Fenofibrate improves insulin sensitivity in connection with intramuscular lipid content, muscle fatty acid-binding protein, and β -oxidation in skeletal muscle

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Abstract

We investigated the effect of fenofibrate, a peroxisome proliferator-activated receptor- α agonist, on insulin sensitivity including lipid metabolism in skeletal muscle. Six-week-old male Sprague-Dawley rats were divided into two groups: those fed a standard chow (control) or a fructose-rich chow (fructose-fed rats (FFRs)) for 6 weeks. FFRs were treated either with a vehicle or with 30 mg/kg per day of fenofibrate for the last 2 weeks. Insulin sensitivity (M-value) was estimated by the euglycemic hyperinsulinemic glucose clamp method. Fatty acid-binding protein (FABP) in skeletal muscle was measured by ELISA, and the expression of FABP mRNA was analyzed by semi-quantitative RT-PCR. The serum and muscle triglyceride (sTG and mTG) levels and the activity of 3-hydroxyacyl-CoA dehydrogenase (HADH), a β -oxidation enzyme, in muscle were also determined. FFRs showed a lower M-value and higher blood pressure,

sTG and mTG than did the control group. The mTG was correlated positively with sTG and negatively with the M-value. Fenofibrate treatment for 2 weeks did not change blood pressure but significantly improved the M-value, sTG and mTG. FABP content and mRNA in the soleus muscle were significantly elevated in FFRs compared with those in the control group. Fenofibrate treatment further increased FABP. The HADH activity was comparable between the control group and FFRs, but significantly increased by fenofibrate treatment. These results suggest that fenofibrate improves insulin sensitivity not only by lowering serum lipids and subsequent influx of fatty acids into muscles but also by reducing intramuscular lipid content via further induction of FABP and stimulation of β -oxidation in muscles.

Journal of Endocrinology (2002) **174**, 321–329

Introduction

Insulin resistance and accompanying hyperinsulinemia have been reported to play an important role in the onset and progression of hypertension, dyslipidemia and atherosclerosis (Reaven 1988, Kaplan 1989, DeFronzo & Ferrannini 1991, Iimura 1996). It has also been reported that accumulation of lipid content in skeletal muscle can interfere with insulin action and result in insulin resistance (Storlien *et al.* 1991, Goodpasteur *et al.* 1997, Pan *et al.* 1997, Kelley & Mandarino 2000).

Fatty acid-binding proteins (FABPs) are low-molecular-weight cytosolic proteins (14–15 kDa) found in various cell types. The FABP found in skeletal muscle was shown to be identical with heart-type FABP (Peeters *et al.* 1991). FABPs are thought to play a role in solubilization of long-chain fatty acids and consequently in facilitation of the cytosolic transport of long-chain fatty acids to various intracellular organelles, including the mitochondria, peroxisomes, nucleus and lipid droplets (Storch &

Thumser 2000). It has been suggested that FABPs are concerned with mitochondrial and peroxisomal β -oxidation, gene transcription and cell growth/differentiation, and incorporation of fatty acids into triglyceride (Veerkamp & van Moerkerk. 1993, Glatz *et al.* 1995, Borchers *et al.* 1997, Zanotti 1999).

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear receptor superfamily, which are involved in gene regulation and are important regulators of glucose and lipid homeostasis (Schoonjans *et al.* 1997). Whereas thiazolidinediones are insulin sensitizers acting via activation of PPAR γ , fibrates exert lipid-lowering activity via PPAR α . Fibrate treatment regulates lipoprotein metabolism (Staels *et al.* 1998), fatty acid oxidation (Meertens *et al.* 1998) and the cellular transport of fatty acids (Motojima *et al.* 1998) in the liver. Since the PPAR α protein is also expressed in skeletal muscle as well as in the liver (Su *et al.* 1998), fibrates also seem to be associated with intracellular lipid metabolism in skeletal muscles. It is noteworthy that the promoter of muscle

FABP has peroxisome proliferator responsive elements (Treuner *et al.* 1994).

