highly expressed in adipose tissues, but is not abundant in skeletal muscles \(^\text{13, 14}\). However, the activation of PPAR-\(\gamma\) also modifies lipid metabolism and the FA composition of skeletal muscles \(^\text{15}\). Lessard et al. suggested that the proportion of monounsaturated FAs in skeletal muscles is increased by rosiglitazone, a TZD compound \(^\text{16}\). Recently, rosiglitazone was also reported to increase the TG content and SCD indices (C18:1/C18:0 ratio) of rat oxidative muscles \(^\text{17, 18}\), but little is known about the effects of other TZDs, e.g., pioglitazone. Some clinical trials have shown that rosiglitazone worsens cardiovascular conditions when compared with pioglitazone \(^\text{17, 18}\). Pioglitazone has not been reported to worsen the risk of cardiovascular disease and has been used for the treatment of type 2 diabetes, although the binding potency of pioglitazone to PPAR-\(\gamma\) is weaker than that of rosiglitazone \(^\text{19}\). However, little is known about the effects of pioglitazone on TG accumulation, FA composition, and SCD indices in several types of skeletal muscles.

Blood TG uptake and fat metabolite accumulation in adipose tissues and skeletal muscles are regulated by or related to lipoprotein lipase (LPL) activity \(^\text{20}\). LPL is a rate-limiting enzyme for TG hydrolysis that regulates the blood TG concentration. Insulin plays an important role in the biosynthesis of LPL, so LPL expression and its function increases with increasing insulin sensitivity \(^\text{21}\). However, it remains unknown which types of skeletal muscles and other tissues are stimulated by pioglitazone, how pioglitazone stimulates LPL activity and fat metabolism, and even whether pioglitazone stimulates LPL activity at all.

In this study, we investigated the effects of pioglitazone on fat accumulation, FA composition, SCD indices, and LPL activity in various rat tissues.

2 MATERIALS AND METHODS

2.1 Animals and diets

All procedures involving rats were approved by the Experimental Animal Care Committee of Kagawa University.

Seventeen 3-week-old male Wistar rats were purchased from Japan SLC (Shizuoka, Japan). The rats were housed individually at 22 ± 1°C with lights on between 08:00 and 20:00 h and given free access to water and food (CE-2; JapanCLEA, Tokyo, Japan) for 7 days of acclimation. The rats were divided into 2 groups, control group (C, \(n = 9\)) and pioglitazone treatment group (P, \(n = 8\)), and were fed a high-fat and high-sucrose diet for 8 weeks. The diet contained 25.0% (w/w) casein, 0.38% DL-methionine, 14.86% corn starch, 20.0% sucrose, 5.0% cellulose, 5.0% soybean oil, 25.0% beef tallow, 3.5% AIN-76-based mineral mix, 1.0% AIN-76-based vitamin mix, 0.25% choline chloride, and 0.01% butylhydroxytoluene. The relative dietary energy from fat, carbohydrates, and protein was 52.3%, 28.1%, and 19.7%, respectively. The FA composition of the soybean oil was 10.8% palmitic acid, 4.6% stearic acid, 23.8% oleic acid, 53.5% linoleic acid, and 7.2% linolenic acid, and the FA composition of the beef tallow was 3.2% myristic acid, 26.3% palmitic acid, 3.4% palmitoleic acid, 18.5% stearic acid, 45.9% oleic acid, and 2.7% linoleic acid. The rats were given an oral dose of 3 mg/kg (as 5 mL of solution per kg) pioglitazone hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in a 0.5% (w/v) carboxymethylcellulose sodium salt (CMC-Na) solution (P group) and 5 mL/kg of 0.5% CMC-Na (C group) once a day for 8 weeks. The body weight and dietary intake of each rat were monitored daily.

2.2 Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed in the eighth week of the test period. After a 12 h fast, D-glucose (2 g, in 5 mL, per kg) was orally administered, and blood was collected from the tail vein before administration and at 30, 60, 90, and 120 min after. The blood was centrifuged at 6200 × g for 5 min to obtain the plasma, and stored at −80°C until analysis.

