Thyroid hormone (T₃) rapidly activates p38 and AMPK in skeletal muscle in vivo

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Irrcher I, Walkinshaw DR, Sheehan TE, Hood DA. Thyroid hormone (T₃) rapidly activates p38 and AMPK in skeletal muscle in vivo. J Appl Physiol 104: 178–185, 2008. First published October 25, 2007; doi:10.1152/japplphysiol.00643.2007.—Thyroid hormone (T₃) regulates the function of many tissues within the body. The effects of T₃ have largely been attributed to the modulation of thyroid hormone receptor-dependent gene transcription. However, nongenomic actions of T₃ via the initiation of signaling events are emerging in a number of cell types. This study investigated the ability of short-term T₃ treatment to phosphorylate and, therefore, activate signaling proteins in rat tissues in vivo. The kinases investigated included p38, AMP-activated protein kinase (AMPK), and extracellular signal-regulated kinase (ERK) 1/2. Following 2 h of T₃ treatment, p38 and AMPK phosphorylation was increased in both the slow-twitch soleus and the fast-twitch plantaris muscles. In contrast, ERK1/2 was not activated in either muscle type. Neither p38 nor AMPK was affected in heart. However, AMPK activation was decreased by T₃ in liver. ERK1/2 activation was decreased by T₃ in heart, but increased in liver. Possible downstream consequences of T₃-induced kinase phosphorylation were investigated by measuring cAMP response element binding protein (CREB) and thyroid hormone receptor DNA binding, as well as peroxisome proliferator-activated receptor-α coactivator-1 mRNA levels. Protein DNA binding to the cAMP or thyroid hormone response elements was unaltered by T₃. However, peroxisome proliferator-activated receptor-α coactivator-1 RNA expression was increased following 12 h of T₃ treatment in soleus. These data are the first to characterize the effects of T₃ treatment on kinase phosphorylation in vivo. We show that T₃ rapidly modifies kinase activity in a tissue-specific fashion. Moreover, the T₃-induced phosphorylation of p38 and AMPK in both slow- and fast-twitch skeletal muscles suggests that these events may be important in mediating hormone-induced increases in mitochondrial biogenesis in skeletal muscle.

mitochondrial biogenesis; peroxisome proliferator-activated receptor-α coactivator-1; signal transduction; adenosine 5′-monophosphate-activated protein kinase; p38 mitogen-activated protein kinase

THYROID HORMONES (THs) [3,5,3′-triiodo-L-thyronine (T₃), thyroxine (T₄)] regulate the differentiation, growth, and metabolism of virtually every cell in the body (34). In skeletal muscle, T₃ regulates muscle fiber-type and mitochondrial content (3, 27, 31). These effects have largely been attributed to the direct modulation of gene transcription by ligand-dependent activation of TH receptors (TRs), which bind specific DNA sequences termed TH response elements (TREs) in the promoters of TH target genes. Upon binding of T₃, TRs undergo a conformational change that favors the release of co-repressor proteins and the docking of coactivator proteins to the TR-TRE complex. This cofactor switch leads to local chromatin remodeling and increased transcription of T₃-responsive genes (34). Because this classic mechanism of TH action involves the direct modulation of TR-dependent transcription by T₃, it has been referred to as the genomic mechanism of TH action. Recently, TR-independent effects of THs have emerged as an important class of TH actions, and these are termed nongenomic effects of THs (4).

Nongenomic TH effects are typically categorized using two criteria: 1) an effect that is rapid in onset (e.g., <2 h), and 2) an effect that is independent of de novo protein synthesis. Nongenomic actions of THs were first described nearly 30 yr ago, with the observation that T₃ stimulates oxidative phosphorylation in isolated mitochondria within minutes of treatment onset (23, 29, 30). Since that time, numerous nongenomic mechanisms of TH action have been identified. These include the modulation of ion channel activity, intracellular Ca²⁺ mobilization, phospholipase activation, and kinase activation (4). In 1999, it was reported that T₃ and T₄ activate the extracellular signal-regulated kinases (ERK) 1/2, members of the mitogen-activated protein kinase (MAPK) family, and this effect persisted when T₃ entry into the cell was blocked (21). Other kinases known to be nongenomically activated by THs include p38 MAPK, protein kinase C (PKC), phosphatidylinositol 3-kinase (PI3K), protein kinase B (PKB)/Akt, mammalian target of rapamycin (mTOR), and p70S6 kinase (7, 12, 18–20). The downstream consequences of TH-induced kinase activation include increases in gene transcription, protein synthesis, cell proliferation, and angiogenesis (14). Recently, integrin α₅β₁ was identified as a plasma membrane receptor for TH (6). Binding of T₃ to this receptor is necessary for T₃-induced activation of ERK1/2 and the downstream stimulation of angiogenesis and glial cell proliferation (6, 13).

