PPARγ coactivator-1α expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations

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Irrcher, Isabella, Peter J. Adhihetty, Treacey Sheehan, Anna-Maria Joseph, and David A. Hood. PPARγ coactivator-1α expression during thyroid hormone- and contractile activity-induced mitochondrial adaptations. Am J Physiol Cell Physiol 284: C1669–C1677, 2003; 10.1152/ajpcell.00409.2002.—The transcriptional coactivator the peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) has been identified as an important mediator of mitochondrial biogenesis based on its ability to interact with transcription factors that activate nuclear genes encoding mitochondrial proteins. The induction of PGC-1α protein expression under conditions that provoke mitochondrial biogenesis, such as contractile activity or thyroid hormone (T₃) treatment, is not fully characterized. Thus we related PGC-1α protein expression to cytochrome c oxidase (COX) activity in (1) tissues of varying oxidative capacities, (2) tissues from animals treated with T₃, and (3) skeletal muscle subject to contractile activity both in cell culture and in vivo. Our results demonstrate a strong positive correlation (r = 0.74; P < 0.05) between changes in PGC-1α and COX activity, used as an index of mitochondrial adaptations. The highest constitutive levels of PGC-1α were found in the heart, whereas the lowest were measured in fast-twitch white muscle and liver. T₃ increased PGC-1α content similarly in both fast- and slow-twitch muscle, as well as in the liver, but not in heart. T₃ also induced early (6 h) increases in AMP-activated protein kinase (AMPKα) activity, as well as later (5 day) increases in p38 MAP kinase activity in slow-twitch, but not in fast-twitch, muscle. Contractile activity provoked early increases in PGC-1α, coincident with increases in mitochondrial transcription factor A (Tfam), and nuclear respiratory factor-1 (NRF-1) protein expression, suggesting that PGC-1α is physiologically important in coordinating the expression of the nuclear and mitochondrial genomes. Ca²⁺ ionophore treatment of muscle cells led to an approximately threefold increase in PGC-1α protein, and contractile activity induced rapid and marked increases in both p38 MAP kinase and AMPKα activities. 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) treatment of muscle cells also led to parallel increases in AMPKα activity and PGC-1α protein levels. These data are consistent with observations that indicate that increases in PGC-1α protein are affected by Ca²⁺ signaling mechanisms, AMPKα activity, as well as posttranslational phosphorylation events that increase PGC-1α protein stability. Our data support a role for PGC-1α in the physiological regulation of mitochondrial content in a variety of tissues and suggest that increases in PGC-1α expression form part of a unifying pathway that promotes both T₃- and contractile activity-induced mitochondrial adaptations. Peroxisome proliferator-activated receptor-γ (PPARγ) coactivator-1α (PGC-1α) is a recently discovered nuclear-encoded transcriptional coactivator that plays a pivotal role in glucose metabolism, mitochondrial biogenesis, muscle fiber specialization, and adaptive thermogenesis (17, 19, 38). PGC-1α was originally cloned from a brown fat cell cDNA library and was found to transcriptionally activate PPARγ and thyroid hormone receptor-β (TRβ) on the uncoupling protein-1 (UCP-1) promoter (23). In muscle cells, forced expression of PGC-1α increased mtDNA copy number and stimulated mitochondrial proliferation, providing the first experimental evidence that PGC-1α was directly involved in upregulating organelle biogenesis (36). The process and regulation of mitochondrial biogenesis are complex, in part because it requires the cooperative induction of gene expression from two functionally independent genomes. The mechanisms involved are partly ascribed to the activation and binding of transcription factors to specific DNA sequences within the promoter regions of genes encoding mitochondrial proteins (16). A number of important transcription factors have been identified, including mitochondrial transcription factor A (Tfam), the nuclear respiratory factor-1 and -2 (NRF-1 and NRF-2), PPARα and PPARγ, and specificity protein 1 (Sp1; Refs. 16 and 25). In response to mitochondrial biogenesis inducing-stimuli, mRNAs encoding these proteins change before, or coincident with, increases in the expression of their target genes, consistent with their function in mediating the characteristic adaptations (12, 37). However, the collaborative effort that is required between these regulatory proteins and the diversity that exists be-
between the promoter regions of nuclear genes encoding mitochondrial proteins (20) calls for the presence of unifying regulators of mitochondrial biogenesis. The preponderance of evidence to date implicates PGC-1α as the most important of these.

