

Endurance Training in Humans Leads to Fiber Type-Specific Increases in Levels of Peroxisome Proliferator-Activated Receptor- γ Coactivator-1 and Peroxisome Proliferator-Activated Receptor- α in Skeletal Muscle

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The peroxisome proliferator-activated receptor (PPAR)- γ coactivator-1 (PGC-1) can induce mitochondria biogenesis and has been implicated in the development of oxidative type I muscle fibers. The PPAR isoforms α , β/δ , and γ control the transcription of genes involved in fatty acid and glucose metabolism. As endurance training increases skeletal muscle mitochondria and type I fiber content and fatty acid oxidative capacity, our aim was to determine whether these increases could be mediated by possible effects on PGC-1 or PPAR- α , - β/δ , and - γ . Seven healthy men performed 6 weeks of endurance training and the expression levels of PGC-1 and PPAR- α , - β/δ , and - γ mRNA as well as the fiber type distribution of the PGC-1 and PPAR- α proteins were measured in biopsies from their vastus lateralis muscle. PGC-1 and PPAR- α mRNA expression increased by 2.7- and 2.2-fold ($P < 0.01$), respectively, after endurance training. PGC-1 expression was 2.2- and 6-fold greater in the type IIa than in the type I and IIx fibers, respectively. It increased by 2.8-fold in the type IIa fibers and by 1.5-fold in both the type I and IIx fibers after endurance training ($P < 0.015$). PPAR- α was 1.9-fold greater in type I than in the II fibers and increased by 3.0-fold and 1.5-fold in these respective fibers after endurance training ($P < 0.001$). The increases in PGC-1 and PPAR- α levels reported in this study may play an important role in the changes in muscle mitochondria content, oxidative phenotype, and sensitivity to insulin known to be induced by endurance training. *Diabetes* 52:2874–2881, 2003

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COX4, cytochrome c oxidase 4; CPT-1, carnitine palmitoyltransferase-1; MHC, myosin heavy chain; PGC-1, peroxisome proliferator-activated receptor- γ coactivator-1; PPAR, peroxisome proliferator-activated receptor.

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Skeletal muscle is a major player in glucose homeostasis under basal conditions and in response to insulin and exercise. The peroxisome proliferator-activated receptor (PPAR)- γ coactivator-1 (PGC-1) is expressed in several tissues, including skeletal muscle and brown adipose tissue. It has been reported to increase mitochondria biogenesis, oxidative metabolism, and both basal and insulin-stimulated glucose uptake (1–5). Recently, PGC-1 has also been implicated in the formation of oxidative skeletal muscle type I fibers in mice (6). The PPAR isoforms α and β/δ have been reported to increase the transcription of genes involved in muscle fatty acid oxidation, such as carnitine palmitoyltransferase-1 (CPT-1) and FAT/36, and of uncoupling protein-2 and -3 (7–10). PPAR- γ has been implicated in fatty acid synthesis and glucose metabolism (for review, see 11). The regulation of gene transcription by the PPAR isoforms involves, in part, ligand-mediated interactions with PGC-1 (2,12). Because of their important roles in the control of energy metabolism and insulin sensitivity, PGC-1 and PPARs are candidate factors in the cause of type 2 diabetes (1,9) and drug targets for its therapeutic treatment.

Endurance training improves skeletal muscle fatty acid oxidation capacity (13) and insulin sensitivity (14), therefore making it an important intervention for the treatment of type 2 diabetes. In rats, PGC-1 mRNA and protein levels are increased after a single bout of exercise (15,16) as well as after several days of training (17). In humans, Pilegaard et al. (18) observed a transient increase in PGC-1 mRNA levels after a single bout of exercise but no significant differences in steady-state PGC-1 mRNA levels after 4 weeks of leg-extension training. Furthermore, Tunstall et al. (19) found no change in PGC-1 mRNA levels after 9 days of cycling training. Until now, the effect of a whole-body endurance training program, performed over several weeks, on human PGC-1 mRNA and protein levels has not been evaluated.

