PGC-1β is downregulated by training in human skeletal muscle: no effect of training twice every second day vs. once daily on expression of the PGC-1 family

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PGC-1β is downregulated by training in human skeletal muscle: no effect of training twice every second day vs. once daily on expression of the PGC-1 family. J Appl Physiol 103: 1536–1542, 2007. First published August 9, 2007; doi:10.1152/japplphysiol.00575.2007.—We hypothesized that the peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) family of transcriptional coactivators (PGC-1α, PGC-1β, and PRC) is differentially regulated by training once daily vs. training twice daily every second day and that this difference might be observed in the acute response to endurance exercise. Furthermore, we hypothesized that expression levels of the PGC-1 family differ with muscular fiber-type composition. Thus, before and after 10 wk of knee extensor endurance training, training one leg once daily and the other leg twice daily every second day, keeping the total amount of training for the legs equal, skeletal muscle mRNA expression levels of PGC-1α, PGC-1β, and PRC were determined in young healthy men (n = 7) in response to 3 h of acute exercise. No significant difference was found between the two legs, suggesting that regulation of the PGC-1 family is independent of training protocol. Training decreased PGC-1β in both legs, whereas PGC-1α was increased, but not significantly, in the leg training once daily. PRC did not change with training. Both PGC-1α and PRC were increased by acute exercise both before and after endurance training, whereas PGC-1β did not change. The mRNA levels of the PGC-1 family were examined in different types of human skeletal muscle (triceps, soleus, and vastus lateralis; n = 7). Only the expression level of PGC-1β differed and correlated inversely with percentage of type I fibers. In conclusion, there was no difference between training protocols on the acute exercise and training response of the PGC-1 family. However, training caused a decrease in PGC-1β mRNA levels.

peroxisome proliferator-activated receptor-γ coactivator-1; peroxisome proliferator-activated receptor-γ coactivator-1β, peroxisome proliferator-activated receptor-γ coactivator-1-related coactivator; adaptation; glycogen

REGULAR EXERCISE HAS BEEN shown to be successful as a treatment for a variety of disorders and diseases, including Type 2 diabetes (26). The exact molecular pathways responsible for these effects have, however, remained elusive. Our laboratory has previously shown a difference in the outcome of endurance training employing different training protocols on separate legs, because an improved adaptation to training occurred in the leg training twice daily every second day vs. the leg training once daily (12).

The peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1) family of transcriptional coactivators comprises PGC-1α, PGC-1β, and PGC-1-related coactivator (PRC) (4, 20, 29). They have recently emerged as important players in the regulation of cellular and systemic metabolism, with emphasis on mitochondrial oxidative metabolism and maintenance of glucose, lipid, and energy homeostasis (8, 19). Both PGC-1α and PRC have previously been shown to be upregulated in skeletal muscle by acute exercise (6, 27, 31, 35), whereas PGC-1β was unaffected by acute exercise (23). PGC-1α has furthermore been shown to be upregulated in skeletal muscle by long-term endurance training (11, 16, 30, 32, 34) and downregulated by inactivity (37) or denervation (14), although its upregulation by training is not consistent in all cases (27, 38), which may be due to differences in training protocols and biopsy sample timings. The involvement of the PGC-1 family in regulation of skeletal muscle metabolism and muscle type composition has furthermore been corroborated by overexpression of PGC-1α in transgenic mice (21), as well as in cell culture studies (24), where overexpression of PGC-1α induced a phenotype shifted toward an oxidative fiber type.

