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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1029 – 1036
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Long-term exercise increases the DNA binding activity of peroxisome proliferator–activated receptor γ in rat adipose tissue

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Received 25 September 2006; accepted 6 March 2007

Abstract

The aim of the present study was to examine the effect of 8 weeks of voluntary wheel running on the gene expression, at the protein level, of 2 enzymes involved in lipogenesis (fatty acid synthase [FAS] and diacylglycerol acyl transferase 1), 2 proteins involved in lipolysis (hormone-sensitive lipase [HSL] and perilipin), and 3 transcription factors mediating the induction of genes involved in lipid metabolism (the α, γ, and δ members of the peroxisome proliferator–activated receptor, or PPAR, family) in rat liver, gastrocnemius muscle, epididymal fat, and subcutaneous fat. Proteins were measured through Western blot analysis in the tissues of 11 trained and 14 untrained rats. The trained rats had lower FAS in the liver; higher FAS, HSL, and perilipin in epididymal fat; and higher HSL in subcutaneous fat. In addition, the trained rats had higher total protein concentrations in both fat depots. No significant differences in the liver, muscle, or adipose tissue PPAR contents were found between groups. However, the DNA binding activity of PPARγ, measured through an enzyme immunoassay-based method, was higher in both fat depots of the trained rats. Our findings suggest that long-term wheel running had significant effects on the concentrations of proteins playing key roles in lipogenesis and lipolysis in rat liver and adipose tissue. These effects may be due to PPAR activation rather than induction, rendering the transcriptional regulation of target genes more economical and flexible. The activation of PPARγ with exercise may mediate its beneficial effect on insulin sensitivity.
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1. Introduction

Excess energy and fat intake, combined with low levels of physical activity in modern societies, have led to an increased prevalence of the metabolic syndrome, characterized by obesity, type 2 diabetes mellitus, hypertension, cardiovascular disease, and dyslipidemia. Part of the connection among these conditions has been attributed to a cross talk between the adipose tissue and organs or tissues such as the liver and skeletal muscle [1-3].

Exercise is a powerful modifier of the manifestations of the metabolic syndrome in the direction of health enhancement [4]. This is achieved especially through alterations in metabolism caused by modifications in the activity and/or quantity of specific proteins. However, the mechanisms by which exercise alters lipid metabolism are unclear, as data regarding the effect of exercise training on key proteins involved in lipogenesis and lipolysis are limited and controversial.

Key proteins of lipogenesis are fatty acid synthase (FAS), which catalyzes the synthesis of fatty acids, and diacylglycerol acyl transferase 1 (DGAT1), which catalyzes the final step in triacylglycerol synthesis. Conversely, lipolysis depends on hormone-sensitive lipase (HSL), which catalyzes the committed step in triacylglycerol degradation, and perilipin, which controls access to the lipid droplets in the adipocytes. There is rapidly accumulating knowledge on the roles of the peroxisome proliferator–activated receptors (PPARs), a family of transcription factors having fatty acids and lipid-derived compounds as natural ligands [5] and inducing proteins that play crucial roles in lipid metabolism. In particular, PPARα induces enzymes involved in fatty acid oxidation [5], whereas PPARγ plays important roles in

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0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.03.011

adipogenesis and insulin sensitivity [6]. Overexpression of PPARδ increases lipid catabolism by up-regulating genes involved in this process or by inducing a pattern of muscle remodeling (increase in oxidative fibers) that is reminiscent of that promoted by endurance training [7,8].

The few results on the effect of exercise training on the FAS content of liver are controversial, with one study showing no difference between trained and untrained rats [9], one showing decreased FAS in trained rats fed a high-starch diet but no difference in trained rats fed a high-fructose diet [10], and one showing decreased FAS in lean trained rats but no difference in obese trained rats [11]. Askew et al [12] found decreased FAS activity in rat liver after exercise training and no difference in the adipose tissue. On the contrary, Faulconnier et al [13] found decreased activity of the enzyme in rat adipose tissue and no difference in the liver with exercise training.