Tunstall et al. (19) observed no change in PPAR- α mRNA levels in human muscle after 9 days of endurance

TABLE 1
Primer sequences, PCR size, and annealing temperatures used for the PCR

Gene	Sequence 5'-3'	Size (bp)	Temperature
PGC-1	sense TCA GTC CTC ACT GGT GGA CA anti TGC TTC GTC GTC AAA AAC AG	351	62
PPAR- α	sense TGA ACG ATC AAG TGA CAT TGC anti TGG GAA GAG AAA GAT ATC G	368	56
PPAR- β/δ	sense ATG GAG CAG CCA CAG GAG GAA GCC anti GCA TGA GGC CCC GTC ACA GC	201	58
PPAR- γ	sense GAC CAC TCC CAC TCC TTT anti CGA CAT TCA ATT GCC ATG AG	257	62
GLUT-4	sense CTT CGA GAC AGC AGG GGT AG anti ATG ACT GTG GCT CTG CTC CT	180	60
CPT-1	sense TGA GGT GCT CTC GGA ACC CT anti CTC GCC TGC AAT CAT GTA GG	163	60
Troponin-1	sense GGA GTC TGA GAA GTT CGA CCT GAT anti TGG CTG ATG CGG TTG TAC A	80	60
COX4	sense CAT GTG GCA GAA GCA CTA TGT GT anti GCC ACC CAC TCT TTG TCA AAG	80	60
β -Actin	sense ACT GAC TAC CTC ATG AAG AT anti CGT CAT ACT CCT GCT TGC TGA T	535	56

training. However, Horowitz et al. (20) found, still in human muscle, that the protein content of PPAR- α as well as of some of its target enzymes, such as medium and very-long-chain acyl CoA dehydrogenases, are increased after 12 weeks of endurance training (20). The effect of several weeks of endurance training on the other PPAR isoforms has not been reported.

Human skeletal muscle consists of predominantly three muscle fiber types, commonly distinguished by their myosin heavy chain (MHC) isoforms (21). The mitochondria content and oxidative capacity are the highest in the slow oxidative type I, lower in the fast oxidative-glycolytic type IIa, and lowest in the fast glycolytic type IIx fibers. The inverse is observed for the glycolytic capacity of these fibers. The PGC-1 protein content in mice has been found to be higher in type I-rich as compared with type II-rich muscles (6). The fiber type distribution of PGC-1 and of PPAR- α has, until now, not been studied in humans.

The aim of this study was to determine the effect of 6 weeks of running endurance training on whole-muscle PGC-1; PPAR- α , - β/δ , and - γ mRNA expression; and fiber-type content of the PGC-1 and PPAR- α proteins in healthy males. In addition, the expression of several nuclear encoded genes, known to increase after endurance training and suggested to be regulated by PGC-1, such as troponin I-slow (TnI-slow), cytochrome c oxidase 4 (COX4), and GLUT4, and by PPAR- α , such as CPT-1, were also measured.

RESEARCH DESIGN AND METHODS

Seven healthy men, age (mean \pm SD) 34 ± 5 years, mass 78 ± 8 kg, maximal oxygen consumption ($\dot{V}O_{2max}$) 54 ± 4 ml \cdot kg $^{-1}$ \cdot min $^{-1}$, participated in the study, which was approved by the local medical society ethical committee. All participants gave their informed consent and agreed to a muscle biopsy followed by a test to measure aerobic capacity.

Measurement of oxygen consumption. $\dot{V}O_{2max}$ was measured using a Quark B2 metabolic cart (Cosmed, Rome, Italy) while the participants ran on a treadmill (Technogym Runrace, Gambettola, Italy). The participants began running at 7.2 km/h with a 1% gradient. The speed was increased by 1.8 km/h every 3 min until the participant could not maintain the speed. The duration of the tests lasted between 10 and 15 min. Heart rate (Polar) and oxygen consumption were measured continually throughout the test. $\dot{V}O_{2max}$ was calculated as the highest value averaged over a 30-s period.

Muscle biopsy technique. Skeletal muscle samples were obtained under local anesthesia (Xylocaine, 1% plain) from the belly of the vastus lateralis muscle using a percutaneous needle biopsy technique (22) modified to include suction. A single incision was made in the skin, and two muscle samples were taken from a single insertion of the biopsy needle. After the first biopsy cut, the needle was rotated and a second cut was performed. One muscle sample was mounted in embedding medium and frozen in isopentane, previously cooled to its freezing point. The other muscle sample was immediately frozen in liquid nitrogen and used for RNA extraction.

Training program. The endurance training program comprised three supervised sessions per week, which included two interval sessions separated by a constant intensity session. Each session commenced with a warm-up consisting of ~ 10 min of light running followed by stretching. The interval sessions consisted of five to six intervals at an individual intensity corresponding to 70–80% of $\dot{V}O_{2max}$. A 1-min recovery was performed between each test at an intensity of 50% of $\dot{V}O_{2max}$. The duration of these intervals ranged between 1 and 3 min for each participant. The number of repetitions and their intensity were gradually increased over the training period so that during week 6, the participants were completing $6 \times 80\%$ repetitions. For example, during the first week, $5 \times 70\%$ repetitions were performed; during the second week, it was $6 \times 70\%$; and during the third week, it was $5 \times 75\%$. This pattern was followed so that during week 6, the participants were completing $6 \times 80\%$ repetitions. A 1-min recovery was performed between each test at an intensity of 50% of $\dot{V}O_{2max}$. The duration of these intervals ranged between 1 and 3 min for each participant. All intensities were controlled by individual heart rates that were measured during the duration of each session using a Polar heart rate monitor. The constant intensity session consisted of running for 40 min at an intensity of 60% of $\dot{V}O_{2max}$.