Only one study to date has examined nongenomic effects of T₃ in skeletal muscle cells. D’Arezzo et al. (12) showed that T₃ rapidly increases the intracellular pH of rat L₆ skeletal muscle cells by activating plasma membrane Na⁺/H⁺ exchangers. This effect was mediated through the activation of a signaling cascade involving phospholipase C activation, inositol triphosphate accumulation, intracellular Ca²⁺ mobilization, and phospholipase of PKC and ERK1/2. These findings have yet to be confirmed in vivo, and no other studies have investigated nongenomic effects of TH in skeletal muscle in vivo or in vitro. Thus our goal was to characterize the activation of kinases in rat slow- and fast-twitch skeletal muscle in vivo. Using whole body injection of T₃ in adult rats, we found that T₃ rapidly activates kinases in a skeletal muscle fiber-type- and tissue-specific manner.
specific fashion. Thus the emerging nongenomic actions of THs do occur in whole animals, and these effects may contribute to the profound physiological effects of THs in a variety of tissues.

METHODS

Materials. T3 was purchased from Sigma-Aldrich (Oakville, Ont, Canada), and primers for PCR were from Sigma Genosys (Oakville). TRIZol and Superscript II RT were purchased from Invitrogen (Burlington, ONT, Canada), and other PCR reagents, including Go Taq polymerase, were obtained from Promega (Madison, WI). The cAMP response element (CRE) and TRE oligonucleotides for electrophoretic mobility shift assays (EMSAs) were from Dalton Chemical (Toronto, Ont, Canada). Radioactivity (γ32-P[γATP] and nitrocellulose membranes were purchased from GE Healthcare (Piscataway, NJ). Antibodies for phospho-p38 (no. 9211), total-p38 (no. 9212), phospho-AMPK-α (no. 2531), total-AMPK-α (no. 2532), and cAMP response element binding protein (CREB) (no. 9192) were purchased from Cell Signaling Technology (Beverly, MA). The enhanced chemiluminescence kit was from Santa Cruz Biotechnology.

Animal care and treatments. All procedures involving animals were approved by the York University Animal Care Committee. Adult male Sprague-Dawley rats (300–400 g; Charles River, St. Constance, Quebec, Canada) were given a single intraperitoneal injection of T3 (0.4 mg/kg body wt) dissolved in vehicle (0.9% NaCl-1,2-propanediol; 40:60 vol/vol) or vehicle alone. This concentration of T3 has been used previously to induce phenotypic alterations in mitochondrial content and function in vivo (10, 11, 17, 25, 27). Rats were anesthetized after 2, 4, 6, or 12 h of T3 treatment, and tissues were excised, quick frozen in liquid nitrogen, and stored at −80°C for later analyses. To measure T3 serum concentrations, blood was withdrawn from the saphenous vein by standard venipuncture techniques from animals at 1 h preinjection, and at 2, 4, 6, 10, and 23 h postinjection. Blood samples were collected free flow into a blood collection sample tube. The collected blood samples were centrifuged at room temperature for 5 min to separate serum from red blood cells. Immediately following, serum was removed and stored at −20°C until they were analyzed for T3 concentration using an immunoassay test kit (ICN Pharmaceuticals).