We found only 3 studies on the effect of long-term exercise on HSL in adipose tissue and 1 in muscle. Nomura et al [14] found increased HSL in rat adipocytes after exercise training, and Enevoldsen et al [15] found higher HSL in retroperitoneal but no difference in mesenteric fat of trained rats. In contrast, De Glisezinski et al [16] found decreased HSL with training in obese humans. Concerning muscle, Enevoldsen et al [15] found no difference in muscle HSL between trained and sedentary rats.

Data on the effect of exercise training on PPARs are contained in 6 studies. These have reported increases in the PPARα [1,17], PPARγ [18], and PPARδ contents [7,19] of skeletal muscle; increase in the PPARα content and DNA binding activity of PPARα in the heart [20]; and no difference in PPARγ of adipose tissue [18].

There seem to be no data on the effect of long-term exercise on the DGAT1 contents of the liver, skeletal muscle, and adipose tissue, as well as on the perilipin content of adipose tissue.

We have recently examined the effect of exercise training on the fatty acid composition of phospholipids and triacylglycerols in rat liver, skeletal muscle, and adipose tissue [21]. Along with several differences in the fatty acid profile of these tissues, we found lower triacylglycerol concentrations in skeletal muscle and subcutaneous fat of trained compared with untrained animals, as well as higher activities of 2 key enzymes of fatty acid oxidation, namely, carnitine palmitoyltransferase and 3-hydroxyacyl coenzyme A dehydrogenase in muscle. These findings, along with the limited and controversial data on the aforementioned protagonists of lipolysis and lipogenesis, prompted us to further explore the effect of exercise training on lipid metabolism in the tissues of the same animals.

Thus, the original aim of the present study was to investigate the effects of 8 weeks of voluntary wheel running, a stress-free exercise model, on the concentrations of PPARα, PPARγ, PPARδ, FAS, DGAT1, HSL, and perilipin in rat liver, gastrocnemius (a muscle involved in the particular exercise), and adipose tissue at 2 different sites (epididymal and subcutaneous fat). An additional aim, which emerged in the course of the study to explore the mechanism behind the observed effects, was to examine the influence of exercise on the DNA binding activity of PPARγ.

2. Materials and methods

2.1. Animals

Thirty-five male Wistar rats were purchased at the age of 7 weeks from Charles River Laboratories (Sulzfeld, Germany) and were housed under controlled environmental conditions (21°C, 12:12-h light-dark cycle starting at 6:00 AM). The rats had free access to water and standard rodent chow (R/M-H V1535, Ssniff, Soest, Germany), the energy of which was derived by 58% from carbohydrate, 9% from fat, and 33% from protein. The animals were maintained according to the European Union guidelines for the care and use of laboratory animals, as well as the Public Health Service Policy on Humane Care and Use of Laboratory Animals. The study design was approved by the institutional ethics committee.

2.2. Training

The animals were divided randomly into an exercise group (n = 20) and a control group (n = 15). The members of the exercise group were housed individually in cages equipped with wheels, in which they exercised ad libitum for 8 weeks, whereas the members of the control group were housed individually in plain cages. The running activity of the exercise group was recorded continuously through the DasyLab 5.0 data collection system from Datalog (Mönchengladbach, Germany). Upon completion of the training period, the animals that had run at least 2 km/d (n = 11) were selected as the trained group.

2.3. Tissues

The 11 trained animals and the 14 untrained animals (1 died during the experimental period) were decapitated at approximately the same time of day (2:00-4:00 PM) in a counterbalanced order. Wheels and food had been removed from the cages 12 and 6 hours earlier, respectively, to minimize the influence of the last exercise bout and the last feeding on the biochemical parameters of interest. The liver, gastrocnemius medialis muscle of the right hind limb, epididymal fat, and subcutaneous fat from the buttock area were then removed as quickly as possible. The muscle was ridden of visible fat, nerves, and fasciae, and all tissues were immediately immersed in liquid nitrogen. Blood was collected promptly and left to clot at room temperature. Upon clotting, the blood was centrifuged at 1500*g* for 10 minutes. Serum was separated and stored at −80°C. Tissues were pulverized with mortar and pestle in liquid nitrogen and were also stored at −80°C until analysis.