RNA extraction and real-time quantitative PCR. Total RNA was extracted, and oligo-dT primed first-strand cDNA was synthesized as reported previously (23). Real-time PCR was performed using a Lightcycler rapid thermal cycler system; a Lightcycler-DNA Fast-Start Master SYBR Green I mix for the quantification of PGC-1, PPAR- β/δ , TnI-slow, COX4, GLUT4, and CPT-1; and a Lightcycler-DNA Master SYBR Green I mix for the quantification of PPAR- α , PPAR- γ , and β -actin (Roche Diagnostics, Rotkreuz, Switzerland), according to the manufacturers' instructions. β -Actin was used as a control to account for any variations due to efficiencies of the reverse transcription and PCR and has previously been shown to remain stable after training (24). The primer sequences are presented in Table 1. For confirming the amplification specificity, the PCR product was subjected to a melting curve analysis and following this, agarose gel electrophoresis. PCR products were purified using a QIAGEN purification kit (QIAGEN AG, Basel, Switzerland), and the DNA sequence was confirmed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Immunofluorescence. Immunofluorescence experiments were performed as reported previously (25). The PGC-1 (sc-5816; Santa Cruz Biotechnologies, San Diego, CA) and PPAR- α (Dr. David Bell, The University of Nottingham, U.K.) antibodies, respectively, were diluted 1:200 and 1:500 in PBS containing 0.5% BSA and 0.1% Na $_2$ S $_2$ O $_3$ and were visualized separately, using a donkey

TABLE 2
Subject characteristics

Variable	Pretraining	Posttraining
$\dot{V}O_{2\max}$ (ml · kg ⁻¹ · min ⁻¹)	55.5 ± 4.3	61.8 ± 6.2*
Type I (%)	51 ± 10	69 ± 12*
Type IIa (%)	33 ± 13	21 ± 8*
Type IIx (%)	16 ± 12	9 ± 5*

*Different from pretraining values ($P < 0.05$).

anti-goat (IgG) and a goat anti-mouse (IgG) antibody, both conjugated with Alexa Fluor 594 (Molecular Probes Europe, Leiden, the Netherlands) at a concentration of 1:500, for 60 min at room temperature. Muscle fiber composition was determined by counting the number of positive fibers stained with antibodies obtained from the Development Studies Hybridoma Bank, University of Iowa, raised against the human slow type I MHC (A4.84) and the fast IIa MHC (N2.261), respectively (25). The muscle fibers that were not stained were deemed to be MHC type IIx fibers. To determine the saturation point of the fluorescence signals, we performed preliminary experiments using serial dilutions of the primary and secondary antibodies. For the PGC-1 antibody, which has previously been used for Western blot analysis (15), SAOS2-GR(+) cells were infected with an adenovirus-expressing human *PGC-1* (26) (Fig. 5) and were used to determine incubation conditions and the specificity of the antibody. A negative control for the PPAR- α antibody was performed by incubating the sections with the preimmune serum, which resulted in no signal. Negative controls, using only the secondary antibody, were also performed in parallel and on the same glass slide as the experimental samples. In addition, pre- and posttraining sections were placed on the same slide and analyzed in duplicate. Sections were viewed and photographed using a Zeiss Axiophot I microscope mounted with an Axiocam color CCD camera. The specific fluorescence within each fiber was quantified using the Zeiss KS400 V3.0 program (25). Approximately 310 ± 45 fibers were scanned for each participant.

Statistics. Group data were presented as mean ± SE. Paired *t* tests were used to determine any changes in $\dot{V}O_{2\max}$ and gene expression changes after training. A 2 (training status, between factor) × 3 (fiber types, within factor) factor ANOVA was used to compare the influence of training status and fiber type on PGC-1 and PPAR- α protein expression, respectively. When a significant interaction between training status and fiber type was observed, stratified analysis was used to locate the significant differences. To be specific, one-way repeated measures ANOVA followed by contrasts were used to compare the

percentage changes in PGC-1 and PPAR- α , respectively, between type I, IIa, and IIx muscle fibers, for the groups pre- and posttraining. The α level of significance for the paired *t* tests was set at 0.05. The overall α level for the ANOVA was set at 0.05, and the Sharpener Bonferroni method was used to adjust the individual α level to significance of $P < 0.0167$ when multiple testings were performed in the stratified analysis.

