Original Article

Regulation of Skeletal Muscle Peroxisome Proliferator-Activated Receptor γ Expression by Exercise and Angiotensin-Converting Enzyme Inhibition in Fructose-Fed Hypertensive Rats

Takayuki KAWAMURA, Kazunori YOSHIDA**, Akira SUGAWA *, Makoto NAGASAKA, Nobuyoshi MORI, Kazuhisa TAKEUCHI *, and Masahiro KOHZUKI

The purpose of this study was to examine the effects of chronic exercise training and angiotensin-converting enzyme (ACE) inhibition on peroxisome proliferator-activated receptor γ (PPARγ) expression in fat and skeletal muscle in fructose-fed spontaneously hypertensive rats (SHR). SHR were fed a fructose-rich diet over 16 weeks of either exercise training (Ex group: 20 m/min, 0% grade, 60 min/day, 5 days/week), ACE inhibitor administration (TM group: temocapril, 10 mg/kg/day), or a combination of both treatments (TM + Ex group). The systolic blood pressure was reduced exclusively in the temocapril-treated groups. Serum leptin level was positively correlated with the ratio of epididymal fat weight to body weight (p < 0.001). Exercise training significantly upregulated the PPARγ expression in all tissues, which was attenuated by temocapril. PPARγ expression was significantly upregulated in skeletal muscles in the Ex group, and temocapril administration attenuated this effect in the Ex + TM group. The level of PPARγ protein was significantly higher in the extensor digitorum longus muscle than in the soleus muscle. Both TM and Ex prevented the fructose diet-induced transitions of fiber type. These data suggested that PPARγ expression is tissue-specific, and that alterations in PPARγ expression in the skeletal muscle induced by either or both treatments may have contributed to reducing the fat mass via the regulation of metabolic homeostasis. Changes in muscle morphology were independent of PPARγ expression, and the higher proportion of type I fiber might also explain some of the beneficial impact of exercise and ACE inhibition on energy metabolism.

*(Hypertens Res 2004; 27: 61–70)*

**Key Words:** peroxisome proliferator-activated receptor γ, exercise, epididymal fat, skeletal muscle

Introduction

Obesity, defined as a state of pathologically increased adipose mass, is a major public health problem and is associated with increased risk of developing diabetes, hypertension, and hyperlipidemia (1). It is now well recognized that the adipose tissue acts as an endocrine organ by releasing many cytokines and hormones that may play roles in linking obesity to these other complications (2).

One of the many adipocyte-derived products, peroxisome proliferator-activated receptor γ (PPARγ), is thought to be an important regulator of adipocyte differentiation, cardiovascular function, lipid metabolism, and insulin action through its coordinated effects on gene transcription (3). Curiously enough, PPARγ is also located within the nuclei of myocytes (4), and skeletal muscle PPARγ protein expression is associated with the expressions of genes involved in fatty from the Department of Internal Medicine and Rehabilitation Science, and "Department of Medicine, Division of Nephrology, Endocrinology, and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan, and "Furukawa City Hospital, Furukawa, Japan.

Address for Reprints: Masahiro Kohzuki, M.D., Ph.D., Department of Internal Medicine and Rehabilitation Science, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980–8574, Japan. E-mail: kohzuki@mail.cc.tohoku.ac.jp

Received July 3, 2003; Accepted in revised form September 18, 2003.
acid transportation and metabolism (5, 6). PPARγ exists as two major isoforms, γ1 and γ2. PPARγ1 seems to be the predominant isoform expressed in skeletal muscle, whereas both isoforms are present in fat tissue (7). PPARγ2 is identical to γ1 with the exception that it has an extra 30 N-terminal amino acids. Despite the structural difference, no clear functional differences between these two isoforms have been identified.

Endurance exercise training helps to reduce fat mass and cell size (8, 9) and elicits many skeletal muscle adaptations, including an increased capacity for oxidative metabolism of fatty acids and carbohydrates. These adaptations have been associated with the upregulation of genes involved in fatty acid uptake and oxidation (10). Many of the key genes for fatty acid uptake and oxidation are under the regulation of PPARγ. Recently, it has been shown that PPARγ coactivator-1 (PGC-1) is highly expressed in type I muscle fiber, which features high mitochondrial content and oxidative capacity, and is likely to be a physiological regulator for fiber type specification (11).