2.4. Western blot analysis

The 7 proteins of interest were measured by protein immunoblot (Western blot) analysis in as many of the 4 tissues harvested as these proteins are known to be expressed. Thus, we measured PPARα, FAS, and DGAT1 in the liver; PPARα, PPARγ, PPARδ, FAS, DGAT1, and HSL in muscle; as well as PPARγ, PPARδ, FAS, DGAT1, HSL, and perilipin in the 2 fat depots. In addition, we measured perilipin in muscle to estimate the presence of adipocytes in the tissue.

Thirty milligrams of tissue were homogenized manually on ice with a glass homogenizer (Kontes, Vineland, NJ) in 19 volumes (in the case of liver and muscle) or 9 volumes (in the case of the fat depots) of 50 mmol/L potassium phosphate buffer (pH 7.4) containing 1 mmol/L EDTA, 1% Triton X-100, 1 mmol/L 2-mercaptoethanol, 10 μg/mL antipain, 20 μg/mL leupeptin, and 1 μg/mL pepstatin (all from Sigma, St Louis, MO). The homogenates were centrifuged at 1500*g* for 15 minutes at 4°C, and total protein concentration was determined in the supernatant by the use of a Bradford reagent from Sigma, with bovine serum albumin as standard.

An aliquot of tissue extract containing 30 μg of protein was mixed with an equal volume of Laemmli buffer (62.5 mmol/L Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate, 5% 2-mercaptoethanol, 25% glycerol, 0.01% bromophenol blue), heated in boiling water for 4 minutes, and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis along with a mixture of molecular-weight standards from Bio-Rad (Hercules, CA). The polyacrylamide content of the gels was 5% for FAS and 8% for the other proteins. Electrophoresis was performed in a Mini-Protean 3 Cell (Bio-Rad).

After electrophoresis, the proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad) in a Mini Trans-Blot Electrophoretic Transfer Cell (Bio-Rad). After treating with blocking buffer (PBS containing 0.1% Tween-20 and 1% casein) for 1 hour at room temperature, the membranes were incubated with pertinent primary polyclonal antibodies for 1 hour at room temperature. These were anti-PPARα (sc-9000, Santa Cruz, Santa Cruz, CA, diluted 1:200 with blocking buffer), anti-PPARγ (sc-7196, Santa Cruz, 1:200), anti-PPARδ (sc-1983, Santa Cruz, 1:100), anti-FAS (sc-20140, Santa Cruz, 1:200), anti-DGAT1 (sc-26173, Santa Cruz, 1:100), anti-HSL (a kind gift of Constantine Londos, 1:200), and anti-perilipin (a kind gift of Constantine Londos, 1:1000). The antibodies had been raised in rabbit or goat against the rat isoforms of the above proteins. The membranes were then incubated with horseradish peroxidase–conjugated secondary antibodies (diluted 1:3000 with blocking buffer), which were either antirabbit immunoglobulin G (Bio-Rad) or antigoat immunoglobulin G (Santa Cruz), as appropriate, for 30 minutes at room temperature. The target proteins were detected by a colorimetric method with the use of the Opti-4CN substrate kit (Bio-Rad), which produced a colored precipitate through the action of horseradish peroxidase. Protein bands appeared violet on a clear background.

The membranes were photographed and the protein bands of interest were quantified with the Gel Analyzer software from Biosure (Athens, Greece). All values were normalized to the signal of one sample, which was included in each gel to correct for blot-to-blot variation. Values were expressed as arbitrary units per tissue mass. The method was linear with a correlation coefficient of 0.98, and the coefficient of variation (CV) was 10%.