RESULTS

The participant data are presented in Table 2. Endurance training increased $\dot{V}O_{2\max}$ by 10% and type I fibers by 18% ($P < 0.05$). The increase in type I fibers was accompanied by a decrease in both type IIa and IIx fibers by 12 and 7%, respectively ($P < 0.05$).

Analysis of mRNA expression indicated that PGC-1 and PPAR- α mRNA levels increased by 2.7- and 2.2-fold ($P < 0.01$), respectively, whereas PPAR- β/δ and PPAR- γ mRNA did not change after endurance training ($P > 0.05$; Fig. 1). *Tn1-slow*, *CPT-1*, *COX4*, *GLUT4*, and *mRNA* increased by 40, 75, 72, and 59%, respectively ($P < 0.01$; Fig. 2). β -Actin mRNA did not change with training (pre- versus post-training values; 5.9 ± 0.9 vs. 6.3 ± 0.7 AU; $P > 0.05$).

Immunofluorescence staining was performed using antibodies that distinguish the different fiber types and with antibodies against PGC-1 and PPAR- α . This enabled the identification of PGC-1 and PPAR- α in the different fiber types. Figure 3 shows a representative immunofluorescence stain of PGC-1 and PPAR- α before and after endurance training. It can be observed that the level of expression of PGC-1, both before and after endurance training, is the highest in type IIa fibers, lower in the type I fibers, and the lowest in the type IIx fibers, whereas the level of PPAR- α was higher in type I than in both the type II fibers (Figs. 3 and 4).

Quantification of the images indicated that before endurance training, PGC-1 protein levels in the type IIa fibers were 2.2- and 6-fold greater than in the I and IIx fibers,

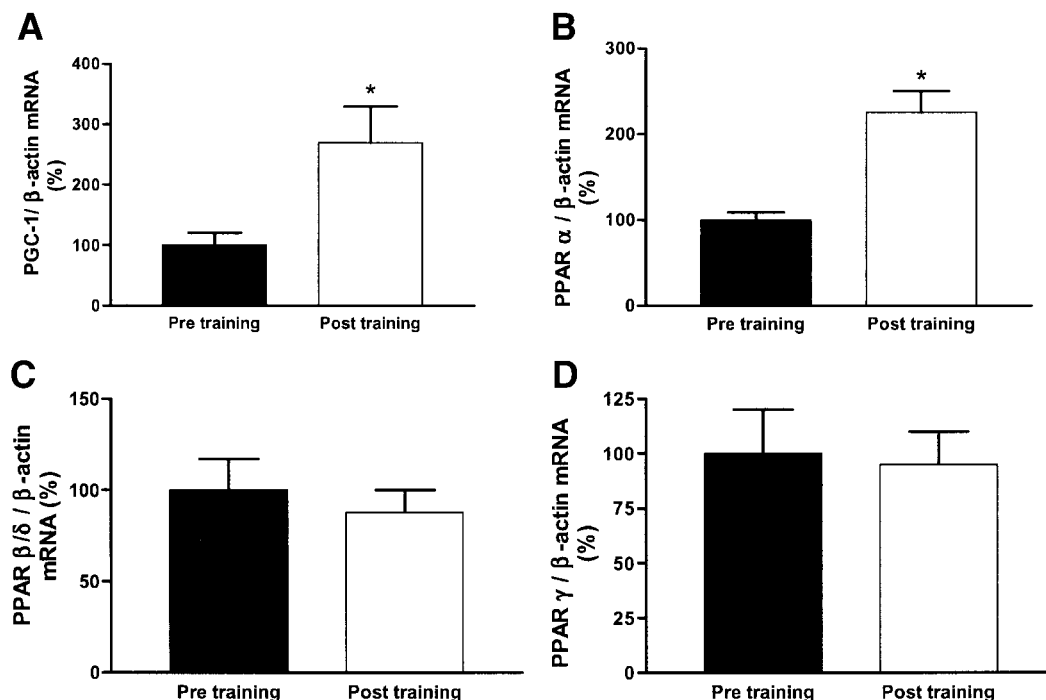


FIG. 1. The effect of 6 weeks of endurance running training on PGC-1 (A), PPAR- α (B), PPAR- β/δ (C), and PPAR- γ (D) mRNA. *Significantly different from pretraining levels ($P < 0.01$).

