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# Effects of Pioglitazone on Fat Accumulation and Fatty Acids Profile in Rat Muscles

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## Abstract

Insulin resistance of muscle is a characteristic of obesity and type 2 diabetes, but little is known about fatty acids metabolism in skeletal muscle with insulin resistance. We here investigated the effects of repeated administration of PPAR-γ agonist pioglitazone on fat accumulation, fatty acids composition, and desaturation index in rat muscles, serum and liver. Thirteen male Wistar rats aged 4 weeks divided into control (Ct, n=7) or pioglitazone (P, n=6) group, and then all rats were fed a high-fat and high-sucrose diet for 8 weeks. Vehicle or pioglitazone (1mg/kg) was orally administered daily to rats in the Ct group or P group, respectively. At 8$^{th}$ week of the test period, oral glucose tolerance test (OGTT) was performed after 12 hours fasting. At the end of the treatment period, serum, liver, intra-abdominal fat and skeletal muscles were kept at −80°C until analysis. Fasting serum and OGTT plasma glucose concentrations were not different between the two groups, although serum adiponectin concentration was significantly higher in the P group than in the Ct group. Stearoyl CoA desaturase (SCD) index was slightly higher, while arachidonic acid percentage was significantly lower in the P group than in the Ct group specifically in plantaris and tibial anterior muscles. These results indicated that repeated administration of pioglitazone could not improve insulin resistance, but the increase of serum adiponectine concentration and SCD index of plantaris and tibial anterior muscles were observed.

Keyword : Pioglitazone, Muscle, Fat accumulation, Fatty acids profile, Stearoyl CoA desaturase

## Introduction

Insulin resistance of muscle is a characteristic of obesity and leads the development of type 2 diabetes. Some data suggested that accumulation of intramuscular triglyceride (IMTG) contributes to the development of insulin resistance[1, 2]. However, well-trained athletes also contain high IMTG contents despite preserved insulin sensitivity. The molecular mechanisms linking IMTG accumulation and impaired insulin sensitivity have not yet fully clarified, although this metabolic paradox indicates that the total amount of IMTG might not directly impair insulin action. Various studies demonstrated that specific lipid intermediates, such as the pattern of intramuscular saturated and unsaturated fatty acids (FA), ceramide, and diacylglycerol may link to the insulin signaling cascade[3-6]. In accordance with such an assumption, increased intracellular amounts of saturated FA and lipid intermediates in muscle were related to insulin resistance, whereas polyunsaturated long-chain FA were associated with improved insulin sensitivity[7, 8]. Thus, considerable evidence suggests that the relation of saturated and unsaturated long-chain FA in muscle may substantially contribute to the development of insulin resistance.

Stearoyl-CoA desaturase 1 (SCD1) is the rate-limiting enzyme responsible for the conversion of the saturated FA, like palmitic acid (C16:0) and stearic acid (C18:0) to the unsaturated FA, like palmitoleic acid (C16:1) and oleic acid (C18:1), respectively. Some researchers suggested that up-regulation of muscular SCD1 activity might protect against free FA-induced insulin resistance[9, 10]. Endurance exercise is reported to stimulate SCD1 activity[11]. In our previous study[12], a single bout of endurance exercise enhanced the ratio of FA (oleic acid/stearic acid) in liver, but the increase of SCD1 index in liver has not yet clarified to improve insulin resistance.

In contrast to the mentioned results described above, SCD1 knockout mice were protected from insulin resistance[13]. Furthermore, increased SCD1 mRNA expression and FA desaturation index (C18:1/C18:0) were found in obese human muscle with insulin resistance. Diet-induced weight loss was accompanied by a reduction of muscle SCD1 protein levels[14]. Considering these controversial data, the role of SCD1 and desaturase activity toward insulin resistance remains unclear in rats and humans.