2.5. DNA binding activity of PPARγ

The DNA binding activity of PPARγ was measured in muscle, epididymal fat, and subcutaneous fat (the PPARγ gene is not expressed in the liver) by using the TransAM PPARγ transcription factor assay kit by Active Motif (Rixensart, Belgium). This enzyme immunoassay-based method quantifies PPARγ activity by using a 96-well plate to which an oligonucleotide containing the peroxisome proliferator response element has been immobilized. Active PPARγ in the samples binds this peroxisome proliferator response element and is detected through the use of an antibody directed against PPARγ, which does not cross-react with PPARα or PPARδ. Addition of a secondary antibody conjugated to horseradish peroxidase provides a colorimetric readout that is quantified by photometry.

Fifty milligrams of muscle or 150 mg of adipose tissue was homogenized manually on ice with a Dounce homogenizer in 200 or 75 μL, respectively, of lysis buffer (lysis buffer AM1 containing dithiothreitol (DTT) and protease inhibitor cocktail, all from Active Motif). After standing for 30 minutes on ice, the homogenates were centrifuged at 14 000*g* for 30 minutes at 4°C. The supernatant of each muscle homogenate and the infranatant of each adipose tissue homogenate (below the fat cake) were removed and 10 μL of them were used for the assay according to the manufacturer's instructions. The specificity of the method was verified by the use of a wild-type consensus oligonucleotide as a competitor for PPARγ binding and a mutated consensus oligonucleotide. Addition of the former decreased the signal dramatically, whereas addition of the latter had little effect on the signal. To avoid day-to-day variations, all samples were measured on the same day. The CV of the method was 9%.

2.6. Leptin assay

Serum leptin was measured as an index of adaptation to training and an index of fat mass change. Measurement was performed by enzyme immunoassay through the use of a kit from Mediagnost (Reutlingen, Germany). The CV of the method was 2.8%.

2.7. Statistics

Values are expressed as the mean ± SE. The distribution of all dependent variables was examined by the Shapiro-Wilk test and was found not to differ significantly from

normal. Significant differences between untrained and trained animals were detected by 2-sided unpaired Student *t* tests. To determine the meaningfulness and magnitude of the effects of exercise, effect sizes (ESs) were calculated as the difference between means divided by the SD of the untrained group. The level of statistical significance was set at α = .05. The SPSS version 12.0 (SPSS, Chicago, IL) was used for all analyses.

3. Results

The running activity of the trained rats was 5.2 ± 0.4 km/d. The total protein concentration in the epididymal fat of the trained rats was significantly higher than that of the untrained rats (12.3 ± 0.8 vs 9.4 ± 0.6 mg/g, P = .005, ES = 1.41). This was also the case for the subcutaneous fat (16.0 ± 1.6 vs 12.1 ± 1.0 mg/g, P = .041, ES = 1.05). There were no significant differences between groups in the total protein concentrations in the liver or muscle. The trained rats had significantly lower serum leptin concentration compared with the untrained rats (388 ± 59 vs 942 ± 114 pg/mL, P < .001, ES = −1.30).

3.1. Liver proteins

There was no significant difference between groups in the liver PPARα and DGAT1 content (Fig. 1). The trained rats had significantly lower FAS content compared with the untrained rats (P = .030, ES = −0.76).

3.2. Muscle proteins

There were no significant differences between trained and untrained animals in the PPARα, PPARγ, PPARδ, DGAT1, and HSL contents of the muscle (Fig. 2). FAS and perilipin were not detected in either the trained or the untrained animals.

3.3. Epididymal fat proteins

The 2 groups did not differ significantly in the PPARγ (either PPARγ1 or PPARγ2), PPARδ, or DGAT1 contents of

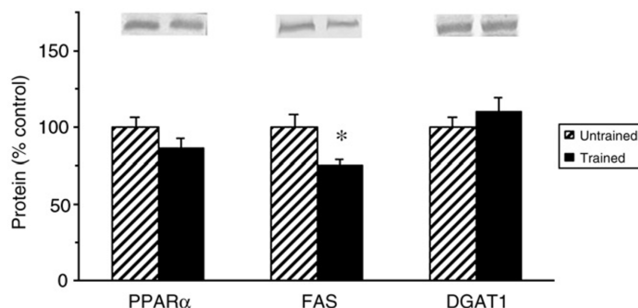

Fig. 1. Mean and SE of the PPARα (55 kd), FAS (>230 kd), and DGAT1 (87 kd) contents of the liver of untrained and trained rats. Values are normalized relative to the control group. Representative bands from Western blots are shown above the bars. *P < .05, significantly different from untrained rats.