Stimuli such as endurance exercise training and thyroid hormone treatment have been shown to induce mitochondrial biogenesis (8, 14, 34). The increased mitochondrial content that is brought about by endurance training attenuates muscle fatigue during submaximal exercise, leading to an improved work capacity (16). Defining the underlying cellular mechanisms resulting in this adaptive response requires an understanding of the initial signaling events involved, as well as their downstream targets that activate the transcription of nuclear and mitochondrial genes (16). Although PGC-1α is a vital component of this process, only one study to date has investigated the regulation of PGC-1α protein expression in response to an exercise stimulus (3). Increases in PGC-1α mRNA as a result of exercise have also recently been reported (13, 30, 31), but it is established that the relationship between PGC-1α mRNA and protein expression is complex in skeletal muscle, because the final protein level is subject to posttranslational modifications that affect its stability (22). Thus we investigated whether contractile activity could modify PGC-1α protein expression, as well as some of the potential signaling mechanisms involved. In addition, PGC-1α is a strong coactivator of TRβ (23), and this is likely to be important in mediating some of the effects of thyroid hormone (T₃) on mitochondrial biogenesis. There is some evidence that T₃ can induce the mRNA expression of PGC-1α in liver (32), suggesting that PGC-1α may autoregulate its own expression via TRβ. Thus we also wished to investigate the role of T₃ in mediating PGC-1α protein expression, the potential tissue specificity of this response, as well as some possible common signaling mechanisms mediating both T₃- and contractile activity-induced mitochondrial biogenesis. Here, we report that both contractile activity and T₃ treatment induce PGC-1α protein expression, which occurs coincident with increases in transcription factor expression, and oxidative capacity in a variety of tissues.

METHODS

Materials. Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin (PS), 3,3′,5-triiodothyronine (T₃), A-23187, 5-aminoimidazole-4-carboxamide-1-β-D-ribonucleoside (AICAR), and cytochrome c from horse heart were purchased from Sigma (Oakville, ON, Canada). Horse serum (HS) was purchased from Invitrogen (Burlington, ON, Canada). The PGC-1α antibody was obtained from Calbiochem (La Jolla, CA) and the phospho-p38 and p38 MAP kinase, as well as the phospho-AMP-activated protein kinase (AMPKα) and AMPKα antibodies, were from New England Biolabs (Mississauga, ON, Canada). The AMPK antibody reacts with both the α1- and α2-isofoms of AMPK. The NRF-1 antibody was a kind gift from Dr. R. C. Scarpulla. The Tfm and cytochrome c antibodies have been previously described (12, 28). Nitrocellulose membrane (Hybond N) and the chemiluminescence kits for Western blot analyses were obtained from Amersham Pharmacia Biotech (Baie D’Urfé, QC, Canada).

In vivo contractile activity. In vivo stimulation was performed as previously described (29). Briefly, male Sprague-Dawley rats were anesthetized with pentobarbital sodium (60 mg/kg body wt), and electrodes were sutured unilaterally beside the common peroneal nerve. Electrode wires were passed subcutaneously from the thigh to the base of the neck, where they were attached to an external stimulator unit secured to the back of the animal. After a 1-wk recovery period, the tibialis anterior (TA) and the extensor digitorum longus (EDL) muscles were stimulated (10 Hz, 0.1 ms duration) 3 h/day for 3, 5, 7, and 10 days. The contralateral limb was used as a nonstimulated control muscle for all animals (n = 4–8/day).

T₃ treatment and tissue extraction. Male Sprague-Dawley rats were injected intraperitoneally with either T₃ (0.4 mg/kg) or vehicle (0.9% NaCl-propylene glycol: 40:60 vol/vol) for 5 consecutive days, as done previously (7, 26). A subgroup of animals (n = 3) was injected with one dose, and the tissues were removed 6 h later. Twenty-four hours after the fifth T₃ treatment, or after the 10 Hz stimulation time point, animals were anesthetized with pentobarbital sodium (40 mg/kg), and selected hindlimb muscles and tissues were removed, quick frozen, and stored in liquid N₂. Total protein extracts from frozen tissue powders were made (29) for subsequent protein and enzyme activity measurements.