A pharmacological intervention affecting insulin sensitiv-

ity may help to further elucidate this paradoxical phenomenon. Thiazolidinediones (TZDs), which stimulate peroxisome proliferator receptor-γ (PPAR-γ), are known to improve muscular insulin sensitivity[15]. It was suggested that the activation of PPAR-γ may also modify intracellular lipid metabolism and the profile of intramuscular long-chain FA[16]. In fact, muscle-specific PPAR-γ deletion did not alter the total IMTG content[17], whereas the relative amount of intramuscular monounsaturated FA was increased after PPAR-γ activation using TZDs[18]. Rosiglitazone, a kind of TZDs, resulted in an increased muscular SCD1 expression in diabetic fatty rats[19]. As concomitant changes in muscular FA profile were not yet evaluated, it remained unclear whether the effect on SCD1 indeed results in a PPAR-γ-dependent modification of intracellular long-chain FA pattern. Further, TZDs are reported to improve hepatic insulin resistance in rats, but little is well-known about the relationship between hepatic insulin sensitivity and FA profile in liver with TZDs treatment.

In this study, we investigated the effects of repeated administration of pioglitazone, another effective PPAR-γ agonist on glucose tolerance, fat accumulation, FA profile, and desaturation index in rat serum, liver, and muscles.

## Materials and Methods

### Test samples

Pioglitazone hydrochloride was purchased from Wako Pure Chemical Industries, Ltd (Tokyo, Japan).

### Animals and diets

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

Thirteen male Wistar rats aged 3 weeks were purchased from Japan SLC (Shizuoka, Japan) and acclimatized to the housing conditions for a week before the experiment. All the rats were individually housed at 22°C ± 1°C with lights on from 0800 to 2000 hours. Rats were divided into two group; control (Ct, n=7) or pioglitazone (P, n=6) group on the bases of body weight, and were fed a high-sucrose and high-fat diet. The diet contained 25.0% (w/w) casein, 0.38% DL-methionine, 14.86% cornstarch, 20.0% sucrose, 5.0% cellulose, 5.0% soybean oil, 25.0% beef tarrow, 3.5% AIN-76-based mineral mix, 1.0% AIN-76-based vitamin mix, 0.25% choline chloride, and 0.001% butylhydroxytoluene. The diets respectively provided 52.3%, 28.1%, and 19.7% of energy as fat, carbohydrate, and protein. 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, while the fatty acids composition of the beef tarrow was 3.2% myristic acid, 26.3% palmitic acid, 3.4% palmitoleic acid, 18.5% stearic acid, 45.9% oleic acid, 2.7% linoleic acid. Rats in the P and Ct groups received one oral dose of 1 mg/5 ml/kg pioglitazone dissolved into 0.5% (w/v) carboxymethylcellulose sodium salt (CMC) solution and 5 ml/kg CMC once a day for 8 weeks, respectively. The body weight and dietary intake of each rat were monitored daily.

### Oral glucose tolerance test

At 8$^{th}$ week during the treatment period, oral glucose tolerance test was performed. After a 12-hours fasting period, D-glucose (2g/kg) was orally administrated and blood was collected from tail vain before and 30, 60, 90, and 120 min after the administration. The blood was centrifuged at 6,200 × *g* rpm for 5 min to obtain plasma, and then stored at −80°C until analysis.

### Sampling of blood and tissues

At the end of the test period, all the rats were sacrificed by decapitation after 12 hours fasting. The blood was collected and centrifuged at 6,200 × *g* for 15 min to obtain serum. The liver, perirenal fat, and muscles (soleus, plantaris, gastrocnemius, tibial anterior and extensor digitorum longus) were rapidly removed, weighed, and frozen in liquid nitrogen. The serum and the all tissues were then stored at −80°C until analysis.

### Analysis

The plasma OGTT glucose concentration and the serum concentrations of glucose, free FA, and triglyceride were measured by using commercial kits (Wako Pure Chemical Industries, Ltd). The serum adiponectin concentration was measured by using commercial ELISA kit (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). The glycogen contents of the liver and plantaris muscles were determined according to the method of Lo *et al.*[20] and total lipids in the liver and muscles (soleus, plantaris, gastrocnemius, and tibial anterior) were extracted by the method of Folch *et al.*[21] The triacylglycerol (TG) concentrations of total lipids in the liver and muscles were measured by using a Triglyceride E-Test kit (Wako Pure Chemical Industries, Ltd). The FA compositions of the serum and TG of the liver, and muscles (soleus, plantaris, gastrocnemius, and tibial anterior) were determined by gas