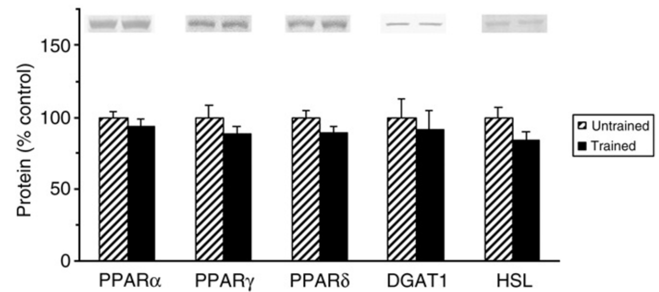

Fig. 2. Mean and SE of the PPARα (55 kd), PPARγ (55 kd), PPARδ (50 kd), DGAT1 (87 kd), and HSL (84 kd) contents of the gastrocnemius muscle of untrained and trained rats. Values are normalized relative to the control group. Representative bands from Western blots are shown above the bars.

epididymal fat (Fig. 3). The trained rats had significantly higher FAS (P = .016, ES = 1.21), HSL (P = .032, ES = 0.91), and perilipin contents (P = .020, ES = 1.01).

3.4. Subcutaneous fat proteins

There were no significant differences between groups in the PPARγ (either PPARγ1 or PPARγ2), PPARδ, FAS, DGAT1, and perilipin contents of the subcutaneous fat (Fig. 4). In contrast, the trained rats had significantly higher HSL content (P = .005, ES = 1.64).

3.5. DNA binding activity of PPARγ

The data presented thus far posed the following apparent contradiction: given the ample evidence that PPARs regulate lipid metabolism, how can there be changes in key proteins involved in lipid metabolism in adipose tissue when there were no changes in PPARs? To address this issue, we examined the possibility that the transcriptional activity rather than the amount of PPARs changed in response to

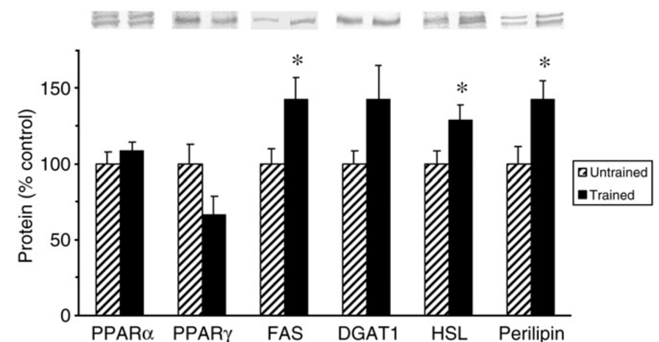

Fig. 3. Mean and SE of the PPARγ (52 and 55 kd), PPARδ (50 kd), FAS (>230 kd), DGAT1 (87 kd), HSL (84 kd), and perilipin (60 and 64 kd) contents of the epididymal fat of untrained and trained rats. Values are normalized relative to the control group. Representative bands from Western blots are shown above the bars. Peroxisome proliferator–activated receptor γ was calculated as the sum of the 2 bands corresponding to the PPARγ1 and PPARγ2 isoforms expressed in adipose tissue [15]. *P < .05, significantly different from untrained rats. Borderline significant differences (.05 < P <.1) are also noted.