2.3 Sampling of blood and tissues

At the end of the test period, all of the rats were sacrificed by decapitation after a 12 h fast. Blood was collected and centrifuged at 6200 × g for 15 min to obtain the serum. The liver, intra-abdominal adipose tissues (perirenal, epididymal, and mesenteric), brown adipose tissue (BAT), and skeletal muscles (soleus, gastrocnemius, and extensor digitorum longus, EDL) were quickly removed, weighed, and frozen in liquid nitrogen. The serum, liver, perirenal adipose tissue, BAT, and muscles were then stored at −80°C until analysis.

2.4 Analysis

Plasma OGTT glucose levels and serum levels of glucose, non-esterified FAs (NEFAs), TGs, and total cholesterol were measured using commercial kits (Wako Pure Chemical Industries, Ltd.). Serum adiponectin levels were measured using a commercial enzyme-linked immunosorbent assay.
230:2, v/v. The TG concentrations in the total lipids from the liver and skeletal muscles were determined using gas chromatography. The liquid in the TG extract was evaporated under a stream of nitrogen gas and then transmethylated with a methanol and sulfuric acid (230:2, v/v) mixture. The FA methyl esters were extracted using hexane and separated using a gas chromatograph (GC-2014; Shimadzu, Kyoto, Japan) equipped with a 30-m capillary column (Ulbon HR-30 M; Shimadzu). The column temperature was 210°C, and the carrier gas was helium at a flow rate of 0.65 mL·min⁻¹. The methyl esters of individual FAs were identified by comparing their retention times to those of pure methyl esters and then quantified using their peak areas. The LPL activity in the perirenal adipose tissue, BAT, and skeletal muscles was measured using the method of Nilsson-Ehle and Schotz. The LPL activity assay was performed by incubating the extracts with the substrate at 37°C for 30 min and measuring the NEFAs released during the incubation calorimetrically using a kit (NEFA-C Test Wako; Wako Pure Chemical Industries, Ltd.). One unit of LPL catalyzed the release of 1 µmol NEFA per hour.

2.5 Statistical analysis

Each value is expressed as the mean ± standard error. Differences between the groups were evaluated using Student’s t-test. A difference of p < 0.05 was considered statistically significant. All statistical analyses were performed using a commercially available software package (Excel Statistics 2008; SSRI, Tokyo, Japan).

3 RESULTS

3.1 Body weight, dietary intake, and tissue weight

The body weight, dietary intake, and tissue weight of the rats are shown in Table 1. Body weight gains and dietary intake were not significantly different between the C and P groups. Relative hepatic weight was significantly lower in the P group than in the C group. The relative weight of the perirenal adipose tissue and BAT was significantly higher in the P group than in the C group. There was no significant difference between the groups for the other tissues.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Effects of pioglitazone administration on body weight, food intake, tissue weights in rats.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Body weight and food intake</td>
<td></td>
</tr>
<tr>
<td>Initial (g)</td>
<td>87.6 ± 2.2</td>
</tr>
<tr>
<td>Final (g)</td>
<td>279.7 ± 7.1</td>
</tr>
<tr>
<td>Gain (g)</td>
<td>188.4 ± 7.1</td>
</tr>
<tr>
<td>Food intake (g/day)</td>
<td>12.1 ± 0.3</td>
</tr>
<tr>
<td>Food efficiency (mg/g)</td>
<td>283.0 ± 4.4</td>
</tr>
<tr>
<td>Organ and tissue weights</td>
<td></td>
</tr>
<tr>
<td>Liver (mg/g)</td>
<td>32.3 ± 0.7</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td></td>
</tr>
<tr>
<td>Perirenal (mg/g)</td>
<td>25.5 ± 0.7</td>
</tr>
<tr>
<td>Epididymal (mg/g)</td>
<td>27.7 ± 1.3</td>
</tr>
<tr>
<td>Mesenteric (mg/g)</td>
<td>21.7 ± 1.0</td>
</tr>
<tr>
<td>Intra-abdominal (mg/g)</td>
<td>74.9 ± 2.6</td>
</tr>
<tr>
<td>BAT (mg/g)</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>Hindlimb muscle</td>
<td></td>
</tr>
<tr>
<td>Soleus (mg/g)</td>
<td>0.8 ± 0.0</td>
</tr>
<tr>
<td>Gastrocnemius (mg/g)</td>
<td>2.0 ± 0.0</td>
</tr>
<tr>
<td>EDL (mg/g)</td>
<td>0.8 ± 0.0</td>
</tr>
</tbody>
</table>