Angiotensin converting enzyme (ACE) inhibitors not only decrease high blood pressure, but also improve insulin-stimulated glucose uptake (12, 13) and are associated with molecular and morphological changes in skeletal muscle (14–17). The combination of ACE inhibition and exercise training has been shown to have additive effects on improvement of energy metabolism in insulin-resistant rats (14). We previously reported that the combination of exercise training and ACE inhibition caused a significant increase of tumor necrosis factor-alpha (TNF-α) protein content in epididymal fat (EPI) (18).

Therefore, it was hypothesized that 1) the exercise training would increase the amount of PPARγ protein and lead to a high percentage of type I fiber in skeletal muscle; 2) in adipose tissue, exercise training would downregulate PPARγ expression, and thereby prevent the accumulation of fat storage; and 3) ACE inhibitor administration would enhance these exercise-mediated changes. To test these hypotheses, we investigated the effect of exercise training and ACE inhibitor treatment on PPARγ expressions in skeletal muscles and epididymal fat tissues in spontaneously-hypertensive rats (SHR) fed a high fructose diet to induce insulin resistance (15, 19).

Methods

Experimental Animals

Fifty-two 8-week-old male SHR (Charles River Japan, Atsugi, Japan) were randomly assigned to a control (C, n = 10), fructose-control (F, n = 11), temocapril-treated (TM, n = 11), exercise-trained (Ex, n = 10), or combined temocapril-treated and exercise-trained (TM + Ex, n = 10) group. Rats were maintained in a humidity- and temperature-controlled room with a 12-h light-dark cycle (7:00 AM on and 7:00 PM off) throughout the study. All procedures conformed to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Tohoku University School of Medicine Animal Use and Care Committee. The rats were fed either a starch- or fructose-based chow according to methods described previously (18). Animals in the temocapril-treated groups were administered an ACE inhibitor, temocapril (Sankyo Co., Tokyo, Japan), at a dose of 10 mg/kg/day orally in 0.5% carboxymethylcellulose (Wako Pure Chemical Industries, Ltd., Osaka, Japan) between 9:00 AM and 10:00 AM for 16 weeks. This dose (10 mg/kg/day) was shown to be more effective at reducing blood pressure in SHR than a lower dose (3 mg/kg/day) (20). Animals in the exercise-trained groups were run five days a week at 20 m/min for 60 min on a rodent treadmill for 16 weeks. This speed (20 m/min) corresponds to 70% \( \text{Vo}_{2 \text{max}} \) in SHR (21). The systolic blood pressure (SBP) was measured by the indirect tail-cuff method as described previously (22). After 5 h of fasting and 24 h without exercise and/or drug administration, rats were decapitated and blood was collected for biochemical analysis. The heart, soleus muscle (SOL), extensor digitorum longus muscle (EDL), and EPI were dissected and weighed. Tissues were snap frozen on dry ice and stored at -80°C until further analysis.

Blood Analysis

Serum triglyceride (TG) levels were measured by a standard technique using a SYNCHRON Clinical System CX7 autoanalyzer (Beckman Coulter Inc., Fullerton, USA). Plasma free fatty acid (FFA) concentrations were measured by an enzyme colorimetric method (NEFA; Boehringer Mannheim, Indianapolis, USA). Serum leptin levels were determined by radioimmunoassay using commercial kits (Linco, St. Louis, USA).

Western Blot Analysis for PPARγ

Protein extracts of skeletal muscles and fat tissues were homogenized in an ice-cold extract buffer (pH 7.5) containing 20 mmol/l Tris-HCl, 1 mmol/l EDTA, 0.1 mmol/l phenylmethylene-sulfonly fluoride, and protease inhibitor cocktail (Sigma Chemical Co., St. Louis, USA), and centrifuged (10,000 rpm) at 4°C for 30 min. The supernatant was collected and transferred to a new tube. The protein concentration was determined by the Bradford method (23) with a Coomassie® Plus protein assay reagent (Pierce Chemical Co., Rockford, USA). Proteins extracted from tissues were mixed with 2 – Laemmli buffer (62.5 mmol/l Tris, pH 6.8, 2% SDS, 5% 2-mercaptoethanol, 25% glycerol, 0.01% Bromophenol Blue), boiled for 5 min, subjected to 10% SDS-polyacrylamide gel electrophoresis, and transferred onto a polyvinylidene difluoride (PVDF) membrane (Immun-Blot® PVDF membrane; Bio-Rad Laboratories Inc., Hercules, USA) in a semi-dry apparatus Trans-Blot® SD cell; Bio-Rad
Table 1. SBP and BW in Rats before and after the 16-Week Intervention Period