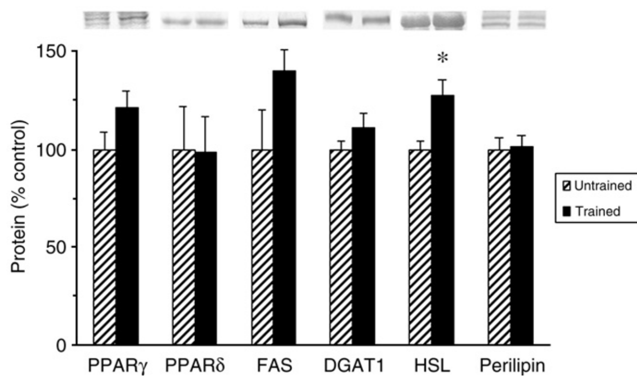

Fig. 4. Mean and SE of the PPARγ, PPARδ, FAS, DGAT1, HSL, and perilipin contents of the subcutaneous fat of untrained and trained rats. Values are normalized relative to the control group. Representative bands from Western blots are shown above the bars. Peroxisome proliferator–activated receptor γ was calculated as the sum of the 2 bands corresponding to the PPARγ1 and PPARγ2 isoforms expressed in adipose tissue [15]. *$P < .05$, significantly different from untrained rats.

exercise. To this end we used a relatively novel quantitative assay, as described under Materials and methods, to measure the DNA binding activity of PPARγ only, because the PPARα gene is not expressed in adipose tissue and no kit for PPARδ was commercially available. For the sake of comparison, we measured the DNA binding activity of PPARγ in muscle as well as in the 2 fat depots.

There were no significant differences between trained and untrained rats in the DNA binding activity of PPARγ in muscle (Fig. 5). In contrast, the trained rats had significantly higher activity compared with the untrained rats in both epididymal (P = .012, ES = 1.45) and subcutaneous fat (P = .010, ES = 2.00).

4. Discussion

The purpose of this study was to explore the effect of exercise training on the gene expression of 4 proteins playing protagonistic roles in lipogenesis and lipolysis (FAS, DGAT1, HSL, and perilipin) and 3 transcription factors (PPARα, PPARγ, and PPARδ) involved in lipid metabolism in rat liver, skeletal muscle, and 2 fat depots. To our knowledge, this is the first report on the effect of exercise training on liver DGAT1 and PPARα, as well as adipose tissue perilipin and PPARδ. Another novelty of the study is the investigation of the effect of exercise training on protagonists of lipogenesis and lipolysis in parallel.

Exercise training had a profound effect on the serum leptin concentration (2.4-fold reduction). Because the serum leptin concentration is positively related to fat mass [22], our finding suggests a decrease in total body fat mass with training. Attesting to this are our previous findings of decreased triacylglycerol concentrations in the subcutaneous fat and gastrocnemius muscle of the same animals [21]. In addition, leptin's reduction can be considered as an adaptation to exercise training [13] because leptin has been reported to be lowered by exercise training in rats in an additive manner to body weight or body fat reduction [23-25]. Combined with the increase of carnitine palmitoyl-transferase and 3-hydroxyacyl coenzyme A dehydrogenase activities in the gastrocnemius muscle of the trained rats [21], the leptin data attest to the effectiveness of the exercise stimulus used.

The lower liver FAS content found in the trained rats is in accordance with the data of Fiebig et al [10] in trained rats fed a high starch diet and with those of Fiebig et al [11] in trained lean rats. However, the same studies found no change with exercise training in rats fed a high fructose diet [10] and obese rats [11]. The partial discrepancy of our results with those of Fiebig et al [10] suggests that the effect of exercise may depend on the diet. Similarly, the partial discrepancy of our results with those of Fiebig et al [11] suggests that the effect of exercise may depend on whether the rats are lean or obese. Finally, Barakat et al [9] reported no differences in liver FAS between treadmill-trained and untrained rats. This difference from our data may be due to the different exercise mode used.

No differences between untrained and trained rats were found in any of the proteins measured in the gastrocnemius muscle, suggesting a resistance of the muscle to changes in the gene expression of the specific proteins with exercise training. The absence of differences in DGAT1 is in accordance with the results of Ikeda et al [26] who found no difference in DGAT1 messenger RNA of rat gastrocnemius muscle after 8 weeks of exercise training.