Cell culture and contractile activity in muscle cells. Cell culture of rat L₆E₉ muscle cells and electrical stimulation of murine C₂C₁₂ skeletal muscle cells were done as previously described (5, 11). Briefly, cells were propagated in DMEM supplemented with 10% FBS and 1% PS and then differentiated in DMEM supplemented with 5% heat-inactivated HS and 1% PS. Stimulation of C₂C₁₂ skeletal muscle cells was performed successively for 2 and 4 days (3 h/day; 5 Hz, 55 V) with an intermittent 21-h period of quiescence. Total protein extracts were prepared 21 h after the last stimulation period (5). In the experiment to measure p38 MAP kinase and AMPKα activation, cells were stimulated for 3 h only, and extracts were made immediately following. L₆E₉ muscle cells were treated continuously with A-23187 (1 μM) or AICAR (1 mM) for 48 h. Total protein extracts were prepared immediately following.

Western blotting. Total protein extracts from cultured cells or tissues were electrophoresed through SDS-polyacrylamide gels and electrophoblotted onto nitrocellulose membranes. Blots were blocked (1 h) with 5% milk in ×1 TBST (Tris-buffered saline/Tween-20; 25 mM Tris·HCl, pH 7.5, 1 mM NaCl, and 0.1% Tween-20), followed by overnight incubation with antibodies diluted in blocking buffer directed toward PGC-1α (1:1,000), Tfm (1:1,000), NRF-1 (1:500), cytochrome c (1:750), phospho-p38 (1:400), p38 MAP kinase (1:400 diluted in 5% BSA/TBST), phospho-AMPKα (1:400 diluted in 5% BSA/TBST), or AMPKα (1:1,000 diluted in 5% BSA/TBST). After 3 × 5 min washes with TBST, blots were incubated at room temperature (1 h) with the appropriate secondary antibody conjugated to horseradish peroxidase. Blots were then washed again 3 × 5 min with TBST, visualized with enhanced chemiluminescence, and quantified using SigmaGel (Jandel, San Rafael, CA). To compare PGC-1α levels among tissues, a standard heart sample was run on each gel and all tissue values were then compared with the heart sample that was assigned a value of 1.

Cytochrome c oxidase activity. Cytochrome c oxidase (COX) activity was measured as previously described (12). The enzyme activity was determined as the maximal rate of oxidation of fully reduced cytochrome c, measured by the
change in absorbance at 550 nm on a Beckman DU-64 spectrophotometer.

**Statistics.** Experiments using C2C12 or L6E9 muscle cells were analyzed using a one-way ANOVA, followed by Tukey's post hoc analysis, to determine individual differences. Paired Student's *t*-tests were used for comparison of data obtained for the stimulated and contralateral control muscles, whereas unpaired *t*-tests were done to compare T3- and vehicle-treated animals. All data are expressed as means ± SE, and differences were considered significant if *P* < 0.05.

**RESULTS**

**Protein expression during contractile activity of muscle cells in culture.** Stimulation of C2C12 cells in culture for 2 and 4 days resulted in a pattern of protein expression that is consistent with the early adaptive responses (i.e., activation of signal transduction pathways and upregulation of transcription factor expression) within skeletal muscle that precede mitochondrial biogenesis. Although no change in cytochrome c protein expression was evident over 2 and 4 days, increases in NRF-1, Tfam, and PGC-1α protein expression occurred progressively, reaching values that were 1.5-, 1.4-, and 1.8-fold above those in nonstimulated cells, respectively (*P* < 0.05; Fig. 1, A and B). We also evaluated the activation of the p38 MAP kinase and AMPKα pathways, which have been implicated in PGC-1α protein expression (22, 39). Three hours of contractile activity led to 3.0- and 2.6-fold increases in p38 MAP kinase and AMPKα activities, respectively (*P* < 0.05; Fig. 1C).