<table>
<thead>
<tr>
<th>Group</th>
<th>SBP (mmHg)</th>
<th>BW (g)</th>
<th>ΔBW gain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
</tr>
<tr>
<td>C</td>
<td>178 ± 13</td>
<td>222 ± 3</td>
<td>192 ± 2</td>
</tr>
<tr>
<td>F</td>
<td>179 ± 1</td>
<td>239 ± 4</td>
<td>191 ± 2</td>
</tr>
<tr>
<td>TM</td>
<td>175 ± 2</td>
<td>152 ± 4*</td>
<td>189 ± 1</td>
</tr>
<tr>
<td>Ex</td>
<td>175 ± 2</td>
<td>236 ± 7**</td>
<td>192 ± 3</td>
</tr>
<tr>
<td>TM+Ex</td>
<td>174 ± 1</td>
<td>146 ± 4***</td>
<td>189 ± 2</td>
</tr>
</tbody>
</table>

Values are the means ± SEM for 10–11 rats/group. Groups are control (C); fructose-sedentary (F); temocapril-treated (TM); exercise-trained (Ex); and combined temocapril-treated and exercise-trained (TM + Ex). SBP, systolic blood pressure; BW, body weight. *p < 0.05 vs. TM; **p < 0.01 vs. F, TM; Ex, respectively.

Table 2. Heart and Epididymal Fat Wet Weights in Rats after the 16-Week Intervention Period

<table>
<thead>
<tr>
<th>Group</th>
<th>Heart Wet Wt (g)</th>
<th>Heart Wt/BW (mg/g)</th>
<th>EPI Wet Wt (g)</th>
<th>EPI Wt/BW (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.49 ± 0.03</td>
<td>3.96 ± 0.05</td>
<td>5.26 ± 0.19*</td>
<td>13.73 ± 0.60</td>
</tr>
<tr>
<td>F</td>
<td>1.38 ± 0.01</td>
<td>3.90 ± 0.05</td>
<td>4.41 ± 0.15</td>
<td>12.43 ± 0.42</td>
</tr>
<tr>
<td>TM</td>
<td>1.07 ± 0.02**</td>
<td>3.33 ± 0.07**</td>
<td>3.70 ± 0.10*</td>
<td>11.49 ± 0.31</td>
</tr>
<tr>
<td>Ex</td>
<td>1.47 ± 0.04###</td>
<td>4.14 ± 0.06###</td>
<td>3.21 ± 0.20**</td>
<td>8.99 ± 0.44###</td>
</tr>
<tr>
<td>TM + Ex</td>
<td>1.19 ± 0.03**###</td>
<td>3.31 ± 0.08###</td>
<td>2.92 ± 0.12###</td>
<td>8.15 ± 0.35###</td>
</tr>
</tbody>
</table>

Values are the means ± SEM for 10–11 rats/group. Groups are control (C); fructose-sedentary (F); temocapril-treated (TM); exercise-trained (Ex); and combined temocapril-treated and exercise-trained (TM + Ex). EPI, epididymal fat; Wt, weight; BW, body weight. *p < 0.05 vs. TM; **p < 0.01 vs. F, TM; Ex, respectively; ***p < 0.001 vs. F, TM, Ex, respectively.

Histological Analysis

Serial transverse cross sections (10 μm thick) near the midbelly portion of the soleus muscle were cut in a microtome cryostat at -24°C, mounted on slide glasses, and air dried. The muscle fiber type was determined by a myofibrillar adenosine triphosphatase (mATPase) staining method (24) with modifications. The proportions of each fiber type were calculated based on the method described by Takada et al. (25). Images were captured by an optical microscope (BX 51; Olympus Optical Co., Ltd., Tokyo, Japan) connected to a CCD video camera (CS 900; Olympus Optical Co., Ltd.) under the same microscope objective (10 ×).

Statistical Analysis

All data were presented as the mean ± SEM. Significant differences between groups were assessed by a factorial analysis of variance (ANOVA). Tukey’s post hoc tests were used to analyze differences among means when significant differences were found. Regression analyses were used to correlate variables of interest. Values of p < 0.05 were considered to indicate statistical significance.