Also in agreement with our data, Enevoldsen et al [15] found no difference in the muscle HSL concentration between endurance trained and untrained rats. To test the possibility that part of the HSL measured in the muscle was coming from adipocytes present along with the muscle fibers, we assayed perilipin, a protein that is almost exclusively expressed in adipocytes and has been used as a specific adipocyte marker [27]. The detection of no perilipin

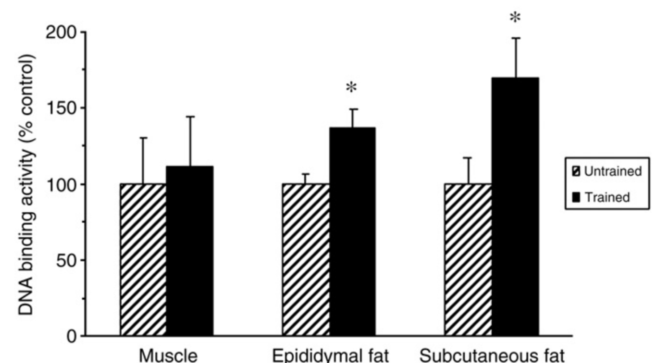

Fig. 5. Mean and SE of the DNA binding activity of PPARγ in gastrocnemius muscle, epididymal fat, and subcutaneous fat of untrained and trained rats. Values are normalized relative to the control group. *$P < .05$, significantly different from untrained rats.

in gastrocnemius suggests the absence of any substantial adipocyte population. This is supported by the very low triacylglycerol content of this muscle, as described in our previous report [21]: 1.33 mmol/L in the untrained rats and 0.33 mmol/L in the trained rats, corresponding to 0.11% and 0.03% by weight, respectively.

Although short-term exercise is known to increase HSL activity [28,29], the effect of long-term exercise is far from clear. The training mode used in the present study caused an increase in the HSL content of both visceral (epididymal) and subcutaneous fat. These data agree with Nomura et al [14] who found increased HSL in rat epididymal adipocytes after 8 weeks of exercise training and, partly, with Enevoldsen et al [15] who found higher HSL in retroperitoneal but not mesenteric fat of trained rats. As also noted by Enevoldsen et al [15], regional differences in adaptations and, in turn, regional differences in lipolysis among intra-abdominal fat depots after exercise training may be the reason for the observed discrepancy. Finally, our data, and those of Enevoldsen et al [15] and Nomura et al [14], differ from those of De Glisezinski et al [16] who found decreased HSL with training in obese humans. This finding may be related to the different species or to obesity.

Although exercise training increased the HSL content of both fat depots examined in the present study, it had a different effect on perilipin, which increased with training in epididymal fat but did not change in subcutaneous fat. This difference, along with the significant increase of FAS (found also by Barakat et al [9] in epididymal fat of trained rats) and the borderline significant increase of DGAT1 in epididymal fat, may explain the decreased triacylglycerol concentration in subcutaneous but not epididymal fat with training, which we found in our previous study [21]. That is, by restricting access to the lipid droplets, perilipin may moderate the lipolytic action of HSL, whereas the increased FAS and DGAT1 may enhance lipogenesis, thus counterbalancing the enhanced lipolysis.

We found no differences between trained and untrained rats in any of the 3 members of the PPAR family in the liver, skeletal muscle, or adipose tissue. The finding of no difference in muscle PPARα contrasts with the increase reported in bicycle-trained women by Horowitz et al [1], but is in agreement with Tunstall et al [30] and Schmitt et al [31] who found no effect of training on the PPARα messenger RNA content of human skeletal muscle. Likewise, the finding of no difference in muscle PPARγ contrasts with the increase reported in the soleus and extensor digitorum longus muscles of treadmill-trained fructose-fed hypertensive rats by the only relevant study [18], although we agree on no change in adipose tissue PPARγ. The different muscles examined and the different exercise mode may have contributed to the discrepancy between the 2 studies. Concerning muscle PPARδ, the absence of difference between trained and untrained rats in the present study contradicts the increased expression found in mouse plantaris muscle with swimming training in the study of Luquet et al [7].