Some studies have shown that fibrates also improved glucose tolerance or increased insulin sensitivity in humans (Jones *et al.* 1990, Inoue *et al.* 1994, Yong *et al.* 1999, Idzior-Walus *et al.* 2000) and rodents (Matsui *et al.* 1997, Guerre-Millo *et al.* 2000, Ye *et al.* 2001). However, in most of these studies, a gold standard assessment such as the euglycemic hyperinsulinemic glucose clamp method was not used. The mechanisms of these effects are also unclear. We therefore investigated the effect of fenofibrate, a highly selective PPAR α activator, on glucose homeostasis and its relationship to intramuscular lipid content, muscle FABP and muscle β -oxidation in fructose-fed rats (FFRs) as an animal model of insulin resistance and hypertension.

Materials and Methods

Protocol 1

As a preliminary study, 12-week-old male Sprague–Dawley rats ($n=15$) obtained from Charles River Japan Inc. (Yokohama, Japan) were used. The care of the animals was in strict accordance with the guiding principles of the Physiological Society of Japan. All rats were fed a standard rat chow containing 60% vegetable starch, 5% fat and 24% protein (Oriental Yeast Co., Tokyo, Japan). They were maintained on a 12 h light:12 h darkness (0700 h–1900 h) cycle and with freely available water and chow. Insulin sensitivity was estimated by the euglycemic hyperinsulinemic glucose clamp method, and soleus muscle was prepared under sodium pentobarbital anesthesia. The lipid content in the soleus muscle was extracted and measured.

Protocol 2

Animals Male Sprague–Dawley rats of 6 weeks of age were used for all experiments. Prior to any manipulation, all rats were fed standard rat chow. The rats were divided into two groups at the start of the study: those fed a standard rat chow (control group, $n=10$) or those given a fructose-rich chow containing 60% fructose, 5% fat, and 20% protein (#78463; Teklad, WI, USA) for 6 weeks. FFRs were treated by oral gavage either with a vehicle (2.5% gum arabic solution; FFR group, $n=10$) or with 30 mg/kg per day of fenofibrate (FF), a PPAR α -selective agonist (Kaken Pharmaceutical Co., Tokyo, Japan) in 2.5% gum arabic solution (FFR+FF group, $n=7$) for the last 2 weeks. The control group was also treated with the same vehicle by oral gavage during the treatment period.

Blood pressure and pulse rate measurements Systolic blood pressure and pulse rate were measured in all

conscious rats using an indirect tail-cuff method (BP-98A; Softran, Tokyo, Japan) on a 37 °C preheated cloth jacket for 10 min. An average of five recordings was taken as each individual value.

Determination of serum variables Blood samples were collected from the tail vein after 12 h deprivation of food. Serum was prepared by centrifugation at 2000 *g* for 15 min at 4 °C and stored at –20 °C until use for measurement of serum insulin and lipids. Rat serum insulin was measured with an enzyme immunoassay kit (Morinaga Seikagaku, Yokohama, Japan). Serum levels of triglyceride and free fatty acid were determined by enzymatic colorimetric methods using commercially available test kits (Wako Chemicals, Osaka, Japan) and a U-1100 spectrophotometer (Hitachi Seisakusyo, Tokyo, Japan).

Euglycemic hyperinsulinemic glucose clamp technique At the end of the treatment period, rats were anesthetized i.p. with sodium pentobarbital (50 mg/kg). The right common carotid artery and the right jugular vein were exposed and cannulated with a polyethylene tube (PE50; Becton Dickinson and Co., Sparks, MD, USA) for collection of blood samples and administration of the infusate respectively. Each rat was placed in a foam plastic jacket that allowed movement of all four limbs and forward vision after overnight fasting (approximately 12 h). At the start of the glucose clamp, fasting blood glucose was measured by the glucose oxidase method using an ExacTech 2A glucose analyzer (MediSense, Waltham, MA, USA). The initial load of insulin (25 mU/kg of Humalin R, U-40; Shionogi Pharmaceutical Co., Osaka, Japan) was infused by a bolus, and this was followed by a constant infusion of insulin at a rate of 4 mU/kg per min for 154 min. During the glucose clamp, 12.5% glucose solution was infused as needed to maintain blood glucose at the fasting level. Ten microliters of arterial blood were sampled at 7 min intervals for determination of blood glucose. The average of the rate of glucose infusion for the last 35 min was taken as an index of insulin sensitivity (M-value; mg/kg per min) (Higashiura *et al.* 1999).