Recently, it was shown that overexpression of PGC-1β in skeletal muscle cell culture selectively induced the expression of myosin heavy chain IIA (MHC IIA) mRNA compared with PGC-1α overexpression (24), showing that more than just one member of the PGC-1 family is involved in skeletal muscle fiber-type control and thus possibly training adaptation. The possible involvement of all the PGC-1 family members in skeletal muscle fiber-type control is also evidenced by the observations that the PGC-1 family is expressed at different levels in different skeletal muscle fibers in rodents, with PGC-1α being more highly expressed in skeletal muscle consisting mainly of type 1 fibers (14, 21) and PGC-1β being expressed more highly in skeletal muscle consisting mainly of type 2 fibers (14). Loss of PGC-1α further implicates PGC-1α in skeletal muscle adaptation as PGC-1α knockout mice exhibit impaired skeletal muscle strength, reduced exercise capacity, and decreased fatigue resistance (18). PGC-1α has been implicated in control of pyruvate dehydrogenase kinase 4, suggesting a direct link between levels of PGC-1α and glyco-
gen (5, 22, 40) and overexpression of PGC-1α has been shown to increase the levels of glycogen in skeletal muscle cell culture (24). It has, however, not been examined whether glycogen levels affect the expression of the PGC-1 family, but several reports suggest a link between substrate availability and PGC-1α expression, mediated by AMP-activated protein kinase (AMPK), because AMPK activation has been shown to be associated with an increase in PGC-1α expression (17, 33). AMPK has, however, been shown to be dispensable for exercise induction of PGC-1α (13). All members of the PGC-1 family have been shown to upregulate mitochondrial biogenesis as well as to regulate a large variety of mitochondria-specific genes (10).

Our laboratory has previously reported on a study where subjects performed one-legged or two-legged knee extensor endurance training for 10 wk following a protocol where one leg trained twice daily every second day and the other trained once daily (12); thus training one leg markedly more in a low-glycogen state than the other, with both legs receiving the same total amount of training over the 10-wk study period. This resulted in a marked similar increase in maximal power output (P_max) in both legs, whereas the leg training twice daily every second day ended up with twice as long a time to exhaustion (T_exh) compared with the leg training once daily (12). Furthermore, there was a similar increase in 3-hydroxyacyl-CoA dehydrogenase (HAD) enzyme activity in the two legs, whereas citrate synthase (CS) increased markedly more in the leg training twice daily every second day, suggesting that training twice daily every second day induced a more oxidative skeletal muscle phenotype.

The rationale for this study is that the superior increase in oxidative capacity and T_exh seen when training twice daily every second day compared with training once daily, might be explained by differences in factors known to be majorly involved in the control of skeletal muscle oxidative metabolism and training adaptation. Thus we hypothesized that changes in the expression levels of the PGC-1 family of transcriptional coactivators might be the underlying cause of the difference seen between the two differentially trained legs and that the difference in training protocol would differentially regulate the PGC-1 family of transcriptional coactivators. We hypothesized that this effect might be apparent in the acute response to exercise of legs trained either once daily or twice daily every second day. Furthermore, we hypothesized that muscles with different fiber-type compositions differ in their expression levels of these coactivators.

**MATERIALS AND METHODS**

**Study 1: training study.** Seven healthy untrained young men [age 26 ± 1 yr (mean ± SE), body mass 84 ± 3 kg, and BMI 24.7 ± 0.1 kg/m²] were recruited to the study. All volunteers underwent a medical examination and a standard set of blood tests. Purpose and possible risks of the study were explained to the participants before written consents were obtained. The study protocol was approved by the local Ethical Committee of Copenhagen and Frederiksberg Communities and was performed in accordance with the Declaration of Helsinki. The study was carried out as previously described (28). Briefly, the subjects were instructed not to perform any vigorous exercise 24 h before the experiment and to report to the laboratory after an overnight fast. Biopsies were obtained from three different muscle groups: triceps brachii caput medialis (triceps), triceps surae pars soleus (soleus), and quadriceps pars vastus lateralis (vastus) as described below. The biopsies used were the same as in a previously published study (12).

**Study 2: fiber-type study.** Seven healthy untrained young men [age 26 ± 1 yr (mean ± SE), body mass 84 ± 3 kg, and BMI 24.7 ± 0.1 kg/m²] were recruited to the study. All volunteers underwent a medical examination and a standard set of blood tests. Purpose and possible risks of the study were explained to the participants before written consents were obtained. The study protocol was approved by the local Ethical Committee of Copenhagen and Frederiksberg Communities and was performed in accordance with the Declaration of Helsinki. The study was carried out as previously described (28). Briefly, the subjects were instructed not to perform any vigorous exercise 24 h before the experiment and to report to the laboratory after an overnight fast. Biopsies were obtained from three different muscle groups: triceps brachii caput medialis (triceps), triceps surae pars soleus (soleus), and quadriceps pars vastus lateralis (vastus) as described below. The biopsies used were the same as in a previously published study (28).**
were at minimum 3 cm apart. Visible connective tissue and blood contamination were removed before the biopsies were frozen in liquid nitrogen and subsequently stored at −80°C until further analysis. The biopsies used were the same as in previously published studies (12, 28).