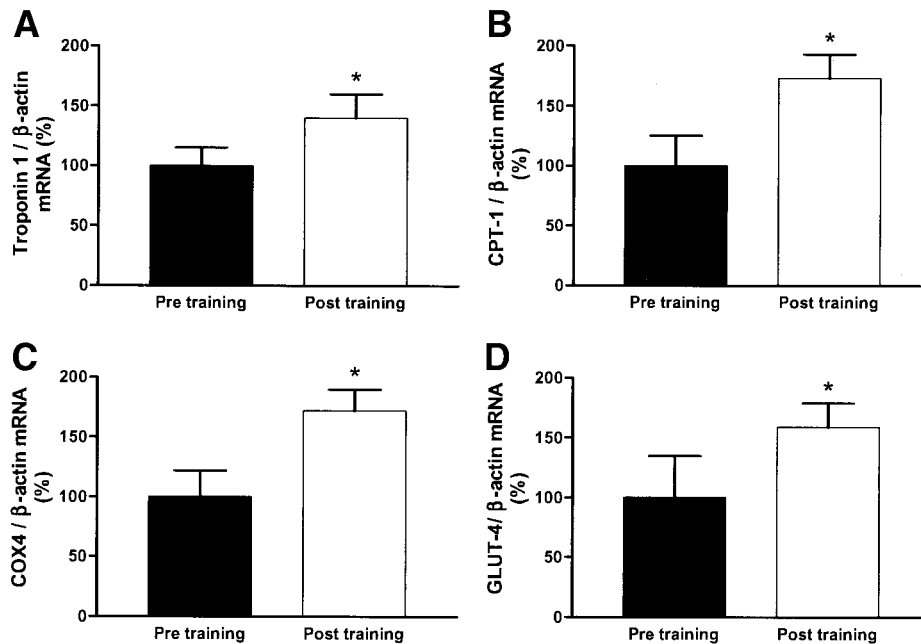


FIG. 2. The effect of 6 weeks of endurance running training on troponin 1 (A), CPT-1 (B), COX4 (C), and GLUT4 (D) mRNA. *Significantly different from pretraining levels ($P < 0.01$).

respectively ($P < 0.001$), whereas PGC-1 was 2.5-fold greater in the type I than in the type IIx fibers ($P < 0.001$). After endurance training, PGC-1 protein content increased by 2.8-fold in the type IIa fibers, an effect that was significantly greater than the 1.5-fold increase measured in both the type I and IIx fibers ($P < 0.015$; Fig. 4). In contrast, before endurance training, PPAR- α levels were 1.9-fold greater in the type I than in the IIa and IIx fibers ($P < 0.001$). No differences in the PPAR- α protein contents were observed when comparing the type IIa with the type IIx fibers ($P > 0.05$). After endurance training, PPAR- α protein content increased by 3.0-fold in the type I fibers, an effect that was significantly greater ($P < 0.015$) than the 1.5-fold increase in both the type IIa and IIx fibers ($P < 0.01$).

DISCUSSION

In the present study, we examined the effect of 6 weeks of endurance training on the skeletal muscle gene expression of PGC-1 and of PPAR- α , - β/δ , and - γ in humans. We also studied, in the different muscle fiber types, the distribution of PGC-1 and PPAR- α proteins before and after the 6-week endurance training period. Two findings of our study are novel. First, we showed that several weeks of whole-body endurance training in humans induces a pronounced increase in skeletal muscle PGC-1 and PPAR- α mRNA and their encoded proteins. Second, we showed that before and after endurance training, PGC-1 protein content is greatest in type IIa, lower in type I, and lowest in type IIx fibers, whereas PPAR- α protein content is greater in the type I than in the type IIa and IIx fibers of the vastus lateralis muscle.

Our observation of an increase in PGC-1 mRNA after 6 weeks of endurance training supports previous observations made in rats (15,17) but is in contrast with studies performed in humans (18,19). These conflicting results are most likely explained by the differences in the training

duration and intensity. Our results suggest that 6 weeks of moderate- to high-intensity running endurance training is required to stimulate an increase in PGC-1 mRNA. More important, we showed for the first time that this same training program can induce increases in the PGC-1 protein content in human skeletal muscle. The increase in PGC-1, in the vastus lateralis muscle of our participants after endurance training, was paralleled by a shift in fiber composition toward a more oxidative type I muscle with an increase in the percentage of type I fibers and a decrease in the percentage of type IIa and IIx fibers. This was supported by an increase in the mRNA expression of TnI-slow, a gene expressed mostly in slow oxidative muscle fibers (27) and suggested to be regulated by PGC-1 (6). The greater percentage of type I fibers measured in the vastus lateralis muscle after endurance training is in support of previous observations made in endurance-trained humans (28,29). Our observation of an increase in PGC-1, in association with an increase in the percentage of type I muscle fibers and TnI-slow mRNA, supports previous findings of Lin et al. (6), who showed that overexpression of PGC-1 in the plantaris, a muscle consisting of predominantly type II fibers, up to the level found in the soleus, a muscle consisting of predominantly type I fibers, induced a type II to type I fiber conversion as well as an induction of TnI-slow mRNA. These findings support the idea that PGC-1 possibly controls the slow oxidative type I muscle phenotype (6). Our findings also suggest that a naturally caused induction of endogenous PGC-1 activity may lead to similar phenotypes as experimentally raised levels of PGC-1, which may be important not only for increasing the content of type I fibers but also for improving insulin sensitivity.