**Contractile activity in vivo stimulates PGC-1α protein expression.** Chronic contractile activity in vivo results in an increase in mitochondrial content and cellular respiration (14, 16). Thus we investigated whether PGC-1α protein expression could be induced by chronic contractile activity and whether these changes occurred coincident with increases in COX activity, a typical marker of mitochondrial biogenesis. Contractile activity increased PGC-1α protein expression by 1.5-fold (*P* < 0.05) by 5 days of stimulation (Fig. 2, A and B). This increase was maintained at 7 days (*P* < 0.05) and was 1.3-fold higher (*P* < 0.05) than control values at 10 days of stimulation. The changes in PGC-1α protein expression occurred coincident with significant increases (*P* < 0.05) in COX activity between 5 and 10 days of stimulation (Fig. 2C). To fortify a potential role for Ca2+ (35) in mediating the contractile activity-induced increase in PGC-1α protein, we treated rat L6E9 muscle cells with the Ca2+ ionophore A-23187, as done previously (11). Consistent with this possibility, A-23187 treatment resulted in an approximate 3.0-fold increase (*P* < 0.05) in PGC-1α protein expression (Fig. 2D).

**AICAR-induced AMPKα activation mimicks the contractile activity-induced PGC-1α protein expression.** To evaluate whether AMPKα activation is involved in PGC-1α protein expression, we treated rat L6E9 muscle cells for 48 h with AICAR, an established AMPK activator (6). Coincident 2.6- and 2.5-fold increases (*P* < 0.05) in PGC-1α protein expression and AMPKα kinase activation occurred in cells treated with AICAR for 48 h. There was no effect of AICAR on p38 MAPK activity (Fig. 3, A and B).

**PGC-1α protein expression is tissue specific.** The expression of PGC-1α was measured under steady-state conditions in six different tissues possessing a wide
range of oxidative capacities. The highest constitutive levels of PGC-1α protein were found in the heart (Fig. 4A). The slow-twitch red soleus muscle had a PGC-1α content that was ~60% of that found in heart (Fig. 4A). Intermediate PGC-1α levels were measured in the fast-twitch muscles plantaris and red gastrocnemius, whereas the lowest PGC-1α content was found in the liver and in the fast-twitch white section of gastrocnemius (Fig. 4A).

T₃ treatment increases PGC-1α protein expression. To investigate a role for PGC-1α in T₃-induced mitochondrial adaptations, we assessed changes in PGC-1α protein expression in animals treated for 5 days with either T₃ or vehicle in both slow-twitch and fast-twitch muscle types, as well as in tissues of varying metabolic activities (i.e., heart and liver). T₃-treatment did not
increase PGC-1α protein expression in the heart (Fig. 4, B and C) but induced approximate 1.7-, 1.5-, and 1.3-fold changes (P < 0.05) in PGC-1α protein expression in the soleus, plantaris, and liver, respectively. Changes in COX activity were also assessed in these tissues as an index of mitochondrial adaptations. Figure 4D shows that T₃ significantly increased (P < 0.05) COX activity by 1.3- to 1.8-fold in all tissues examined.

**T₃ treatment activates AMPKα in a time-dependent and tissue-specific manner.** Because PGC-1α protein expression can be induced by both contractile activity and by T₃ treatment, we investigated the possibility that a common link between these stimuli could be mediated by AMPKα. T₃ treatment for 6 h induced a 5.4-fold increase (P < 0.05) in AMPKα activity in slow-twitch, but not fast-twitch, muscle (Fig. 5A). This effect of T₃ on AMPKα was an early, transient response because the activation was no longer observed after 5 days of T₃ treatment (Fig. 5B).