As mentioned under Results, an explanation for the lack of changes in the PPAR content of adipose tissue despite the presence of changes in key proteins involved in lipogenesis and lipolysis with exercise could be the activation rather than induction of PPARs. This was indeed found to be the case with PPARγ. To our knowledge, this is the first report of an effect of exercise on the activity of PPARγ. What could activate this transcription factor in the trained rats? PPARγ is known to be activated by fatty acids [32], whose concentrations in plasma increase immediately with exercise [33] and may also increase in adipocytes as a result of enhanced lipolysis. Although we have been unable to locate any study that has examined whether the binding of fatty acids increases the DNA binding activity of PPARγ, this has been shown for PPARα [34]. Activation of a transcription factor is more economical and flexible than induction and may thus be preferred by cells as a means of triggering a signal transduction pathway.

Although the DNA binding activity of PPARγ increased significantly with training in both fat depots, FAS increased significantly in epididymal but not in subcutaneous fat. However, the increase was numerically similar in the 2 fat depots (43% and 40%, respectively), suggesting that a larger sample size would probably make the difference significant in subcutaneous fat as well.

The transcriptional activity of PPARγ is an important contributor to insulin sensitivity, as shown by pharmacologic and genetic data summarized by Gurnell [6]. For example, the in vitro binding affinities of PPARγ ligands (including the most widely known thiazolidinediones) correlate closely with their in vitro potencies as insulin sensitizers, and humans with loss-of-function PPARγ mutations exhibit severe insulin resistance. The link between PPARγ and insulin sensitivity seems to be particularly strong in adipose tissue (in which PPARγ gene expression is markedly higher than in muscle). Attesting to this are the findings that mice lacking adipose tissue are refractory to the antidiabetic effects of thiazolidinediones and that adipose-specific PPARγ knockout causes insulin resistance in fat [6]. On the other hand, it is well known that exercise improves insulin sensitivity, although how it does is not clear [35]. Thus, the increase in PPARγ activity in adipose tissue with long-term wheel running may be part of the mechanism for this beneficial effect of exercise. Although the effects of pioglitazone, a thiazolidinedione, have been studied vs exercise and diet [36], we have been unable to locate any study of whether exercise training increases the sensitivity to pioglitazone. This could be tested in a future study in the direction of optimizing interventions to increase insulin sensitivity.

In conclusion, 8 weeks of voluntary wheel running had significant effects on the concentrations of proteins playing key roles in lipogenesis and lipolysis in rat liver, visceral fat, and subcutaneous fat. Exercise training lowered FAS in the liver and increased FAS, HSL, and perilipin in epididymal fat of the trained animals. The higher HSL in subcutaneous fat of the trained rats is in

accordance with the reduced triacylglycerol content of the same tissue in the same animals [21]. In addition, the trained rats had higher total protein concentrations in both fat depots. The ES of exercise training on all these parameters was large according to the classification of Cohen [37] who set the threshold for large ES at 0.8. No differences in the liver, muscle, or adipose tissue PPAR contents were found between groups. However, the DNA binding activity of PPARγ was higher in both fat depots of the trained rats. These findings suggest that the specific type of exercise training may regulate the expression of target genes of these transcription factors through PPAR activation rather than induction, rendering the regulation of transcription more economical and flexible. Finally, the activation of PPARγ with exercise may mediate its well-known beneficial effect on insulin sensitivity.

Acknowledgments

This study was supported by a grant from the Greek Ministry of Education (EPEAEK-Heracletus project). The first author was a recipient of a doctoral fellowship from the same source.

We thank Biosure for providing the image analysis software. We also thank Active Motif for providing kits for the preparation of tissue extracts and the measurement of PPARγ activity.

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