The increase in PPAR- α mRNA and protein content after the 6 weeks of endurance training performed in the present study is in contrast with the results of Tunstall et al. (19), who observed no change in PPAR- α mRNA in

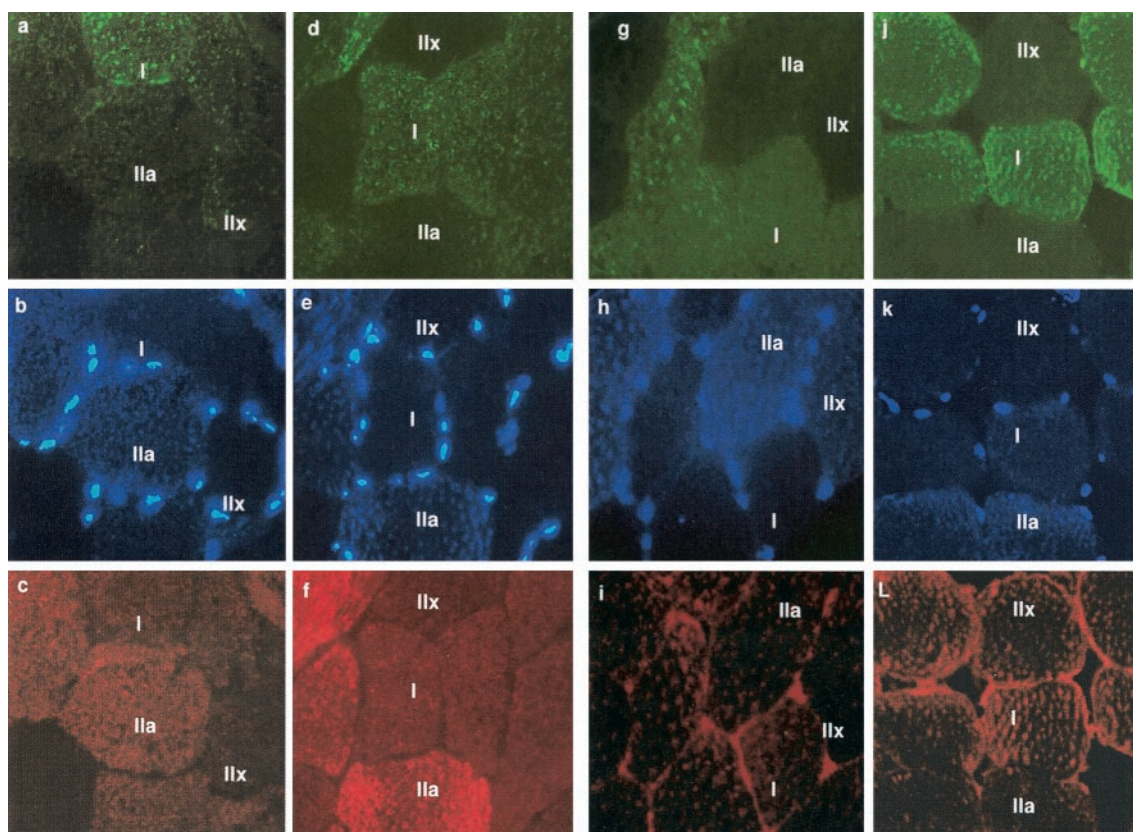


FIG. 3. Triple-immunofluorescent staining of serial sections. For all images, type I muscle fibers are stained green, type IIa muscle fibers are stained blue, and type IIx muscle fibers are unstained. Panels *c* and *f* show PGC-1 before and after endurance training, respectively. Panels *i* and *l* show PPAR- α before and after endurance training, respectively.

humans after 9 days of endurance training, but in agreement with those of Horowitz et al. (20), who observed a twofold increase in PPAR- α protein content after 12 weeks of endurance training. Again, these differences may be due to the duration and intensity of the endurance training performed. The metabolic stress caused by a short training duration of 9 days at an intensity of 63% of $\dot{V}O_{2max}$ may not be sufficient to evoke an increase in PPAR- α gene transcription (19) and, one can speculate, on PPAR- α protein translation. The pretraining PPAR- α levels may be adequate to increase the transcription of genes involved in fatty acid oxidation, such as CPT-1 and FA translocase

(FAT/CD36), and therefore assist, in part, with the increased fat oxidation that was observed after 9 days of training (19). Our results suggest that if a longer training period of at least 6 weeks at intensities between 60 and 80% of $\dot{V}O_{2max}$ is performed, then an increase in PPAR- α mRNA and protein levels is necessary to meet these requirements. Indeed, this is supported by the 73% increase in CPT-1 mRNA observed in the present study.