Table 1 Body weight, food and energy intake, and tissue weights

| | Ct | P |
|---|---|---|
| Body weight and food intake | | |
| Initial (g) | 74.0 ± 3.4 | 77.7 ± 1.5 |
| Final (g) | 269.0 ± 5.8 | 267.5 ± 8.8 |
| Gain (g) | 195.0 ± 5.7 | 189.8 ± 7.8 |
| Food intake (g/day) | 11.6 ± 0.3 | 11.5 ± 0.4 |
| Tissues weights | | |
| Liver (mg/g) | 30.9 ± 0.7 | 29.2 ± 1.1 |
| Perirenal fat (mg/g) | 30.9 ± 1.2 | 31.3 ± 1.1 |
| Epididymal fat (mg/g) | 30.8 ± 1.2 | 34.8 ± 1.6 |
| Mesentric fat (mg/g) | 20.8 ± 1.4 | 20.4 ± 1.0 |
| Intra-abdominal fat (mg/g) | 82.5 ± 3.4 | 86.5 ± 3.1 |
| Soleus muscle (mg/g) | 0.608 ± 0.04 | 0.624 ± 0.03 |
| Plantaris muscle (mg/g) | 1.71 ± 0.06 | 1.75 ± 0.05 |
| Gastrocnemius muscle (mg/g) | 9.23 ± 0.27 | 9.04 ± 0.25 |
| Tibia anteria muscle (mg/g) | 4.22 ± 0.14 | 4.06 ± 0.08 |
| EDL muscle (mg/g) | 0.788 ± 0.026 | 0.773 ± 0.012 |

Values are means ± SE (n=6–7).

Table 2 Serum concentrations of biochemical components

| | Ct | P |
|---|---|---|
| Glucose (mg/dL) | 147.8 ± 6.5 | 135.0 ± 4.4 |
| Triglyceride (mg/dL) | 110.8 ± 22.3 | 86.8 ± 13.9 |
| Free fatty acid (mEq/L) | 1.2 ± 0.1 | 1.5 ± 0.1* |
| Adiponectin (ng/mL) | 5.0 ± 0.3 | 6.1 ± 0.4* |

Values are means ± SE (n=6–7).
The differences were evaluated using Student *t*-test.
A difference of $p<0.05$ was considered statistically significant.
*$p<0.05$, vs Ct gtoup

chromatography. The liquid in the TG extract was vaporized by nitrogen gas and then transmethylated with methanol-sulfuric acid (230:2, v/v). The FA methyl esters were extracted with hexane and separated by gas chromatography (GC-2014 instrument; Shimadzu, Kyoto, Japan) equipped with a 30-m capillary column (Ulbon HR-20 M; Shimadzu). The column temperature was set at 210°C, and the carrier gas was helium at a flow rate of 0.65 mL·min$^{-1}$. The methyl esters of individual FA were identified in the chromatograms by comparing their retention times to those of pure methyl esters, and then quantified by comparing the areas under their peaks.

### Statistical analysis

Each value is expressed as the mean ± SE (n=6–7 rats per group). Differences between 2 groups were evaluated by Student *t*-test. A difference of $p < 0.05$ was considered statistically significant. The all statistical analyses were performed by using a commercially available statistical package (Excel Statistics 2008; SSRI, Tokyo).

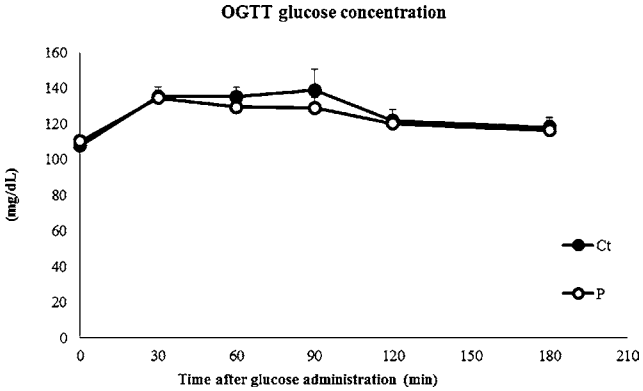


Fig. 1 Effects of repeated administration of pioglitazone (1mg/kg) on oral glucose tolerance test in rat fed high-fat and high-sucrose diet. Values are means and SE for 6–7 rats.

## Results

### Body weight and dietary intake

Body weight and dietary intake of rats are shown in Table 2. The body weight gain and dietary intake were not significantly different between Ct and P groups.