Results

Body Measurements

The results of the SBP and body and organ-weight measurements are shown in Tables 1 and 2. After 16 weeks of treatment, the body weights in both of the groups that received exercise training (Ex and TM + Ex) were significantly higher than those of the TM group. The groups administered TM showed a lower heart mass than the non-TM groups, both in absolute and relative terms. In the TM + Ex group, temocapril treatment significantly prevented the heart weight increase associated with exercise training. Exercise training significantly lowered the absolute and relative EPI weight compared with the other groups. The EPI weight was also lower in the TM than in the F group, but the relative weights were not significantly different between TM and F. No sig-
Table 3. Blood Measurements in Rats after the 16-Week Intervention Period

<table>
<thead>
<tr>
<th>Group</th>
<th>FFA (mmol/l)</th>
<th>Leptin (ng/ml)</th>
<th>TG (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.38 ± 0.08</td>
<td>3.25 ± 0.37</td>
<td>0.47 ± 0.04</td>
</tr>
<tr>
<td>F</td>
<td>1.52 ± 0.07</td>
<td>2.38 ± 0.25</td>
<td>0.60 ± 0.10</td>
</tr>
<tr>
<td>TM</td>
<td>1.63 ± 0.10</td>
<td>1.90 ± 0.18</td>
<td>0.61 ± 0.08</td>
</tr>
<tr>
<td>Ex</td>
<td>1.33 ± 0.09</td>
<td>1.35 ± 0.14*</td>
<td>0.61 ± 0.09</td>
</tr>
<tr>
<td>TM + Ex</td>
<td>1.30 ± 0.04*</td>
<td>1.07 ± 0.12**</td>
<td>0.37 ± 0.03</td>
</tr>
</tbody>
</table>

Values are the means ± SEM for 10–11 rats/group. Groups are control (C); fructose-sedentary (F); temocapril-treated (TM); exercise-trained (Ex); and combined temocapril-treated and exercise-trained (TM + Ex). FFA, free fatty acids; TG, triglycerides. * p < 0.05 vs. TM; ** p < 0.01 vs. F, TM, respectively.

Fig. 1. Detection of PPARγ1 (bottom band) and γ2 (upper band) protein in skeletal muscles and fat tissue. Fifty micrograms of total protein from epididymal fat (EPI) or the extensor dixitorum longus muscle (EDL) were subjected to Western blot analysis. Membranes were incubated with a poly-monoconal PPARγ antibody (at a dilution of 1:200 for EPI; 1:500 for EDL). The control sample was provided by Biotechnology Inc. (Santa Cruz).

Significant differences in SOL or EDL wet weight were seen among groups (data not shown). Temocapril administration alone—but not exercise training alone—significantly reduced SBP.

Blood Analysis

The results of the blood analysis are shown in Table 3. The plasma FFA concentration tended to be low in the exercise-training groups, with a significant difference between TM and TM + Ex (p < 0.05). The serum leptin concentration was significantly lower in the exercise-trained groups than in the F group, but the serum leptin levels of TM were not significantly different between the F group and the exercise-trained groups. When the data for all subgroups were combined, there was a significant correlation between the serum leptin level and the EPI weight per body weight (r = 0.787, p < 0.001).

PPARγ Protein

The effect of the 16-week exercise training or temocapril administration on the expression of PPARγ protein was examined by immunoblotting. The approximate molecular weights of the two isoforms (γ1: 52 kDa; γ2: 55 kDa) were comparable with those of previous studies (26). Due to the lower detection of PPARγ from EPI samples, the primary antibody was applied at a concentration five times higher (1:200) than that for skeletal muscles (1:1,000). It was found that PPARγ1 and γ2 were present in EPI tissue, whereas only γ1 was detected in skeletal muscle tissues (Fig. 1). To minimize the blot-to-blot variation, the PPARγ content was expressed relative to the control SHR (normal diet) sample (arbitrarily set at 100%) run on each gel. Representative Western blots are shown in Fig. 2A. There were no significant differences among groups in PPARγ expression in EPI (Fig. 2B). The Ex group showed the highest concentration of PPARγ protein in the SOL muscle (p < 0.01 compared to the other groups), whereas the TM had the lowest of all. In fact, the Ex group had the highest average PPARγ expression in every type of tissue, and these upregulations were all attenuated by the addition of exercise-training (TM + Ex group). To compare the PPARγ expression between the SOL and EDL muscles, which have been well described in terms of their distinctive muscle fiber types and oxidative capacity, arbitrary units of PPARγ were expressed per μg of protein (Fig. 3). Interestingly, the PPARγ protein expression was lower in the SOL than in the EDL in every group (p < 0.001).