Unlike PPAR- α mRNA, PPAR- β/δ and PPAR- γ mRNA were not changed after endurance training. This is the first time that PPAR- β/δ gene expression has been measured in human skeletal muscle after endurance training. In mouse

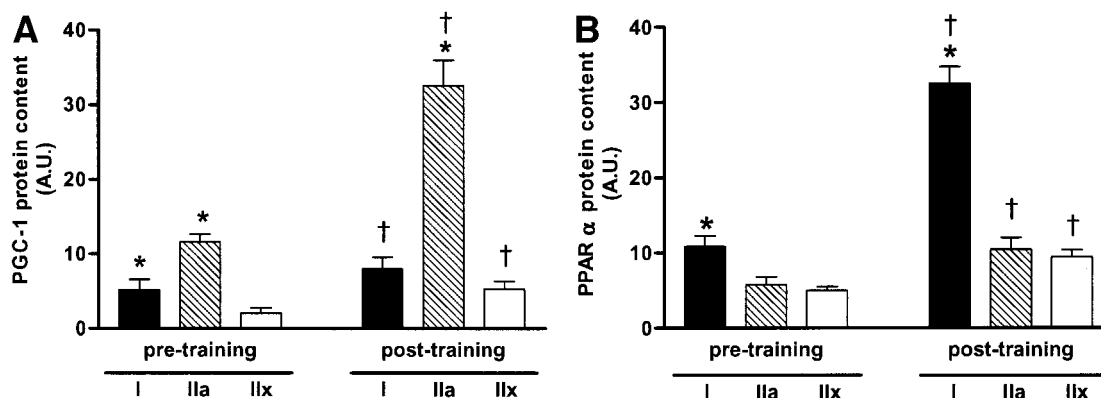


FIG. 4. The expression of PGC-1 (*A*) and PPAR- α (*B*) protein contents in the different fiber types before and after endurance training. ■, type I fibers; ▨, type IIa fibers; □, type IIx fibers. *Significantly different from the other fibers in the same group ($P < 0.0167$); †significantly different from the same fiber in the pretraining group.

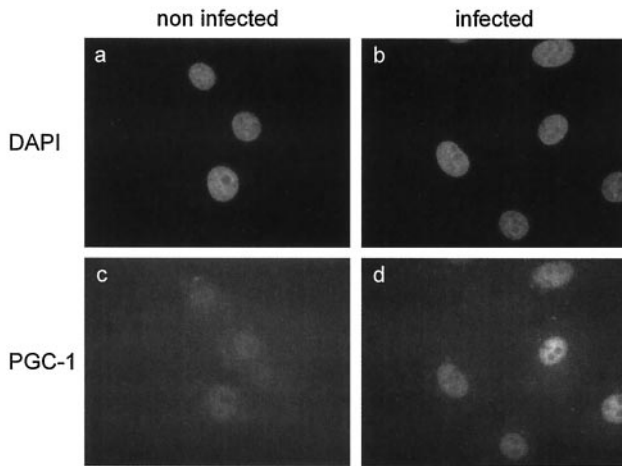


FIG. 5. Immunostaining of SAOS2-GR(+) cells, noninfected (*a* and *c*) and infected with a PGC-1-expressing adenovirus (*b* and *d*). Nuclei DAPI staining in *a* and *b* and staining with the PGC-1 antibody in *c* and *d*.

skeletal muscle, PPAR- β/δ gene expression is severalfold higher than PPAR- α and PPAR- γ (9). In both human and rodent myocytes (9) and in mouse white adipose tissue (12), the activation of PPAR- β/δ increases fatty acid oxidation and induces the expression of lipid regulatory genes. It is possible that the basal level of PPAR- β/δ in those who participated in the present study may have been sufficient to regulate the genes that assist in the increase in fat oxidation commonly observed after endurance training (20,30,31). In addition, even though an increase in PPAR- β/δ mRNA was not observed, its activity would presumably be higher as a consequence, of among others, the increase in PGC-1. In contrast with our results, a small but significant 20% decrease in PPAR- γ mRNA was reported in the study by Tunstall et al. (19) after 9 days of endurance training. However, whether the 20% decrease in PPAR- γ mRNA was paralleled by a decrease in the PPAR- γ protein content was not determined. Again, the discrepancy between these studies may be due to the different training protocols used. The downregulation of PPAR- γ mRNA may be an early but transient response to training. It is possible that after prolonged endurance training, PPAR- γ mRNA returns to its pretraining levels.