**Real-time RT-PCR.** Total RNA was extracted from the muscle tissue with the use of TRIzol according to the manufacturer’s instructions (Invitrogen, Grand Island, NY). The resulting RNA pellet was dissolved in diethylpyrocarbonate-treated water. Reverse transcription (RT) reactions were performed using random hexamers on 2 μg RNA using an RT kit (Applied Biosystems, Foster City, CA) in a reaction volume of 100 μl. The resulting cDNA product was stored at −20°C until further analysis. Primers and probes for PGC-1α were designed (Primer Express version 1.0, Applied Biosystems) from the gene sequences for human PGC-1α as described previously (27); forward primer 5′-CAAGGCTAAACCAACACTTATCTCT-3′, reverse primer 5′-CACACTTAAGGTGCGTCAAATAGTC-3′, and TaqMan fluorescent probe 5′-FAM-TGTCACCAATGACCCCAAGGG-3′ for PGC-1α. PGC-1β, PRC, β-actin, and 18S rRNA were amplified using predeveloped assay reagents (Applied Biosystems). The mRNA levels of PGC-1α, PGC-1β, and PRC as well as the mRNA levels of the endogenous controls, 18S rRNA and β-actin, were determined using real-time RT-PCR using an ABI PRISM 7900 sequence detector (Applied Biosystems). 18S rRNA was used to normalize mRNA expression values in the training study, and β-actin was used to normalize mRNA expression values in the fiber-type study. Comparable results were obtained if values in the training study were normalized to GAPDH instead of 18S (data not shown).

**Statistics.** Data were log transformed to obtain normal distribution. Accordingly, results are presented as geometric means ± SE. All statistics were performed on log-transformed values using SAS 9.1.2 (SAS Institute). The training study was analyzed using mixed-model statistics to detect the effect of training protocol after which the study was split up, and, using mixed model statistics, each training protocol was examined individually with regard to training and time, followed by post hoc t-tests with Bonferroni correction to identify differences between groups at specific time points. A one-way ANOVA was used to detect differences between the three muscle groups in the fiber type study, followed by post hoc t-test with Bonferroni correction. Correlation analyses were performed using Pearson correlation analysis. A P value <0.05 was considered significant.

**RESULTS**

**Effect of training on the acute exercise response of the PGC-1 family mRNA levels.** The effect of training and training protocol on the acute exercise response of the PGC-1 family was assessed by measuring the mRNA levels before, directly after, or 2 h after 3 h of dynamic knee extensor exercise at the same relative intensity before and after training (Fig. 1). Overall there was no difference between training once daily vs. training twice daily every second day in terms of the response of the PGC-1 family to acute exercise. Nor was there a difference between the resting levels of the PGC-1 family in the two legs.

The resting levels of PGC-1α mRNA increased by ~50% with training but only significantly so when training twice daily every second day. Surprisingly, the resting levels of PGC-1β mRNA significantly decreased by ~35% regardless of training protocol. Training had no effect on the resting mRNA levels of PRC.

PGC-1α mRNA levels increased in response to acute exercise, showing a maximal increase of ~10-fold after training and 5-fold before training. The exercise-induced increase in PGC-1α was more pronounced 2 h postexercise after training than before training, when training twice daily every second day (P = 0.043) but not when training once daily (P = 0.074). Acute exercise had no effect on PGC-1β mRNA levels, whereas PRC mRNA levels increased following acute exercise without effect of training.