**The relationship between PGC-1α protein expression and COX activity.** Given the existence of tissue-specific differences in PGC-1α protein levels, we wanted to establish the overall relationship between PGC-1α expression and COX activity, used as an indicator of mitochondrial content. We examined this relationship under steady-state conditions in a variety of tissues (Fig. 6A), as well as under conditions in which COX activity was induced by T₃ (Fig. 6B) and contractile activity in vivo (Fig. 6C). T₃- and contractile activity-evoked changes in PGC-1α protein expression were largely matched by parallel changes in COX activity. A parallelism between COX activity and PGC-1α was also evident among different tissues, with the exception of the slow-twitch soleus muscle and the liver, in which deviations from the line of identity are evident (Fig. 6A). When the 71 pairs of observations from Fig. 6A–C, were combined, a strong, positive correlation of 0.74 was found (P < 0.05), indicating that over 50% of the variance in mitochondrial content, as reflected by COX activity, can be attributed to variations in PGC-1α protein level.

**DISCUSSION**

The transcriptional coactivator PGC-1α has recently emerged as an important regulator of adaptive thermogenesis in adipocytes (23), gluconeogenic capacity in hepatocytes (38), and muscle fiber specialization in skeletal muscle cells (19). Forced overexpression of PGC-1α in cell culture and in vivo using transgenic animals resulted in large increases in mitochondrial content, cellular respiration, and transcription factor gene expression in both heart and skeletal muscle (18, 36). Based on these studies, we hypothesized that conditions that induce mitochondrial biogenesis, such as chronic contractile activity and T₃ treatment, could regulate PGC-1α protein expression and that this would play a pivotal role in influencing mitochondrial content. In this study, we report that PGC-1α protein expression can be modified by both chronic contractile activity and T₃ treatment and that the regulation of PGC-1α protein expression in response to these stimuli occurs before, or coincident with, increases in COX activity, which we used as an index of mitochondrial content because of its requirement for gene products from both the nuclear and mitochondrial genomes.

We show here that 2 and 4 days of contractile activity in cell culture induced time-dependent, coincident elevations in Tfam, NRF-1, and PGC-1α protein expression (Fig. 1). These changes represent a highly favorable environment for the onset of mitochondrial biogenesis in muscle cells because PGC-1α strongly coactivates NRF-1, which transcriptionally activates the promoters of a wide range of nuclear genes encoding mitochondrial proteins, including the important mtDNA transcription factor, Tfam (25). The parallel
increases in Tfam protein ensure the coordinate induction of mtDNA transcription and replication, leading subsequently to the enhanced expression of the 13 mitochondrial proteins that are vital for respiratory chain function. In support of this, we have observed that mtDNA content is increased approximately two-fold by 4 days of contractile activity in this cell culture model (Joseph and Hood, unpublished observations). Cytochrome c, a nuclear-encoded component of the respiratory chain, typically requires a greater length of time for its induction, compared with early responding transcription factors such as NRF-1 and Tfam, during chronic contractile activity in vivo (10, 12) and also during contractile activity in cell culture (5, 37). Our previous work has shown that cytochrome c transcriptional activation and mtDNA content are elevated by 4 days of stimulation in cell culture (5) but that this change is not yet reflected in detectable increases at the protein level at that time (Fig. 1, A and B).

To assess a possible role for PGC-1α in contractile activity-induced increases in mitochondrial content in vivo, we next investigated whether the changes in PGC-1α protein expression could be induced at an early stage of mitochondrial adaptations produced via electrical stimulation of the peroneal nerve for 3, 5, 7, and 10 days. Five days of chronic contractile activity was sufficient to induce PGC-1α protein expression. This increase occurs coincident with the change in Tfam protein expression that occurs between 5 and 7 days of stimulation, which we have previously shown using this experimental model (12). Moreover, we also show that the changes in PGC-1α protein expression parallel increases in COX activity (Figs. 2 and 6). These data indicate that chronic contractile activity in vivo induces the coordinated expression of transcription factors and the transcriptional coactivator PGC-1α, which are important in mediating the parallel responses of the nuclear and mitochondrial genomes required for organelle biogenesis within mammalian skeletal muscle.