Protein extraction. Tissues stored at −80°C were pulverized at the temperature of liquid nitrogen to yield a fine powder. Tissue powders were diluted in muscle extraction buffer (20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM β-glycerophosphate, 1% glycerol, 1% Triton X-100) supplemented with protease and phosphatase inhibitors (1 mM dithiothreitol, 1 mM Na3VO4, 10 μM leupeptin, 1.5 μM pepstatin A, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). Homogenates were rotated end over end for 1 h at 4°C, sonicated (3 × 3 s at 30% power), then centrifuged at 14,000 g for 10 min at 4°C. The supernatant fraction (representing the DNA-binding proteins) was removed, and following the determination of protein concentration, extracts (50 μg protein) were incubated for 30 min at room temperature with 0.025 μg/ml poly(dI-dC) and 62.5 μM pyrophosphate in binding buffer [20 mM Tris (pH 8.0), 1 mM EDTA (pH 8.0), 50 mM NaCl, 10% glycerol, 0.3 mg/ml BSA, and 1 mM dithiothreitol]. Following the addition of [γ32-P]ATP-labeled double-stranded oligonucleotides (40,000 counts) representing the CRE (sense: 5′-GAT TGC GTG AGC TCA GAG AGC TA-3′) or the TRE from the COX2 promoter (sense: 5′-ACG CGG ACA GGT CAT GAA CCC GGA GAC-3′) (5), the reactions were incubated for an additional 30 min at room temperature. To determine the binding specificity of each oligonucleotide, some reactions were incubated with a 100-fold molar excess of unlabeled oligonucleotide simultaneously with 40,000 counts of labeled oligonucleotide. Supershift analysis was performed by including the CREB antibody (10 μl) in both reaction incubations for CRE binding. Binding reactions were subject to electrophoresis (250 V for 1.5 h) through a 5% acrylamide (29:1 acrylamide-bisacrylamide) gel at 4°C, and gels were fixed in acetic acid-methanol-H2O (10:30:60 vol/vol/vol) for 15 min at room temperature, dried at 80°C for 45 min, and then imaged autoradiographically (InstantImager, Packard).

RNA isolation and RT-PCR. Total RNA was isolated from frozen muscle powders (25 mg) using TRIZol reagent, according to the manufacturer’s instructions (Invitrogen). After determining the concentration of each total RNA sample by measuring the absorbance at 260 nm, RNA (1 μg) was reverse-transcribed to cDNA using Superscript II reverse transcriptase. cDNA was amplified by PCR using Taq polymerase and primers for peroxisome proliferator-activated receptor-α coactivator-1 (PGC-1α) (5′-GAC CAC AAA CGA TGA CCC TCC-3′, forward; 5′-CCT GAG AGA GAC TTT GGA GGC-3′, reverse) and GAPDH (5′-GGT GCT GAG TAT GTC GTG GA-3′, forward; 5′-CCT CTG AGT GCC AGT GG-3′, reverse). PCR products were run on a 2% agarose gel and visualized by ethidium bromide staining. Bands were quantified using SigmaGel software.

Statistical analysis. Data are presented as means ± SE. Vehicle- and T3-treated animals were compared at each time point using unpaired Student’s t-tests. Differences were considered statistically significant if P < 0.05.

RESULTS

We first assessed circulating levels of T3 by measuring serum T3 concentration in animals 1 h preinjection, and at 2, 4, 6, 10, and 23 h postinjection. As shown in Fig. 1, a single intraperitoneal injection of T3 (0.4 mg/kg) led to a rapid 50-fold increase in serum T3 levels that reached a peak concentration of ~105 ng/ml by 2 h. Thereafter, T3 levels steadily declined to ~37 and 10 ng/ml 10 and 24 h postinjection, respectively. T3 values remained unchanged in the range of 2 ng/ml in vehicle-injected animals.

Next, to evaluate whether these rapid changes in the concentration of T3 could activate signaling kinases in vivo, we performed Western blotting analyses using phospho-specific antibodies recognizing the phosphorylated form of the signaling kinases of interest. We began by evaluating the effect of T3 on
on the phosphorylation of the p38 MAPK pathway using a p38 antibody that only recognizes p38 when dually phosphorylated on Thr182 and Tyr184, a modification that activates p38. As seen in Fig. 2A, T3 increased p38 phosphorylation approximately twofold at all three time points of treatment in the slow-twitch soleus (SOL) muscle (2 h, 4 h, \( P < 0.05 \); 6 h, \( P < 0.001 \)). In contrast, p38 was only significantly phosphorylated in response to 2 and 4 h of T3 treatment in the fast-twitch plantaris (PL) muscle (\( P < 0.05 \)), while p38 phosphorylation returned to baseline after 6 h of T3 treatment (Fig. 2B). Interestingly, T3 failed to phosphorylate p38 in the heart (HRT) at any time point (Fig. 2C).