Values are means ± SE (n=8-9).

*, **, *** p <0.05, 0.01, 0.001, respectively compared to C group (Student’s t-test)

1 Sum of the perirenal, epididymal, and mesenteric adipose tissues

3.2 OGTT

The OGTT glucose and insulin levels are shown in Fig. 1. The fasting and OGTT plasma glucose levels at 30 min after glucose administration were significantly lower in the P group than in the C group. The OGTT plasma insulin levels at 30, 60, and 120 min after glucose administration were significantly lower in the P group than in the C group.

3.3 Serum biochemical components

The serum biochemical-component levels are shown in Table 2. The serum adiponectin levels were significantly higher in the P group than in the C group. The serum glucose and total cholesterol levels were significantly lower in the P group than in the C group. The TG and NEFA levels were not significantly different between the groups.

3.4 TG content in tissues

The TG content in the liver and skeletal muscles is shown in Fig. 2. The hepatic TG content was significantly lower in the P group than in the C group. In contrast, the TG content in the soleus, gastrocnemius, and EDL muscles was significantly higher in the P group than in the C group.
3.5 FA composition of serum and tissues

The FA composition of the serum, liver, perirenal adipose tissue, BAT, and skeletal muscles is shown in Table 3. The palmitic acid percentage in the serum lipids was significantly higher in the P group than in the C group. The other FA percentages in the serum lipids were not significantly different between the groups. The palmitic and oleic acid percentages in the hepatic lipids were significantly lower, while the stearic, linoleic, and arachidonic acid percentages were significantly higher in the P group than in the C group. The palmitic and stearic acid percentages in the perirenal adipose tissues were significantly lower and the palmitoleic and oleic acid percentages were significantly higher in the P group than in the C group. The palmitic acid percentage in BAT was significantly lower and the stearic and linoleic acid percentages were higher in the P group than in the C group. The palmitoleic and oleic acid percentages in the muscular lipids were significantly higher and the palmitic and stearic acid percentages were significantly lower in the P group than in the C group. The FA desaturation indices, i.e., the C16:1/C16:0 and C18:1/C18:0 ratios, for the lipids in the serum and other tissues are shown in Figs. 3 and 4, respectively. The FA desaturation indices for the muscular lipids were significantly higher in the P group than in the C group. The FA desaturation indices for the perirenal adipose tissues were also significantly higher in the P group than in the C group. The C18:1/C18:0 ratios in the hepatic lipids and BAT were significantly lower in the P group than in the C group. The relationship between TG content and adipose tissue weight with the FA desaturation index C/C18:0 in the soleus muscle, liver, perirenal adipose tissue, and BAT is shown in Fig. 5. In the soleus muscle and liver, the FA desaturation indices were highly correlated with the TG content; however, this tendency was not seen in BAT.

3.6 LPL activity of tissues

The LPL activity of perirenal adipose tissue, BAT, and skeletal muscle is shown in Fig. 6. The perirenal adipose tissue and BAT LPL activity was significantly higher in the P group than in the C group. There was no significant difference in the LPL activity in the muscles.