Skeletal myofibrils differ greatly with respect to their contractile and metabolic capabilities. In humans, the type I fibers are highly oxidative with a slow contraction speed, the type IIa fibers are considered an oxidative-glycolytic fiber with intermediate contraction speed, and the IIx fibers demonstrate a highly glycolytic metabolism and are the most rapidly contracting fibers (21). This study is the first to report the distribution of PGC-1 and PPAR- α proteins in human muscle fiber types. PGC-1 protein levels were the greatest in the type IIa fibers, lower in the type I fibers, and the lowest in the type IIx fibers. As type I fibers have the greatest mitochondrial content, it could have been expected that the content of PGC-1, a coactivator of mitochondria biogenesis, would be the highest in these fibers. It has been suggested that the responsiveness to stimuli of muscle fibers to alter their phenotype would follow as such, type I \leftrightarrow type IIa \leftrightarrow type IIx (32). However, in light of the ability of PGC-1 to induce mitochondrial biogenesis (4), the greater PGC-1 content in the type IIa

fibers observed in the present study may entail part of the mechanism, that when properly activated, promotes mitochondrial biogenesis and the switch of muscles to a type I phenotype. This notion would be in agreement with the findings of Howald et al. (33), who observed, after 6 weeks of endurance training in humans, an increase in mitochondrial density in all three fiber types, with the greatest increase occurring in the type IIa fibers, as compared with the type I and IIx muscle fibers. Our observation of an increase in the gene expression of the mitochondrial marker COX4 supports previous observations made after endurance training (34,35) and suggests that the increase in PGC-1 in the present study resulted in an increased mitochondrial biogenesis. In addition, the high levels of PGC-1 in the type IIa fibers may be indicative that PGC-1 functions in other aspects of muscle metabolism. PGC-1 overexpression in C₂C₁₂ myoblasts increases both basal and insulin-stimulated glucose uptake (5) and has been shown to regulate gene expression of GLUT4, a glucose transporter (5,15). Because obesity and diabetes are associated with a decrease in the population of muscle type I fibers and this change of phenotype has been postulated to play a role in obesity/diabetes-related insulin resistance (36), targeting PGC-1 in obese and diabetic patients may increase their type I fiber phenotype and GLUT4 levels, with a concomitant improvement in insulin sensitivity. Indeed, endurance training has previously been shown to increase the mRNA levels of GLUT4 in both healthy (35,37) and diabetic patients (37), an observation supported by the 59% increase in GLUT4 mRNA in the present study.

PPAR- α content was greater in the type I as compared with the IIa and IIx fibers. Because PGC-1 acts as a coactivator of PPAR- α , the simultaneous increase in PGC-1 and PPAR- α levels in type I fibers would be expected to lead to a robust increase in PPAR- α activity per se. As PPAR- α is associated with the control of genes involved in fatty acid metabolism, this would be, among others, a mechanism contributing to the greater fatty acid oxidative capacity of these fibers (38). In addition, the increase in PPAR- α content in all fiber types would play a role in the improved oxidative capacity after endurance training (39). An increase in PPAR- α activity has been shown to improve muscle insulin responsiveness (40). Therefore, our observed increase in PPAR- α in response to endurance training may be another factor contributing to the improved skeletal muscle insulin sensitivity observed after endurance training (41,42).

In summary, the present results show that in human skeletal muscle, endurance training induces an increase in both PGC-1 and PPAR- α mRNA as well as in PGC-1 and PPAR- α protein contents in the type I, IIa, and IIx fiber types. PGC-1 is expressed the most in type IIa as compared with type I and IIx fibers, whereas PPAR- α is expressed the most in type I as compared with type IIa and IIx fibers. The fiber type differences in PGC-1 and PPAR- α remain after endurance training. The increase in PGC-1 was also associated with a shift toward an increased type I muscle fiber phenotype, which supports the role, previously observed in transgenic mice, that PGC-1 acts as a coactivator of oxidative type I muscle fibers in humans. In addition, endurance training increased the gene expres-

sion of target genes of PGC-1, including troponin-1, COX4, and GLUT4, and of PPAR- α , such as CPT-1. Combined, our results suggest that the increases in muscle mitochondria content, type I muscle fiber phenotype, oxidative capacity, and insulin sensitivity, induced by endurance training, might be mediated by increases in PGC-1 and PPAR- α and their nuclear encoded target genes.

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