To determine the activation status of AMPK in response to T3, we performed Western blotting analyses using an antibody that recognizes AMPK only when phosphorylated at Thr172, a modification that is highly correlated with AMPK enzymatic activity (24). Similar to p38, AMPK was significantly phosphorylated by T3 in both SOL and PL (Fig. 3, A and B). However, there were subtle differences in the timing of AMPK activation, as AMPK was phosphorylated by T3 at 2 h (\( P < 0.01 \)) and 4 h (\( P < 0.05 \)) postinjection, an effect that started to decline by 6 h in SOL, whereas AMPK phosphorylation was seen only at 2 h in PL (\( P < 0.05 \)). These results suggest that the effect of T3 on AMPK activation is more transient than the effect on p38. In HRT, AMPK phosphorylation was not significantly altered by T3 treatment (Fig. 3C), while in the liver (LIV), AMPK phosphorylation was significantly decreased at 4 h of T3 treatment (Fig. 3D).

Using an antibody that selectively recognizes ERK1/2 when dually phosphorylated on Thr202 and Tyr204, Western blotting analyses demonstrated that ERK1/2 phosphorylation was not altered by T3 in SOL or PL (Fig. 4, A and B). In contrast, ERK1/2 phosphorylation was significantly decreased by 2 h of T3 treatment in HRT (\( P < 0.05 \)), with no effect seen at 4 or 6 h (Fig. 4C). In LIV, T3 had no effect on ERK1/2 phosphorylation.
A t2h, b u4t or 6 ho f T3 treatment caused a significant increase in ERK1/2 phosphorylation (P < 0.05, Fig. 4D).

In an attempt to identify downstream targets of T3-induced kinase activation, we performed EMSAs to measure the DNA-binding activity of two transcription factors known to function downstream of these kinases. CREB is activated downstream of p38, whereas TR is a downstream target of both p38 and ERK1/2 (8, 15, 26). Although T3 activated p38 in SOL and PL, there was no change in CRE binding in either muscle following T3 treatment (Fig. 5A). The CRE can be bound by both CREB and activating transcription factor 2, which is also a target of p38 phosphorylation (1). Supershift analysis using a CREB antibody demonstrated that CREB is the predominant CRE binding protein in nuclear extracts from SOL and PL (Fig. 5A, inset, and data not shown). TRE protein binding was also not altered by T3 treatment in any tissue following 4 h of T3 treatment (Fig. 5B).

The phosphorylation of p38 and AMPK in SOL and PL led us to investigate the responsiveness of a downstream target of these kinases. PGC-1α is upregulated by T3 treatment in skeletal muscle (17), but it is not yet clear whether this is mediated transcriptionally or posttranscriptionally. In addition, PGC-1α expression can be induced by either p38 or AMPK (1, 17). We isolated RNA from SOL and PL of rats injected with T3 for 6 or 12 h and performed RT-PCR to measure PGC-1α mRNA. We found that 12 h of T3 treatment resulted in a 1.6-fold increase in PGC-1α mRNA in SOL (P < 0.001), but not in PL (Fig. 6, A and B, respectively). In contrast, 6 h of T3 treatment were insufficient to alter PGC-1α mRNA in either muscle (data not shown).

**DISCUSSION**

Our laboratory’s previous work has shown that 5 consecutive days of T3 treatment in vivo can increase the expression of genes encoding mitochondrial proteins (17, 27), as well as augment the expression of PGC-1α, an important regulator of organelle biogenesis. This effect of T3 was tissue specific (27). Thus the purpose of this study was to investigate the early
events leading to this change in phenotype, with possible implications for the nongenomic actions of T₃.

Several studies have demonstrated that THs (T₃ or T₄), acting via nongenomic mechanisms, can activate kinases such as ERK1/2, p38, PKC, Akt, mTOR, and p70S6K (7, 12, 18–21). These effects occur in several cell lines and lie upstream of diverse cellular and physiological processes. TH-induced activation of ERK1/2 leads to the phosphorylation of several transcription factors and induces angiogenesis and alterations to cellular pH (12, 14). In contrast, p38 activation by T₃ causes hypertrophy of cardiomyocytes (20) and modifies osteoblast activity (18), whereas activation of the PI3K-Akt-mTOR-p70S6K pathway by T₃ in human skin fibroblasts and primary rat cardiomyocytes induces mRNA expression and increases protein synthesis, respectively (7, 19). Interestingly, TRs can act as both effectors of, and participants in, the nongenomic actions of THs. T₄-activated ERK1/2 phosphorylates TRβ1, which facilitates the dissociation of the co-repressor protein-silencing mediator of retinoid and thyroid receptors, while p38 activity is necessary for T₃-induced stabilization of TRβ1 protein (8). While these studies have been invaluable in identifying novel actions of THs, they were all performed in cell culture models and have, therefore, failed to address the in vivo relevance of such phenomena. Thus we set out to measure the activation of four kinases relevant to muscle physiology following short-term T₃ treatment in vivo.