4 DISCUSSION

The repeated administration of pioglitazone increased serum adiponectin levels remarkably and slightly improved glucose tolerance, as reported previously, and it significantly increased fat accumulation in the skeletal muscles, perirenal adipose tissue, and BAT. Pioglitazone also significantly increased the SCD indices for the skeletal muscles and perirenal adipose tissue. As shown in Fig. 5, fat accumulation and SCD indices were highly correlated in the soleus muscle and slightly correlated in the perirenal adipose tissue, but there was no correlation in BAT. Our
findings are in agreement with the results of other studies. Singh Ahuja et al. and Kuda et al. independently demonstrated that a TZD-induced increase in intramuscular SCD1 levels in rats improved IR, and Chabowski et al. demonstrated that the SCD indices in oxidative muscles were increased by PPAR-γ activation.
Fig. 3  Effects of pioglitazone administration on FA desaturation indexes (C16:1/C16:0) of serum and tissue TG Values are means ±SE (n=8-9). *** p < 0.001 compared to C group (Student’s t-test).

Fig. 4  Effects of pioglitazone administration on FA desaturation indexes (C18:1/C18:0) of serum and tissue TG Values are means ±SE (n=8-9). *, **, *** p < 0.05, 0.01, 0.001 compared to C group (Student’s t-test).
Fig. 5  Relationship between FA desaturation indexes (C18:1/C18:0) and TG content in tissues or adipose tissue weights in rats administered with pioglitazone.

Fig. 6  Effects of pioglitazone administration on LPL activities of tissues Values are means ±SE (n=8-9).*,**, p < 0.05,0.01 compared to C group (Student’s t-test).
SCD1 plays an important role in skeletal-muscle lipogenesis and that in other tissues, and is expressed abundantly in oxidative muscles\(^{26}\). Our results initially showed that TG accumulation and SCD indices were increased by pioglitazone not only in oxidative muscle (soleus) but also in glycolytic muscle (EDL) and oxidative-glycolytic muscle (gastronemius), and adiponectin secretion and glucose tolerance improved. However, the present study could not explain the contradictory result that glucose tolerance was improved by the increased accumulation of muscular TGs. Chabowski et al.\(^{16}\) showed that TG accumulation in the soleus and red gastrocnemius muscles was significantly increased by the administration of rosiglitazone or endurance exercise training in rats fed a high-fat diet. Their findings induced by PPAR-\(\gamma\) activation were in agreement with our present findings. Chabowski et al.\(^{16}\) measured the accumulation of muscular fat metabolites (TGs, diacylglycerol (DG), NEFAs, and phospholipids) separately in skeletal muscles and showed that the accumulation of DG and NEFAs in the soleus muscle was significantly decreased by rosiglitazone; however, we did not measure the fat metabolite content in the present study. Several research\(^{5, 29}\), and review articles\(^{3, 30}\) suggested that increased muscular TG accumulation was not directly involved in the disruption of the insulin signaling pathway, and the accumulation of other lipid metabolites (mainly DG) in skeletal muscles was closely related to IR. It is quite well accepted that DG content is increased in IR\(^{29}\) and reduced by endurance exercise\(^{29}\). However, as described previously\(^{50}\), whether the reduction in muscular DG by PPAR-\(\gamma\) activation is closely correlated to the improvement of glucose tolerance needs to be further investigated.

It has been repeatedly suggested that the excessive accumulation of saturated fat in skeletal muscles is an important factor in the development of IR, and lipotoxicity from fat accumulation is specific to saturated FAs\(^{3, 11, 32}\). Gao et al.\(^{31}\) suggested that oleate protects against palmitate-induced IR in L6 myotubes. Peng et al.\(^{32}\) suggested that oleate blocked palmitate-induced IR in muscle cells, and Pinnamaneni et al.\(^{37}\) suggested that increased muscular SCD1 activity resulted in a significant increase in the rate of TG synthesis and protected against FA-induced IR. The pioglitazone-induced increase in the ratio of palmitoleic and oleic acids can be closely associated with the improvement of glucose tolerance in the present study.