Tissue preparation Rats were anesthetized i.p. with sodium pentobarbital (50 mg/kg) and perfused with 150 ml sterile saline through the apex of the left ventricle. Soleus (containing mainly slow-twitch oxidative fibers, type I) and extensor digitorum longus (EDL, containing a mixture of fast-twitch oxidative-glycolytic, type IIa and glycolytic fibers, type IIb) muscles (Ariano *et al.* 1973) were employed as red and white muscle respectively, and were dissected and immediately frozen in liquid nitrogen. Muscle samples were stored at –70 °C until use.

Skeletal muscle triglyceride Total lipid extracts of skeletal muscles were obtained by the method previously

described (Folch *et al.* 1957) using 2:1 (v/v) chloroform–methanol. These extracts were then dried under a nitrogen stream, dissolved in 2:0 ml 100:1 (v/v) chloroform–acetic acid, and applied to a Sep-Pak Silica cartridge (Waters, Milford, MA, USA) for separation of phospholipids. The fraction including triglyceride was eluted with 14 ml additional 100:1 chloroform–acetic acid (Hamilton & Comai 1984). A known volume of the fraction was evaporated to dryness using nitrogen. Triglyceride content was determined spectrophotometrically using a test kit as stated above.

Muscle FABP content All steps were performed on ice. Muscles were cut and homogenized (2:5%, w/v) in ice-cold PBS (pH 7.4) using a Polytron (Kinematica, Lucerne, Switzerland). Tissue homogenates were centrifuged at 15 000 *g* for 15 min at 4 °C to remove cell debris. The tissue content of cytosolic FABP was measured with a sandwich ELISA kit (HyCult Biotechnology, Uden, Netherlands) using murine monoclonal IgG antibodies directed against purified FABP. Total protein concentration was determined with a bicinchoninic acid kit (Protein Assay No. 23225; Pierce, Rockford, IL, USA) using BSA as a standard (Smith *et al.* 1985).

RNA extraction and analysis In all steps of the procedure, 0.1% diethyl pyrocarbonate (DEPC)-treated water was used. Total RNA was isolated according to the modified method as previously described (Chomczynski & Sacchi 1987) using Trizol reagent (Life Technologies, MD, USA) and dissolved in DEPC-treated water for direct use or stored in 70% ethanol at –70 °C. Semi-quantitative RT-PCR was performed to assess mRNA expression. The PCR primers used were an oligo (dT) first-strand primer and two pairs of specific primers based on previously reported sequences: 5′-TTGCACCATGG CGGACGCCTTT-3′ and 5′-AGTGACGGGGCAGC CAGGTCACGCCTCCTT-3′ for muscle FABP (Van Nieuwenhoven *et al.* 1994), and 5′-CTGATCCACA TCTGCTGGAAGGTGG-3′ and 5′-ACCTTCAACAC CCCAGCCATGTACG-3′ for β -actin (Wu *et al.* 2000) as a housekeeping gene and internal control. The PCR product from an RNA template was obtained with Ready-To-Go RT-PCR Beads (Amersham Pharmacia Biotech, Tokyo, Japan) containing Moloney murine leukemia virus reverse transcriptase and Taq DNA polymerase. The reaction mixture contained 2 μ g total RNA, the first-strand primer, one pair of specific primers, and DEPC-treated water in a total volume of 50 μ l. A one-step protocol for RT-PCR was performed using a PCR Thermal Cycler SP (TP400; Takara Shuzo Co., Shiga, Japan) according to the following scheme: (i) 15 min first-strand synthesis at 42 °C; (ii) 5 min inactivation of reverse transcriptase and denaturation at 95 °C; (iii) optimized cycles depending on the target (FABP of the soleus, 16 cycles; FABP of the EDL, 22 cycles; β -actin of

both muscles, 22 cycles) of 30 s at 95 °C (denaturation), 30 s at 59 °C (annealing), 30 s at 72 °C (elongation); and (iv) finally 7 min at 72 °C (final extension). Preliminary experiments confirmed that each PCR product was performed within the linear phase of the PCR amplified reaction. Each sample was electrophoresed on 1.5% agarose gel and stained with ethidium bromide. The intensity of the ethidium bromide luminescence was detected by charge coupled device (CCD) imaging (COMPACT BIS; Amersham Pharmacia Biotech) and analyzed using SigmaGel software (SPSS Inc., Chicago, IL, USA).