### Oral glucose tolerance test

The OGTT glucose concentrations are shown in Fig.1. No significant difference was observed between the Ct and P groups in any time points.

### Tissue weights

Tissue weights are shown in Table 3. No significant difference was observed in the liver, intra-abdominal fat, and muscles.

### Serum components

Serum components are shown in Table 4. Serum concentrations of free FA and adiponectin were significantly higher in the P group than the Ct group. Serum concentrations of glucose and triglyceride were not significantly different between the two groups.

### Glycogen and triglyceride contents in tissues

Glycogen and TG contents in the liver and skeletal muscles are shown in Table 5. Glycogen and TG contents in the plantaris muscle were significantly higher in the P group than in the Ct group. The glycogen and TG contents in the liver were not significantly different between the two groups. TG contents in the other muscles were also not significantly different.

Table 3 Glycogen and TG contents in tissues

| | Ct | P |
|---|---|---|
| Glycogen content | | |
| Liver (mg/g) | 17.9 ± 4.1 | 24.4 ± 5.6 |
| Plantaris muscle (mg/g) | 0.260 ± 0.02 | 0.348 ± 0.02* |
| Triglyceride content | | |
| Liver (mg/g) | 34.9 ± 2.7 | 31.1 ± 4.2 |
| Soleus muscle (mg/g) | 36.4 ± 9.6 | 48.2 ± 9.8 |
| Plantaris muscle (mg/g) | 9.1 ± 0.9 | 14.3 ± 2.1* |
| Tibia anteria muscle (mg/g) | 15.5 ± 1.0 | 17.0 ± 2.0 |
| Gastrocnemius muscle (mg/g) | 19.0 ± 2.4 | 14.8 ± 1.7 |

Values are means ± SE (n=6-7).
The differences were evaluated using Student *t*-test.
A difference of $p<0.05$ was considered statistically significant.
*$p<0.05$, vs Ct gtoup

Table 4 FA profile of serum lipids

| | Ct | P |
|---|---|---|
| Fatty acid[1] | | |
| C14:0 | ND | ND |
| C16:0 | 29.3 ± 0.7 | 29.9 ± 0.7 |
| C16:1 | ND | ND |
| C18:0 | 24.9 ± 0.9 | 26.8 ± 1.0 |
| C18:1 | 17.5 ± 1.3 | 15.8 ± 0.8 |
| C18:2 | 6.8 ± 0.9 | 9.0 ± 0.8 |
| C18:3 | ND | ND |
| C20:4 | 21.4 ± 1.1 | 18.5 ± 0.8 |
| Desaturation index | | |
| C16:1/C16:0 | ND | ND |
| C18:1/C18:0 | 0.72 ± 0.1 | 0.60 ± 0.0 |

Values are means ± SE (n=6-7).
[1]Number of carbon atoms : number of double bonds; ND, not detected

## FA compositions of serum and tissues

FA compositions of serum and skeletal muscles are shown in Tables 6-9. Stearoyl-CoA desaturase (SCD) index, the ratio of oleic acid/stearic acid, and palmitoleic acid/palmitic acid of serum and the tissues are also shown in Tables 6-9. The FA profile of lipids in the liver, soleus muscle, and gastrocnemius muscle were not significantly different between the two groups. The arachidonic acid percentage of lipids in the plantaris and tibial anterior muscles were significantly higher in the P group than in the Ct group.

## Discussion

We here investigated the effects of repeated administration of PPAR-γ agonist pioglitazone (1mg/kg) for 8 weeks on glucose tolerance, fat accumulation, and FA profile in rat serum, liver and muscles. Especially, it was investigated whether

Table 5 FA profile of liver lipids

| | Ct | P |
|---|---|---|
| Fatty acid[1] | | |
| C14:0 | 0.7 ± 0.1 | 0.8 ± 0.3 |
| C16:0 | 23.4 ± 0.4 | 24.0 ± 1.2 |
| C16:1 | 2.4 ± 0.2 | 2.4 ± 0.5 |
| C18:0 | 18.2 ± 1.0 | 19.6 ± 2.0 |
| C18:1 | 31.2 ± 1.0 | 28.7 ± 1.7 |
| C18:2 | 8.4 ± 0.3 | 9.0 ± 0.6 |
| C18:3 | 0.1 ± 0.1 | ND |
| C20:4 | 15.6 ± 0.7 | 15.4 ± 1.7 |
| Desaturation index | | |
| C16:1/C16:0 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| C18:1/C18:0 | 1.8 ± 0.1 | 1.6 ± 0.3 |