Characteristics of Muscle Fibers

Figure 4 shows the distribution of the soleus muscle fiber types for each group. The composite ratio of type I fibers of the soleus muscle was significantly decreased in the F group (76.7% of total fibers), while both the Ex and TM groups maintained the fraction of type I fiber up to the ratio seen in the control group (Fig. 5). There was no further increase in the type I fiber ratio in the TM + Ex group.

Discussion

The SHR has been proposed as a model of the insulin resistance syndromes on account of its insulin resistance, hypertriglyceridemia, abdominal obesity and hypertension (27, 28). SHR have been used as a model of insulin resistance syndromes because they shown insulin resistance, hypertriglyceridemia, abdominal obesity, and hypertension (27, 28). Although fructose feeding has been shown to aggravate these conditions (17, 19, 29), little is known about the underlying mechanisms. Exercise and ACE inhibitors are fre-
subsequently prescribed for the control of hypertension and its complications. Given the critical role of obesity in the development of metabolic disturbances, adipocyte-derived products such as leptin, TNF-α, and PPARγ have been proposed to play key roles in the regulation of peripheral fuel storage, mobilization, and energy homeostasis (2). We and others have demonstrated that either exercise, ACE inhibition, or a combination of both interventions can change the expression levels of leptin and TNF-α (9, 17, 18, 30). The present study, therefore, was focused on whether and how tissue levels of another key factor, PPARγ, could be influenced by exercise, ACE inhibitor administration, or both.

The results from this study demonstrated for the first time that chronic exercise training increased PPARγ protein in the skeletal muscles, but not in adipose tissue. The increase in PPARγ content may contribute to the enhanced expression of genes involved in the transport and oxidation of fatty acids in the skeletal muscles. There have been only two studies that examined PPARγ expression in rat or human skeletal muscle after an endurance exercise program (10, 31). In rats, 8 weeks of exercise training did not influence the expression of PPARγ mRNA in either the SOL or EDL (31). In humans, changes in PPARγ mRNA in the vastus lateralis muscle after a single bout of exercise or after 9 days of exercise training for 60 min per session had no influence on the gene (10). In humans, there was no difference in PPARγ mRNA expression in the vastus lateralis muscle between a group performing a single bout of exercise and a group performing 9 days of exercise training for 60 min per session (10). In vivo studies on lipid metabolism (32) and on glucose mRNA and protein (33) have suggested that changes at the mRNA level do not always parallel the content of protein in the same samples. Therefore, measurement of PPARγ at the protein level, rather than at the mRNA level, may provide more precise information regarding the physical adaptations to long-term interventions. The results of several previous investigations support the present observation that chronic exercise training was able to increase the content of PPARγ protein in tissues. For example, the gene expression of PGC-1, a transcriptional co-activator of PPARγ, was shown to be upregulated by prolonged low-intensity swimming (34).
so, skeletal muscle PPARα protein expression and fatty acid oxidative enzymes were doubled after completion of a 12-week exercise program (35). Therefore, we speculate that post-transcriptional regulation is important in the regulation of the PPARγ protein abundance. A question might be raised as to whether PPARγ leads to accumulation of muscle lipids, which reduce muscle oxidative capacity in human obesity and type 2 diabetes (36). Unfortunately, neither muscle lipid content nor oxidative capacity in skeletal muscle was measured in this study, but the high PPARγ protein expression in exercise-trained SHR, which have an elevated fat oxidative capacity (37), would be able to mediate the potential negative influence of muscle lipid on insulin-stimulated glucose metabolism (38).

The circulating leptin level was positively correlated with EPI weight irrespective of the PPARγ protein levels in any of the tissues examined. Leptin can directly stimulate the fatty acids oxidation and glucose uptake in non-adipose tissues (39), and intracerebroventricular injection of leptin upregulates PPARγ expression (40), while PPARγ activation downregulates leptin expression in adipose tissue (41). We speculate that the physiologic level of circulating leptin is proportional to the adipose tissue mass, and the effects of leptin on fatty acids metabolism in skeletal muscle are mediated by other molecules, such as 5'AMP activated protein kinase (42).