To confirm a broader role for PGC-1α in regulating steady-state mitochondrial content, we compared the levels of PGC-1α protein and COX activity among a variety of untreated tissues, as done previously (15). Analyses of the data from the heart, white gastrocnemius, plantaris, and red gastrocnemius muscles revealed a close parallelism between PGC-1α protein content and COX activity (Fig. 6A). However, exceptions to this relationship were observed in the liver and in the slow-twitch soleus muscle. Assuming that COX activity accurately represents mitochondrial content in these tissues, the data obtained from liver suggest that PGC-1α activation via posttranslational modifications, rather than the absolute amount of PGC-1α protein, is more important in determining the steady-state mitochondrial content in this tissue. In contrast, because the elevated level of PGC-1α is not matched by an equivalent COX activity in the soleus muscle, these data suggest the possibility that a high level of PGC-1α repressor activity (17) exists in this tissue type.

To some degree, our data at the protein level mirror the relative tissue differences in PGC-1α mRNA expression, which have been reported previously. In general, PGC-1α mRNA has been reported to be highest in heart, followed by muscle and liver (2, 9, 23). In addition, slow-twitch myofibers possess a greater PGC-1α mRNA content compared with fast-twitch fibers (19), a profile that is reflected at the protein level (Fig. 4A; 19). The parallelism between PGC-1α protein and mRNA among a variety of tissues suggests that PGC-1α expression is regulated, at least in part, at the transcriptional level. However, recent evidence within some
protein can be induced in the absence of apparent increases at the mRNA level. This occurs by increasing PGC-1α protein stability via p38 MAP kinase-mediated Ser/Thr phosphorylation (22). Whether transcriptional or posttranslational mechanisms may be involved in determining the increase in PGC-1α protein as a result of chronic contractile activity, or T3 treatment, remains to be determined. However, it is established that contractile activity can lead to p38 activation (4, 24). In our muscle cell contraction model, p38 MAP kinase was dramatically activated during a single, 3-h bout of contractile activity (Fig. 1C). Because PGC-1α is a direct downstream target of p38 (22), the resulting phosphorylation and protein stabilization could be important in mediating the increase in PGC-1α protein observed here.

Contractile activity also activates other signaling cascades, including AMPK and Ca^{2+}-regulated kinases and phosphatases (16, 24). Evidence that AMPK is involved in regulating PGC-1α expression was recently demonstrated in mice expressing a muscle-specific, dominant-negative mutant of AMPK (39). Activation of AMPK was shown to be a required signaling event leading to an increase PGC-1α mRNA, as well as calcium/calmodulin-dependent protein kinase IV (CaMKIV) protein expression. Our results here demonstrate that activation of AMPK also invokes changes in PGC-1α at the protein level. Taken together, these data suggest that activation of the p38 MAP kinase and AMPK pathways may serve distinct roles in mediating the contractile activity-induced increase in PGC-1α protein expression.

Recent work has shown that transgenic animals overexpressing a constitutively active form of CaMKIV displayed an increase in PGC-1α transcriptional activation (35). Because Ca^{2+} is a known activator of CaMK in vivo, and cytosolic Ca^{2+} increases during muscle contraction, it is reasonable to hypothesize (19) that Ca^{2+}-mediated signaling may be initially responsible for transcriptional activation leading to the increase in PGC-1α expression during contractile activity, as demonstrated here. Our observations using the Ca^{2+} ionophore A-23187 further support this. Because it appears that CaMKIV is a downstream target of AMPK (39), it is possible that contraction-induced signaling events mediated by Ca^{2+} and AMPK could converge on CaMK to regulate final PGC-1α protein content.

Another mechanism by which PGC-1α is reported to coactivate transcription is via interaction with the TRβ isofrom, an effect that is ligand dependent (23). This may be an important way in which T3 exerts its effects on mitochondrial content within a variety of tissues. Although de novo PGC-1α protein synthesis is not required for this process, we hypothesized that an induction of PGC-1α could serve to amplify this transcriptional effect, leading to more pronounced mitochondrial biogenesis in response to T3. The induction in PGC-1α expression by T3 was expected on the basis of the recent finding that 6 h of T3 treatment induced increases in PGC-1α mRNA expression (32). Although