Values are means ± SE (n=6-7)
[1]Number of carbon atoms : number of double bonds; ND, not detected

Table 6 FA profile of soleus muscle lipids

| | Ct | P |
|---|---|---|
| Fatty acid[1] | | |
| C14:0 | ND | ND |
| C16:0 | 24.4 ± 2.0 | 21.9 ± 0.2 |
| C16:1 | 3.7 ± 0.3 | 4.5 ± 0.5 |
| C18:0 | 13.4 ± 1.3 | 11.8 ± 1.3 |
| C18:1 | 39.3 ± 2.4 | 43.8 ± 1.9 |
| C18:2 | 15.4 ± 1.9 | 14.1 ± 0.9 |
| C18:3 | ND | ND |
| C20:4 | 3.9 ± 0.6 | 3.8 ± 0.7 |
| Desaturation index | | |
| C16:1/C16:0 | 0.16 ± 0.01 | 0.21 ± 0.02 |
| C18:1/C18:0 | 3.22 ± 0.52 | 4.01 ± 0.58 |

Values are means ± SE (n=6-7).
[1]Number of carbon atoms : number of double bonds; ND, not detected

Table 7 FA profile of tibial anterior muscle lipids

| | Ct | P |
|---|---|---|
| Fatty acid[1] | | |
| C14:0 | ND | ND |
| C16:0 | 30.6 ± 0.6 | 31.7 ± 1.3 |
| C16:1 | 2.8 ± 0.2 | 4.1 ± 0.5 |
| C18:0 | 16.9 ± 0.4 | 15.5 ± 1.0 |
| C18:1 | 34.1 ± 1.2 | 35.5 ± 1.6 |
| C18:2 | 10.0 ± 0.2 | 9.6 ± 0.4 |
| C18:3 | ND | ND |
| C20:4 | 5.6 ± 0.5 | 3.6 ± 0.3** |
| Desaturation index | | |
| C16:1/C16:0 | 0.09 ± 0.01 | 0.13 ± 0.02 |
| C18:1/C18:0 | 2.04 ± 0.12 | 2.37 ± 0.26 |

Values are means ± SE (n=6-7).
The differences were evaluated using Student *t*-test.
A difference of $p<0.05$ was considered statistically significant.
**$p<0.01$, vs Ct gtoup
[1]Number of carbon atoms : number of double bonds; ND, not detected

Table 8 FA profile of plantaris muscle lipids

| | Ct | P |
|---|---|---|
| Fatty acid[1] | | |
| C14:0 | ND | ND |
| C16:0 | 30.5 ± 0.8 | 30.0 ± 0.9 |
| C16:1 | 2.2 ± 0.2 | 2.9 ± 0.3 |
| C18:0 | 18.1 ± 0.5 | 17.4 ± 1.3 |
| C18:1 | 27.5 ± 1.6 | 30.4 ± 2.9 |
| C18:2 | 10.8 ± 0.4 | 11.6 ± 0.3 |
| C18:3 | ND | ND |
| C20:4 | 10.8 ± 1.0 | 7.5 ± 0.9* |
| Desaturation index | | |
| C16:1/C16:0 | 0.1 ± 0.0 | 0.1 ± 0.0 |
| C18:1/C18:0 | 1.5 ± 0.1 | 1.9 ± 0.4 |

Values are means ± SE (n=6-7).
The differences were evaluated using Student *t*-test.
A difference of $p<0.05$ was considered statistically significant.
*p<0.05, vs Ct gtoup
[1]Number of carbon atoms : number of double bonds; ND, not detected