Fig. 4. Representative cross-sections of soleus muscle from control (C), fructose-sedentary (F), temocapril-treated (TM), exercise-trained (Ex), and combined TM and Ex (TM + Ex) rats with serial sections stained for fiber type determination. Sections from the same muscle were assayed for myosin adenosine triphosphatase activity after different preincubation pH treatments. The type I fiber stained dark after acid treatment (pH 4.50–4.55) but stained light after the alkaline treatment (pH 10.9). Conversely, the type IIa fiber stained light after the acid treatment but dark after alkaline treatment. The type IIb fiber stained intermediate after the acid treatment, and dark after the alkaline treatment. Scale bars: 100 µm.

![Muscle fiber types](image)

Fig. 5. The ratio of the muscle fiber composition (Type I, IIa, and IIb) of the soleus muscle for 8–10 rats/group. Each bar corresponds to either control (C, checkered bar), fructose-sedentary (F, solid black bar), temocapril-treated (TM, diagonally striped bar), exercise-trained (Ex, open bar), and combined temocapril-treated and exercise-trained (TM + Ex, horizontally striped bar). *** p < 0.001 vs. other groups.

Given that ACE inhibition and exercise training improve the state of insulin resistance through various mechanisms, we sought to clarify whether the effect of these treatments is associated with upregulation of PPARγ in tissues from fructose-fed SHR. However, when the hypothesis was tested, we found the opposite—that is, the trend toward increasing PPARγ protein content in tissues by exercise training was abolished by TM, especially in the SOL in this study (Fig. 2). This phenomenon could be explained by the possibility that temocapril might ameliorate glucose utilization by activating other pathways, not by upregulating PPARγ expression in skeletal muscle or adipose tissue. That is, ACE inhibition itself has been shown to improve insulin resistance through several pathways, such as an increase in peripheral blood flow, or the maintenance of the ratio of type I fiber types in the SOL of fructose-fed SD rats (15) or of SHR (the present experiment). ACE inhibition has also been associated with an increase in glucose transporter (GLUT)-4 and an increase in the amount of insulin substrate receptor-1 protein in the skeletal muscle of SHR (17). Another possible explanation is that the temocapril-induced reduction in PPARγ expression may be related to the preservation of bradykinin and/or the elevation of the serum adiponectin level, both of which improve insulin sensitivity (43, 44). It is unknown whether PPARγ can interact with the tissue renin-angiotensin system in organs other than those of the cardiovascular system.
system. In fact, the cardiovascular-protective effects by PPARγ activation and ACE inhibition seem to have several similarities. PPARγ is expressed in the cardiovascular system and its activation can block angiotensin II (AII) actions and lower blood pressure (45). Further studies are required to elucidate the role of PPARγ in the renin-angiotensin system in skeletal muscle and adipose tissue.

As shown in Fig. 3, the PPARγ1 content was greater in SOL than in EDL in all groups. There are several lines of evidence suggesting a possible connection between the muscle fiber type and and PPARγ1 expression. In a study on PPARγ expression in various muscle tissues (31), PPARγ mRNA was detected exclusively in diaphragm muscle. PGC-1 is up-regulated by exercise (34) and can promote the fiber type conversion, preferentially to type I fiber type (11). In the present experiment, however, the subsequent fiber type determination in SOL failed to demonstrate a relationship between the amount of PPARγ protein and the type I muscle fiber ratio in SOL. We speculate that the expression of PGC-1 is independent of PPARγ expression.

Unlike the skeletal muscles, which showed differential PPARγ expression among groups, the adipose tissues showed no intragroup differences in PPARγ expression. Since PPARγ is required for adipocyte formation (3), a decline in the intrinsic preadipocyte replication potential as well as by an impaired capacity of preadipocytes to differentiate with aging (46) might have blunted the regulation of PPARγ in EPI by the end of the experiment. Animal studies have also revealed that the preadipocyte differentiation program is impaired with aging, as mRNA levels of β-actin and -tubulin (early differentiation markers), lipoprotein lipase (midway marker), and glycerol-3-phosphate dehydrogenase (late marker) were decreased in preadipocytes cultured from rats of various age (47). Other groups have also reported that PPARγ expression in adipose tissue is downregulated by aging (40, 48). It is thus possible that the total reduction of adipose PPARγ activity masked or became refractory to any interventions in the present study.