**PGC-1 family mRNA levels in different skeletal muscle types.** The expression levels of PGC-1α, PGC-1β, and PRC were also determined in three different skeletal muscle types, triceps, soleus, and vastus, in young healthy men (Fig. 2). Soleus consisted of 68–83% (range) type I fibers, vastus lateralis of 40–56% type I fibers, and triceps of 20–33% type I fibers (28). Only PGC-1β was differentially expressed in human skeletal muscle, with triceps having approximately twice the mRNA expression level compared with vastus and soleus. Accordingly, we found a highly significant negative correlation between PGC-1β mRNA levels and percentage of type I fibers (P = 0.0002), whereas there was no correlation between PRC and percent type I fibers (28). As previously reported, we did not find any correlation between PGC-1α and percent type I fibers (28).

**DISCUSSION**

The present study demonstrated that the muscular expression of all members of the PGC-1 family is regulated either by acute exercise or by training. The main novel findings of this study were that 1) Training once daily vs. training twice daily every second day makes no difference in terms of the effect caused by endurance training on the mRNA expression of the PGC-1 family of transcriptional coactivators, 2) PGC-1β mRNA in skeletal muscle is downregulated by endurance training, and 3) PGC-1β mRNA is preferentially expressed in triceps and correlates negatively with percentage of type I fibers.

**Exercise and the PGC-1 family of transcriptional coactivators.** The increase of PGC-1α in skeletal muscle by acute exercise was in accordance with previous studies in humans (25, 27, 31, 39), rats (6, 9, 14, 36), and mice (1, 2, 13). Other studies have previously found an effect of endurance training on resting PGC-1α levels in humans (16, 30, 32) and rats (9, 11, 14, 34). However, a previous study by Pilegaard et al. (27), employing a protocol similar to the training once daily leg in this study, found no significant increase in resting PGC-1α mRNA levels following 4 wk of training. The present study suggests that PGC-1α mRNA might be more consistently increased by training when training twice a day; however, when comparing training once daily with training twice daily every second day, no significant difference was found. Generally, training adaptation is associated with an increase in muscle glycogen content, and training twice daily every second day induced a significant increase in muscle glycogen content posttraining, whereas the increase in glycogen content was not statistically significant for training once daily (12). Furthermore, overexpression of PGC-1α in skeletal muscle cell culture increases glycogen levels in vitro (24). Despite these observations, we saw no overt difference in the PGC-1α mRNA levels when training once daily vs. training twice daily every second day, which is reminiscent of the finding that there was no difference between the glycogen levels in both legs at rest following the 10 wk of training. Interestingly, only the leg that showed a statistical significant increase in PGC-1α mRNA
also showed a significant increase in glycogen content following training.

In the present study, the PGC-1α mRNA content was downregulated by endurance training, but no changes were observed at the investigated time points following acute exercise. The latter finding is in accordance with a few previous studies showing no effect of acute exercise (14, 23). In a cross-sectional study, others have found a higher level of PGC-1β in trained than in untrained or spinal cord-injured humans (15); however, the difference in expression levels might have taken years to develop. Thus it appears that the kinetics of changing PGC-1α expression by exercise in skeletal muscle is long term, which might be reminiscent of training adaptation and that perhaps the level of PGC-1β changes as training adaptation progresses.

PRC, the last PGC-1 family member, has previously been shown to be upregulated by acute endurance exercise (31). Here we show yet another pattern of regulation of a PGC-1 family member by exercise, because PRC was only regulated by acute endurance exercise and not by long-term endurance training. This finding suggests that PRC may play a role during exercise or in the recovery phase, but that its expression level does not reflect training adaptation to endurance exercise because no change in PRC mRNA levels was observed comparing pre- and posttraining levels at rest.

We previously found that as an effect of training the increase in maximal workload was identical for the two legs; however, time to exhaustion at 90% of actual maximal workload before and after 10 wk of training was much more increased when training twice daily every second day compared with training once a day. This could not be explained by a difference in glycogen content, because the time to exhaustion tests were carried out at a relative high intensity, which did not allow the volunteers to exercise for more than a maximum of 25 min, ensuring that glycogen was not a limiting factor. In light of the findings in this study, it seems that differences in the expression levels of the PGC-1 family members are not a plausible explanation for the difference in T_exh seen when training twice daily every second day compared with training once daily (12).