Enzymatic activities Activities of 3-hydroxyacyl-CoA dehydrogenase (HADH, EC 1.1.1.35) and citrate synthase (EC 4.1.3.7) were measured spectrophotometrically at 37 °C in supernatants of muscle homogenates (Osumi & Hashimoto 1979, Srere 1969). Enzyme activities are expressed in μ mol/min per g wet weight of tissue.

Statistical analysis All numeric variables are expressed as means $\pm$ S.E.M. Group statistical comparisons were assessed by one-way ANOVA and individual comparisons by Fisher post hoc tests. Regression analyses were used to compare relationships. A *P* value of <0.05 was considered statistically significant.

Results

Protocol 1

Intramuscular triglyceride content in the soleus muscle of Sprague–Dawley rats (*n*=15) was negatively correlated with the M-value as an index of insulin sensitivity (*r*= –0.67, *P*<0.05; Fig. 1).

Protocol 2

Physical and biochemical findings (Table 1) There were no inter-group differences in body weight, pulse rate and fasting blood glucose. Systolic blood pressure was significantly higher in FFRs than in control rats. The average rate of glucose infusion during the last 35 min of the glucose clamp, as an index of insulin sensitivity (M-value), was significantly lower in FFRs than in control rats. Fenofibrate treatment for 2 weeks did not change blood pressure in FFRs but significantly improved the M-value in FFRs. A high fructose diet significantly increased serum insulin, triglyceride and free fatty acid levels. Fenofibrate treatment essentially reversed the serum insulin and lipids concentrations. In particular, the serum triglyceride was significantly lower in the FFR+FF group than in the FFR and control groups.

Muscle triglyceride content FFRs showed a significant increase in the triglyceride content of the soleus muscle

Figure 1 Negative correlation between intramuscular triglyceride level and the M-value in Sprague–Dawley rats. The M-value as an index of insulin sensitivity was estimated by the euglycemic hyperinsulinemic glucose clamp method. Intramuscular triglyceride level was measured in the soleus muscle.

compared with that in the control group (Fig. 2A). Both serum triglyceride and free fatty acid levels were significantly correlated with the triglyceride content in the soleus muscle (Fig. 3). The lipid content of the EDL muscle in FFRs maintained its tendency to be higher than that in the control group (5.8 ± 1.0 and $8.6 \pm 1.1 \mu\text{mol/g}$ wet weight of tissue for the control and FFR groups respectively, $P=0.06$; Fig. 2B). Fenofibrate treatment recovered the intramuscular triglyceride to the same level as that of the control group.

FABP in muscle tissue Cytosolic FABP content of the soleus muscle was significantly elevated in FFRs compared with that in the control rats (2.3 ± 0.1 and $3.1 \pm 0.2 \text{ mg/g}$ protein for the control and FFR groups respectively, $P<0.05$). Fenofibrate treatment further increased the FABP content of the soleus muscle ($5.3 \pm 0.5 \text{ mg/g}$

Figure 2 Intramuscular triglyceride levels in the soleus (A) and extensor digitorum longus (EDL) (B) muscles in the control group ($n=10$), fructose-fed rats (FFR) group ($n=10$), and FFR + fenofibrate (FF) group ($n=7$). The triglyceride content of the soleus muscle in FFRs was significantly higher than that in control rats, whereas that of the EDL tended to be higher in FFRs. FF treatment reduced the intramuscular triglyceride content to the control level. Values are means $\pm$ S.E.M. * $P<0.01$ vs control and FFR + FF.

protein, $P<0.01$ vs other groups; Fig. 4A). The FABP content of the soleus muscle was approximately 4-fold higher than that of the EDL muscle in the control group.

Table 1 Physical and biochemical characteristics of rats at 12 weeks of age. Values are means $\pm$ S.E.M.