tissues (e.g., muscle cells) indicates that posttranslational mechanisms are also involved in regulating the final PGC-1α protein content. For example, in response to a hypermetabolic stimulus, increases in PGC-1α protein can be induced in the absence of apparent increases at the mRNA level. This occurs by increasing PGC-1α protein stability via p38 MAP kinase-mediated Ser/Thr phosphorylation (22). Whether transcriptional or posttranslational mechanisms may be involved in determining the increase in PGC-1α protein as a result of chronic contractile activity, or T3 treatment, remains to be determined. However, it is established that contractile activity can lead to p38 activation (4, 24). In our muscle cell contraction model, p38 MAP kinase was dramatically activated during a single, 3-h bout of contractile activity (Fig. 1C). Because PGC-1α is a direct downstream target of p38 (22), the resulting phosphorylation and protein stabilization could be important in mediating the increase in PGC-1α protein observed here.

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we failed to observe an increase in PGC-1α protein expression after 6 h of T₃ treatment (Sheehan and Hood, unpublished observations), significant increases in PGC-1α protein occurred by 5 days in a variety of tissues, with similar increases observed in the slow-twitch soleus, as well as in the fast-twitch plantaris muscles (Fig. 4C). Given the approximate 50% higher endogenous levels of PGC-1α (Figs. 4A and 6), as well as greater levels of thyroid hormone receptor expression in slow-twitch muscle fibers (27, 33), the transcriptional effect of T₃ and the resulting mitochondrial adaptation are expected to be more pronounced in slow-twitch muscle. This provides a potential explanation for the differential effect of T₃ on muscle type-specific mitochondrial adaptations, a result that has been established for many years (34).

We sought to determine whether the effects of T₃ and contractile activity on PGC-1α protein content could be mediated via common mechanisms. Thus we examined the time-dependent effects of T₃ on AMPKα activity because 1) Park et al. (21) have recently shown that combined T₃ and T₂ treatment for 7 days could induce the levels of AMPK isoform subunits and 2) our data clearly implicate AMPKα in mediating increases in PGC-1α protein levels. We found no change in AMPKα protein expression in animals treated with T₃ for either 6 h or 5 days. However, a dramatic increase in AMPKα activation was observed in slow-twitch, but not in fast-twitch, muscle by 6 h (Fig. 5A). This effect was attenuated by 5 days of treatment (Fig. 5B). Thus the increase in PGC-1α protein in slow-twitch muscle at 5 days could be partly the result of sequential and cumulative effects of 5 days of T₃-induced AMPKα signaling. Moreover, it appears that AMPKα activation is more sensitive to changes in T₃ levels in slow- vs. fast-twitch muscle, perhaps related to the lower AMPKα isoform content in slow-twitch muscle (1). The lack of T₃ on AMPKα activation in fast-twitch muscle suggests that the effect of T₃ on PGC-1α protein content may be mediated by more traditional T₃- and AMPKα-mediated increases in PGC-1α transcription. Further work characterizing the PGC-1α promoter is needed to verify this.

In contrast to the T₃-mediated effects in skeletal muscle type types, T₃ had no effect on PGC-1α protein expression in the heart, despite producing a 30% increase in COX activity. In view of the very high levels of endogenous PGC-1α protein in the heart, activation of PGC-1α, rather than its induction, may be a means by which an increase in mitochondrial biogenesis is achieved in this tissue. The lack of increase in PGC-1α protein with T₃ treatment may be beneficial in preventing the deleterious effects that excess PGC-1α protein can have on the morphological and functional capabilities of the heart (18). In addition, the lower COX adaptive response of heart muscle to T₃ compared with other tissues (see Fig. 3D) may be related to the relative proportion of α- and β-TR isoforms. Cardiac muscle expresses very high ratios of the TRα₂ repressor isoform relative to the activating α₁- and β-isoforms (33), and this could serve to reduce the transcriptional response of target genes, such as PGC-1α, to T₃ treatment.

In summary, the present study documents that both contractile activity and T₃ induce increases in PGC-1α protein content, consistent with the established effects of these stimuli on mitochondrial biogenesis. Further, we present evidence to suggest that AMPK activity may be a common signaling intermediate that leads to this increase. However, PGC-1α adaptations are likely mediated by a combination of both transcriptional and posttranscriptional mechanisms in a fiber type- and tissue-specific manner.

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