Both PGC-1α and PGC-1β are known to control expression of CS (24), as well as control mitochondrial β-oxidation (19). In an earlier study, our laboratory found both CS and HAD to
increase following training, with the leg training twice daily every second day having a significantly higher CS increase than the leg training once daily (12). The lack of a similar pattern in the expression levels of PGC-1α, PGC-1β, and PRC mRNA levels and type I fiber composition (right) for 7 subjects. Biopsies from the same subjects are connected, and the muscle type is marked as follows: triceps (■), vastus (●), and soleus (▲). Bold line, result of the linear regression. †P < 0.01 different from triceps. ‡P < 0.001, different from triceps.

Fig. 2. PGC-1α, PGC-1β, and PRC mRNA levels in skeletal muscle biopsies taken from either triceps brachii caput medialis (triceps), triceps surae pars soleus (soleus), and quadriceps pars vastus lateralis (vastus). Data are presented as geometric means ± SE (left) or as correlations between PGC-1α, PGC-1β, and PRC mRNA levels and type I fiber composition (right) for 7 subjects. Biopsies from the same subjects are connected, and the muscle type is marked as follows: triceps (■), vastus (●), and soleus (▲). Bold line, result of the linear regression. †P < 0.01 different from triceps. ‡P < 0.001, different from triceps.

increase following training, with the leg training twice daily every second day having a significantly higher CS increase than the leg training once daily (12). The lack of a similar pattern in the expression levels of PGC-1α and PGC-1β following the training period again suggests that changes in the expression level of the PGC-1 family are not a plausible explanation for the difference seen between the two training protocols. Unfortunately, in this study, we can not exclude the possibility that training in a low-glycogen state, e.g., the second bout of training of the leg training twice a day every second day, gives rise to a temporarily different expression pattern of the PGC-1 family members (12). However, we found no evidence of this in the resting state or in the acute exercise experiments performed after the 10 wk of training.

Skeletal muscle phenotype and PGC-1 family expression. A few studies have shown differential expression of PGC-1α mRNA with either skeletal muscle fiber type or skeletal muscle type. In humans, PGC-1α protein content is higher in type MHC IIA than in type MHC I and MHC IIX fibers (30). In rodents, huge differences in PGC-1α mRNA levels are seen between muscle types with PGC-1α mRNA levels being up to five times higher in soleus (primarily type 1 fibers) than in any other muscle types (14, 21). Our laboratory has previously shown that there is no difference in PGC-1α mRNA levels in humans between the three skeletal muscle types examined in this study (28), which we also find here.

PGC-1β has been demonstrated to have huge differences in mRNA expression levels in different rodent muscle types, with PGC-1β mRNA levels being up to four times higher in extensor digitorum longus (primarily type 2 fibers) than in soleus and gastrocnemius (14). Our finding that PGC-1β mRNA levels are higher in triceps (primarily type 2 fibers) than in both vastus (intermediate with respect to type 1 and 2 fibers) and soleus (primarily type 1 fibers) suggests that similar differences exists in human skeletal muscle.

Using 18S as the reference gene, Koves et al. (14) showed a remarkable difference in PGC-1α expression levels in different rodent muscle types, with PGC-1β mRNA levels being up to four times higher in extensor digitorum longus (primarily type 2 fibers) than in soleus and gastrocnemius (14). Our finding that PGC-1β mRNA levels are higher in triceps (primarily type 2 fibers) than in both vastus (intermediate with respect to type 1 and 2 fibers) and soleus (primarily type 1 fibers) suggests that similar differences exists in human skeletal muscle.
studies may not only rely on species difference but may also be related to the chosen reference gene.

**Conclusion.** This is the first complete assessment of the changes in mRNA levels of the PGC-1 family of transcriptional coactivators induced by either acute exercise or endurance training in humans showing a remarkably different regulation of all three family members by endurance exercise or training. Furthermore, we here show for the first time a possible involvement of PGC-1β in the adaptive response of skeletal muscle to endurance training. Finally, we showed that there was no difference between training protocols on the acute exercise and training response of the PGC-1 family and that training caused a decrease in PGC-1β mRNA levels at rest.

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