	Control (<i>n</i> = 10)	FFR (<i>n</i> = 10)	FFR + FF (<i>n</i> = 7)
Variables			
Body weight (g)	384 $\pm$ 5	388 $\pm$ 4	388 $\pm$ 10
Systolic blood pressure (mmHg)	127 $\pm$ 2	137 $\pm$ 2*	136 $\pm$ 2*
Pulse rate (beats/min)	329 $\pm$ 7	329 $\pm$ 8	317 $\pm$ 6
M-value (mg/kg per min)	16.3 $\pm$ 0.8	12.4 $\pm$ 1.1*	15.9 $\pm$ 0.9 [§]
Fasting blood glucose (mmol/l)	4.7 $\pm$ 0.2	5.0 $\pm$ 0.2	5.1 $\pm$ 0.1
Fasting insulin (pmol/l)	128 $\pm$ 24	343 $\pm$ 31*	107 $\pm$ 23 [‡]
Triglyceride (mmol/l)	1.34 $\pm$ 0.11	2.55 $\pm$ 0.27*	0.68 $\pm$ 0.05 ^{†‡}
Free fatty acid (mmol/l)	1.23 $\pm$ 0.07	1.53 $\pm$ 0.06*	1.03 $\pm$ 0.07 [‡]

FFR, fructose-fed rat; FF, fenofibrate.
* $P<0.01$ vs control; [†] $P<0.05$ vs control; [‡] $P<0.01$ vs FFRs; [§] $P<0.05$ vs FFRs.

Figure 3 Positive correlation between triglyceride content of the soleus muscle and serum triglyceride (A) or free fatty acid (B) in both the control group ($n=10$) and FFR group ($n=10$).

No significant inter-group difference in the FABP content of the EDL muscle was observed (Fig. 4B). The expression of FABP mRNA analyzed by RT-PCR was similar to that of FABP protein in each muscle (Fig. 5).

Enzymatic activities (Table 2) The activities of the β -oxidation enzyme HADH in both muscles were comparable in the control and FFR groups. Fenofibrate increased HADH activity significantly in the soleus muscle (11.5 ± 0.5 and 16.6 ± 1.3 $\mu\text{mol}/\text{min}$ per g wet weight of tissue in the FFR and FFR+FF groups respectively, $P < 0.01$), but not in the EDL muscle (4.1 ± 0.4 and 5.4 ± 0.6 $\mu\text{mol}/\text{min}$ per g wet weight of tissue in the FFR and FFR+FF groups respectively, $P = 0.07$). The activity of the mitochondrial enzyme citrate synthase in muscles was not significantly different among the three groups. The soleus muscle exhibited greater HADH and citrate synthase activities than did the EDL.

Discussion

There are few reports on the effects of fibrates (PPAR α agonists) on insulin sensitivity. Some studies have

Figure 4 Muscle fatty acid-binding protein (FABP) levels in the soleus (A) and EDL (B) muscles in the control group ($n=10$), FFR group ($n=10$) and FFR+FF group ($n=7$). FABP was measured by ELISA. (A) Muscle FABP was elevated by high fructose diet and further induced by FF treatment in the soleus muscle. (B) No significant difference in muscle FABP was observed among the three groups in the EDL muscle. Values are means $\pm$ S.E.M. * $P < 0.01$ vs control; $^{\dagger}P < 0.01$ vs control and FFRs.

demonstrated that bezafibrate normalized impaired glucose tolerance in both human and rodent models of insulin resistance (Jones *et al.* 1990, Inoue *et al.* 1994, Matsui *et al.* 1997). However, bezafibrate activates PPAR α , PPAR γ and PPAR δ with almost comparable EC_{50} values (PPAR α : 50; PPAR γ : 60; PPAR δ : 20 μM in humans, PPAR α : 90; PPAR γ : 55; PPAR δ : 110 μM in rodents, for reference see Brown *et al.* (1999)). Therefore, it is impossible to conclude that PPAR α activation improves glucose metabolism, since PPAR γ agonists are known to be insulin sensitizers. In the present study, fenofibrate was used as a PPAR α -selective fibrate (PPAR α : 18; PPAR γ : 250; PPAR δ : >300 μM , for reference see Brown *et al.* (1999)). Although small-scale studies using human subjects have shown that fenofibrate increased insulin sensitivity as assessed by oral glucose tolerance tests and the serum insulin level (Yong *et al.* 1999, Idzior-Walus *et al.* 2000), the mechanisms remain unclarified. Recently, it has been reported that selective

Figure 5 Muscle FABP mRNA expression in the soleus (A) and EDL (B) muscles in the control group (n=8), FFR group (n=8) and FFR+FF group (n=6). FABP and β-actin (as an internal control) mRNA were analyzed by semi-quantitative RT-PCR. (A) Muscle FABP mRNA in the soleus muscle was significantly elevated in the FFR group compared with that in the control group and further induced in the FFR+FF group. (B) No significant difference in muscle FABP mRNA was observed among the three groups in the EDL muscle. Values are means ± S.E.M. *P<0.05 vs control; †P<0.01 vs control and FFRs.