Muscles, liver, and adipose tissues are important insulin-responsive tissues, and the accumulation of fat metabolites is generally thought to be an important factor related to IR. LPL plays an important role in the hydrolysis of TG-rich lipoproteins and the delivery of blood NEFAs to insulin-responsive tissues. Stimulation LPL activity in muscles is known to increase the concentrations of fat metabolites and worsen IR because of lipotoxicity\(^{33, 34}\). Kageyama et al.\(^{35}\) suggested that pioglitazone selectively increases LPL mRNA expression in white adipose tissues, but not in muscles. The lack of a significant change in LPL production in skeletal muscles caused by pioglitazone could prevent the development of IR because excess NEFAs and TGs in the blood would not be transported to the muscles. This is in agreement with our results, which showed that LPL activity was closely correlated with fat accumulation in BAT and perirenal adipose tissue, but not in the various types of skeletal muscles examined. The LPL activity of the soleus muscle, which contained 3 times more TGs than the other muscles, was not altered by pioglitazone. The serum levels of TGs and NEFAs, serum SCD indices, and LPL activity in the skeletal muscles were not significantly influenced by pioglitazone, indirectly indicating that the uptake of blood TG-derived NEFAs was not stimulated by pioglitazone in the skeletal muscles, and the increased fat accumulation and SCD indices in the skeletal muscles occurred independently. Therefore, the increase in muscular TG content was not caused by the stimulation of muscular LPL activity, but by other mechanisms; however, the detailed mechanisms still need to be elucidated. The increase in LPL activity in the perirenal adipose tissue and BAT can partly explain the increase in adipose tissue weight by pioglitazone, as described by Kageyama et al.\(^{35}\). Some reported results\(^{36-38}\) do not agree with our findings. Dobrzn et al.\(^{36}\) suggested that SCD1 deficiency increased the rate of \(\beta\)-oxidation in oxidative muscles by activating the AMP-activated protein kinase pathway, thus protecting against IR and obesity. Increased SCD1 mRNA expression and SCD indices have been found in IR skeletal muscle from obese humans\(^{37}\). Diet-induced weight loss has been found to accompany a reduction in muscle SCD1 protein levels\(^{38}\). Considering these controversial data, the role of SCD in IR is important in muscles, but the detailed mechanisms still need to be fully elucidated.

SCD1 expression is reportedly abundant and closely related to lipogenesis in the liver\(^{25}\). SCD1 deficiency leads to a decrease in hepatic TG contents and downregulates \textit{de novo} FA synthesis\(^{38, 39}\). In our study, the hepatic weight, hepatic TG content, and hepatic SCD indices were closely correlated, and they were all decreased by pioglitazone. In particular, the stearic acid percentage was significantly increased, while the oleic acid percentage was decreased in the hepatic lipids by pioglitazone. The results from the liver clearly contrasted with those from the skeletal muscles and white adipose tissue. Therefore, the regulation of lipogenesis by pioglitazone was tissue specific. In spite of these contrasting results, Kubota et al.\(^{35}\) suggested that the pioglitazone-induced increased secretion of adiponectin in the blood improved hepatic IR. Toyama et al.\(^{40}\) showed that a high dose of pioglitazone (30 mg/kg) improved IR, but did not alter the serum and liver SCD indices. These findings suggest that the hepatic SCD index is not responsible for the improvements in IR. Therefore,
there are still conflicting findings regarding the effects of pioglitazone on SCD function in the liver. In conclusion, the repeated administration of low levels of pioglitazone for 8 weeks improved glucose tolerance and increased fat accumulation and SCD indices in adipose tissues and oxidative and glycolytic skeletal muscles in rats. The increase in fat accumulation induced by pioglitazone was closely correlated with LPL activity in adipose tissues, but did not correlate with LPL activity in the skeletal muscles.

REFERENCES