Our present findings confirm the results of previous investigations which showed that the proportion of type I fiber in SOL can be altered by either diet, ACE inhibition, or endurance exercise (14, 49). Human and rodent skeletal muscles largely consist of slow twitch oxidative fiber (type I) and fast twitch non-oxidative fiber (type II). Type I fibers are more sensitive and more insulin responsive than fast-twitch fibers (50). A reduction in the fraction of type I fibers, combined with a reduction in GLUT-4 expression in type I fibers, may contribute to skeletal muscle insulin resistance (51), and thus the maintenance of the type I fiber ratio in the skeletal muscle by the two treatments may prevent the development of glucose intolerance.

One might point out that treatment with ACE inhibitors has been shown to slightly elevate the circulating FFA level. Although we did not measure the mechanics of fatty acid circulation in the present study, the circulating level of plasma FFA depends on the lipolysis of circulating lipoproteins, the rate of FFA release from adipose tissue, and the rate at which these fatty acids are taken up and reesterified by tissues (52). According to the fatty acid stealing hypothesis (53), PPARγ may act to promote the movement of FFA into skeletal muscle and other tissues having a high level of PPARγ (54). Other authors have reported that all significantly increased triglyceride content and the activity of lipogenic enzymes in 3T3-L1 adipocytes (55), and in contrast, that rats treated with an AII antagonist exhibited a decrease in adipocyte size (56). Taking these results together, we hypothesize that a slight elevation of blood FFA level might result from the reduction of FFA flux into the adipocytes and skeletal muscles due to lower PPARγ expression; however, this remains to be clarified.

Despite the metabolic and morphologic changes observed in this study, blood pressure was not reduced at the end of the 16-week period of exercise training. Although moderate-intensity exercise has been used to lower blood pressure in hypertensive animals and humans, such a training-induced reduction in blood pressure is dependent on the intensity of exercise training (21, 57). For example, in SHR, low-intensity exercise training (16.6 to 18.3 m/min) for 13 weeks was reported to reduce SBP in SHR (21), while 8 weeks of high-intensity (26.2 m/min) treadmill running increased SBP by an average of 33.9 mmHg (58). These variable effects of exercise training on blood pressure suggest that the present 16-week protocol of training at 20 m/min may have been too intense to induce a significant reduction of SBP in fructose-fed SHR. Moreover, several mechanisms have been proposed to explain fructose-induced hypertension in male rats, including sympathetic nervous system activation, defects in endothelial function, and increased production and/or activity of endothelin-1 or thromboxane A2 (59). Therefore, 16 weeks of moderate-intensity treadmill running might not have been sufficient to counteract the fructose-induced changes and thereby lower blood pressure in fructose-fed SHR.

Although we did not assess glucose utilization in the peripheral tissue, both studies using animal models (14–16) and clinical investigations (12, 13) have demonstrated that ACE inhibitors can ameliorate peripheral insulin resistance. Both skeletal muscle PPARγ1 expression (60) and the composite ratio of type I muscle fiber ratio (61) have been positively correlated with glucose disposal rate, suggesting that exercise- and ACE inhibition-mediated changes in skeletal muscle PPARγ1 protein and an increase in the ratio of type I muscle fiber may account, in part, for the favorable change in the peripheral glucose uptake. In this context, we should note that these results in fructose-fed SHR do not necessarily abrogate the interactive effects of exercise training and ACE inhibition in other insulin-resistant or hypertensive rat strains or humans.

In summary, we found that 16 weeks of endurance exercise training in fructose-fed SHR reduced the EPI weights in accordance with lowered serum leptin concentrations. Ele-
vated PPARγ protein expression was seen in the rats undergoing exercise training alone. On the other hand, in rats receiving both exercise training and temocapril, temocapril downregulated the exercise-induced increase in PPARγ expression in the soleus muscle. Collectively, these results indicate that PPARγ protein expression varies with the type of tissue, and is also affected by diet, endurance training, and ACE inhibition in a tissue-specific manner. Preferential changes in muscle morphology, which are not related to the amount of PPARγ protein, might also explain some of the beneficial impact of exercise and ACE inhibition on energy metabolism.

References

27. Rao RH: Insulin resistance in spontaneously hypertensive rats: difference in interpretation based on insulin infusion rate or on plasma insulin in glucose clamp studies. Diabetes