PPARα agonists such as fenofibrate and Wy14,643 inhibited high fat diet-induced insulin resistance concomitantly with prevention of high fat diet-induced increase in body weight and adipose tissue mass in rodents (Guerre-Millo *et al.* 2000, Ye *et al.* 2001). It is unclear, however, whether the effect of PPARα agonists on insulin sensitiv-

ity is a direct action or a secondary action due to a decrease in body weight and adiposity. We therefore used FFRs as a non-obese insulin-resistant hypertension model (Hwang *et al.* 1987, Reaven *et al.* 1988) and correctly assessed insulin sensitivity by the euglycemic hyperinsulinemic glucose clamp method. The results showed that fenofibrate increased the M-value in FFRs, indicating an improvement in insulin sensitivity without influencing body weight as well as fat mass weight (data not shown).

Skeletal muscle plays important roles not only in insulin-mediated glucose metabolism, which accounts for more than 80% of whole body glucose disposal (DeFronzo *et al.* 1981), but also in the catabolism of fatty acids (Dagenais *et al.* 1976). Studies in rats and humans have shown that muscle triglyceride content is negatively related to insulin action (Storlien *et al.* 1991, Goodpaster *et al.* 1997, Pan *et al.* 1997, Kelley & Mandarino 2000). This finding was also confirmed in the present study (Fig. 1). A high fructose diet for 6 weeks increased muscle triglyceride content, which was correlated with serum lipids, and might cause insulin resistance together with previously reported mechanisms such as an alteration of muscle fiber composition (Higashiura *et al.* 1999) and increased tumor necrosis factor-α in skeletal muscle (Togashi *et al.* 2000). Fenofibrate treatment for 2 weeks might improve insulin sensitivity by lowering muscle triglyceride content in FFRs.

PPARα is highly expressed in the liver, and fibrates exert lipid-lowering activity via activation of PPARα, leading to altered expression of genes involved in lipid and lipoprotein metabolism in the liver (Staels *et al.* 1998). A possible mechanism of the improvement in insulin sensitivity by fenofibrate may be a decrease in influx of fatty acids into muscles via a decrease in circulating lipids. In addition, since Wy14,643, a PPARα agonist, increases total glucose incorporation into both glycogen and lipids in the liver (Ye *et al.* 2001), the liver may be another important site of glucose disposal induced by PPARα stimulation.

In general, the tissue FABP content is related to the rate of fatty acid uptake and/or utilization (Glatz *et al.* 1995). Furthermore, manipulations that will change the rate of tissue fatty acid metabolism appear to be associated with

Table 2 Activities of metabolic enzymes in skeletal muscles. Values are means ± S.E.M.

	HADH (μmol/min per g wet tissue)		Citrate synthase (μmol/min per g wet tissue)	
	Soleus	EDL	Soleus	EDL
Treatment				
Control (n=10)	11.8 ± 1.0	4.4 ± 0.5	23.3 ± 3.2	9.0 ± 1.8
FFR (n=10)	11.5 ± 0.5	4.1 ± 0.4	21.0 ± 3.0	8.2 ± 1.4
FFR+FF (n=7)	16.6 ± 1.3*	5.4 ± 0.6	35.0 ± 7.5	11.2 ± 2.5

HADH, 3-hydroxyacyl-CoA dehydrogenase.
*P<0.01 vs control and FFRs.

concomitant changes in FABP content. A high dietary fat content increased liver-type FABP in the liver and heart-type FABP in the heart (Glatz *et al.* 1995). It has also been reported that the content of heart-type FABP in the rat heart (Glatz *et al.* 1994) and red muscle (Carey *et al.* 1994) was increased in streptozotocin-induced diabetic rats known to increase fatty acid utilization. In the present study, a high fructose diet increased serum triglyceride and free fatty acid levels and also induced muscle FABP content in the soleus muscle. Elevated muscle FABP may facilitate the transport of fatty acids to mitochondria and the catabolism of fatty acids in skeletal muscle (Storch & Thumser 2000). It seems that excess influx of fatty acids into muscles causes accumulation of muscle lipid content because of insufficient elevation of muscle FABP.