Table 9 FA profile of gastrocnemius muscle lipids

| | Ct | P |
|---|---|---|
| Fatty acid[1] | | |
| C14:0 | ND | ND |
| C16:0 | 28.6 ± 0.5 | 29.5 ± 0.7 |
| C16:1 | 2.7 ± 0.1 | 2.5 ± 0.3 |
| C18:0 | 17.1 ± 0.7 | 18.3 ± 0.7 |
| C18:1 | 31.0 ± 1.4 | 29.5 ± 1.3 |
| C18:2 | 11.3 ± 0.2 | 11.4 ± 0.6 |
| C18:3 | ND | ND |
| C20:4 | 9.4 ± 0.4 | 8.7 ± 0.8 |
| Desaturation index | | |
| C16:1/C16:0 | 0.09 ± 0.00 | 0.09 ± 0.01 |
| C18:1/C18:0 | 1.85 ± 0.17 | 1.63 ± 0.13 |

Values are means ± SE (n=6-7).
[1]Number of carbon atoms : number of double bonds; ND, not detected

SCD index of muscle was influenced by administration of pioglitazone.

Our results showed that repeated administration of pioglitazone significantly increased the serum adiponectin concentration and contents of glycogen and TG in plantaris muscle. Futhermore, the SCD index of plantaris muscle was slightly, but not significantly increased (1.3 times) by the repeated administration of pioglitazone. The percentages of stearic acid (C18:0) and oleic acid (C18:1) in the plantaris muscle were not significantly altered between the two groups, but arachidonic acid (C20:4) percentage was significantly lower in the P group. These results from the plantaris muscle indicated that some metabolic pathway from linoleic acid (C18:2) to arachidonic acid (C20:4) could be downregulated, but the details are not be clearfied in the present study. The arachidonic acid percentage of the tibial anterior muscle was also significantly lower and the SCD index was also a little higher in the P group than in the Ct group. However, no alterations were observed in the serum, liver, and the other muscles. It is not well clear the reason why the alteration of FA profile of the tibial anterior muscle was varied at different sites in the present study.

It is reported that increase of the arachidonic acid percentage in the serum and tissues may increase several eicosanoids, which are kinds of prophlogistic lipids mediators[22]. The increase of inflammatory mediators was well known to be associated with the development of obesity and type 2 diabetes. Therefore, administration of low pioglitazone for 8 weeks could prevent muscular insulin resistance, but further study is needed to clarify the effects of pioglitazone on the FA induced insulin resistance.

In the present study, the repeated administration of pioglitazone did not improve glucose tolerance (Fig.1) and fasting serum glucose concentration, although serum adiponectin concentration and glycogen content in the plantaris muscle were significantly increased. Kubota et al.[23] reported that the oral administration of 30 mg/kg pioglitazone for 2 weeks improved fasting glucose concentration and glucose tolerance in *ob/ob* mice, but the administration of 10 mg/kg pioglitazone did not show the similar results above. The dosage of 1 mg/kg pioglitazone in the present study might be low to improve glucose metabolism. It is possible that a higher dosage of pioglitazone can improve glocose metabolism and protect from insulin resistance in rats.

In conclusion, repeated administration of PPAR-γ agonist pioglitazone (1 mg/kg) for 8 weeks could not improve fasting glucose concentration and glucose tolerance in rats, but slightly, but not significantly increased SCD index specifically in the plantaris and tibial anterior muscles in the present study. Further studies using higher dosage of pioglitazone are needed to clarify the relationship between SCD activity and muscle insulin resistance.

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# ラット骨格筋脂肪蓄積と脂肪酸組成に及ぼすピオグリタゾンの影響

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## 要　　約

骨格筋インスリン抵抗性は肥満および２型糖尿病の発症要因であることが知られているが，骨格筋における脂肪酸代謝について明確ではない．本研究では，ラットの骨格筋および肝臓の脂肪蓄積と骨格筋，肝臓，および血清の脂肪酸組成，および脂肪酸不飽和化指標に及ぼすピオグリタゾン（１mg/kg/日）投与の影響についてラットを用いて検討した．８週間のピオグリタゾン投与により，血清アディポネクチン濃度が有意に上昇し，足底筋及び前脛骨筋の脂肪酸不飽和化指標（オレイン酸／ステアリン酸比）が上昇する傾向が示されたが，絶食時血糖値および耐糖能において有意な影響は確認されなかった．また，ピオグリタゾン投与は上記の骨格筋のアラキドン酸含有比を有意に低下させた．以上の結果より，低用量のピオグリタゾンの連続投与は耐糖能を改善しなかったが，血清アディポネクチン濃度と骨格筋不飽和化を上昇することが示された．