PPAR α is highly expressed in tissues with elevated rates of fatty acid catabolism, where it regulates genes involved in fatty acid intake, activation into acyl-CoA esters, degradation via the mitochondrial and peroxisomal β -oxidation pathways and ketone body synthesis (Schoonjans *et al.* 1997). Fibrates induce liver-type FABP (Kaikaus *et al.* 1993) and β -oxidation enzyme HADH (Zhang *et al.* 1992) via PPAR α activation. PPAR α protein is also expressed in skeletal muscle as well as in the liver (Su *et al.* 1998). The promoter of heart-type FABP (equal to muscle FABP) has peroxisome proliferator responsive elements (Treuner *et al.* 1994) as well as that of liver-type FABP (Issemann *et al.* 1992) and HADH (Zhang *et al.* 1992). It has also been reported that heart-type FABP is expressed in skeletal muscle and induced by Wy14,643, a PPAR α activator, suggesting that the gene is regulated by PPAR α (Motojima 2000). We found fenofibrate induced red muscle FABP at the transcriptional level.

It is presumed that FABPs serve as intracellular buffers of fatty acids, protecting cells from deleterious effects of excess free fatty acids by sequestering them inside the ligand cavity (Bernlohr *et al.* 1997). This could include a more specific role in scavenging of excess intracellular lipid content. The present study demonstrated that fenofibrate increased FABP in oxidative red muscle, probably facilitating the transport of fatty acids to mitochondria, and stimulated β -oxidative degradation of fatty acids. Scavenging of intracellular fatty acids in muscle might lead to a decrease in intramuscular triglyceride and result in improved insulin sensitivity.

The effects of fenofibrate on FABP in the soleus (red) and EDL (white) muscles were different in the present study. Red skeletal muscle (containing mainly slow-twitch oxidative fibers) differs from white skeletal muscle (containing fast-twitch glycolytic fibers) in several ways, including capillary density, oxidative enzyme content and mitochondria density. These inherent characteristics of red muscle account for its overall higher respiratory capacity and ability to oxidize fatty acids. Previous assessment of muscle FABP expression suggests that the levels of both protein and mRNA correspond to the oxidative fiber-type

content in a given muscle (Peeters *et al.* 1991). It was also reported that red, but not white, muscle FABP was modulated in streptozotocin-induced diabetic rats known to increase fatty acid utilization (Carey *et al.* 1994). The modulation of muscle FABP may be predominant in red muscle compared with white muscle.

Previous reports showed that PPAR α agonists such as bezafibrate (Si *et al.* 1999, Jonkers *et al.* 2001) and fenofibrate (Yong *et al.* 1999, Idzior-Walus *et al.* 2000) reduced blood pressure. It is speculated that this blood pressure-lowering effect is due to an enhancement of insulin sensitivity or an improvement in endothelial function. In the present study, fenofibrate treatment for 2 weeks improved insulin sensitivity but did not influence blood pressure. This discrepancy is probably due to the treatment period, which was shorter in the present study than in the previous studies (6–24 weeks). It was also reported that TSJN, a Chinese medicine used to treat diabetes mellitus, attenuated blood pressure in FFRs treated for 4 weeks but not those treated for 2 weeks despite improvement in insulin sensitivity (Li *et al.* 2000). Taking this point into consideration, fenofibrate treatment for 2 weeks seems to be sufficient to ameliorate insulin resistance but not to reduce blood pressure.

In conclusion, the results from this study suggest that fenofibrate improves insulin sensitivity not only by lowering serum lipids and subsequent influx of fatty acids into muscles but also by reducing intramuscular lipid content via further induction of FABP and stimulation of β -oxidation in muscles.

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Received 25 February 2002

Accepted 8 April 2002