Employing whole body T₃ injection in adult rats, we found that p38 and AMPK were phosphorylated by 2 h of T₃ treatment in both slow- and fast-twitch skeletal muscles but are unaffected in HRT, while AMPK activation is decreased in LIV. In contrast, ERK1/2 was activated by T₃ in LIV but not skeletal muscle, while ERK1/2 phosphorylation decreased in HRT. These results confirm the ability of short-term T₃ treatment to activate ERK1/2 and p38, while providing the first evidence that short-term T₃ treatment is capable of activating AMPK. Our laboratory previously showed that 6 h of T₃ treatment activated AMPK in muscle (17), and the current results extend this finding to show that 2 h of T₃ treatment are sufficient to activate AMPK in SOL and PL. In addition, we show here for the first time that T₃ can activate these kinases in vivo. These findings represent proof in principle that T₃ has the ability to alter the phosphorylation status of several kinases in multiple tissues in a whole animal setting. However, it should be noted that the serum T₃ concentration elicited by our single dose of hormone reached a level ∼10-fold higher than that seen in hyperthyroid humans (28). Thus it will be impor-

Fig. 4. The effect of short-term (2, 4, or 6 h) T₃ treatment on extracellular signal-regulated kinase (ERK) 1/2 activation, represented by dual Thr202/Tyr204 phosphorylation, was assessed in soleus (A), plantaris (B), heart (C), and liver (D). Representative Western blots and graphical summaries are shown as in Fig. 1. ERK1/2 activation in V-treated rats was normalized to a value of 1.0 for each time point. For ERK1/2 activation, ERK2 was quantified densitometrically. n = 5–9 animals/group for soleus and plantaris; n = 3 animals/group for heart and liver. Open bars, V; solid bars, T₃. P-ERK1/2, phospho-ERK1/2; T-ERK1/2, total-ERK1/2. *P < 0.05.
tant to establish whether changes in serum T3 concentration within the normal physiological or pathological ranges are sufficient to induce such effects.

In addition to showing that T3 can activate kinases in vivo, we have also demonstrated that there is a tissue specificity to T3-induced kinase phosphorylation. While p38 and AMPK are selectively activated in skeletal muscle, the activation of ERK1/2 is unaffected by T3 in this tissue and is instead altered solely in HRT and LIV. The underlying cause of this tissue specificity may be related to the following: 1) the tissue-specific expression of kinases, 2) T3 delivery to peripheral tissues, and 3) the expression of integrin αβ3 receptor. The first possibility is the least likely, as most of the kinases examined are ubiquitously expressed. T3 delivery due to blood flow differences is also unlikely to account for the tissue specificity of T3-induced kinase activation. This assertion is based on the fact that kinase activation among the different tissues (e.g., Figs. 2 and 3) failed to correlate to known blood flow differences, which are highest in HRT, followed by SOL and PL muscles (22). Last, the tissue-specific distribution of the integrin αβ3 receptor is an interesting candidate for mediating the tissue-specific kinase activation by T3. This receptor has been shown to bind T3 with high affinity in a variety of cell lines (14). Moreover, many nongenomic effects of THs, including the activation of ERK1/2, have been shown to be initiated by the binding of T3 or T4 to this receptor (14). An investigation of the tissue distribution of this specific integrin dimer would be useful in helping us understand the tissue specificity of T3-induced kinase activation, as demonstrated here.

As an initial attempt to identify the downstream consequences of T3-induced kinase activation in vivo, we measured the DNA binding activity of two transcription factors that are targets of p38 and ERK1/2. p38, acting through the kinase...
mitogen and stress-activated protein kinase 1, indirectly activates CREB, whereas ERK1/2 directly phosphorylates TRβ1 (15, 26). In addition, p38 activity is required for the nongenomic stabilization of TRβ1 by T3 (8). However, this prior study did not address the effect of p38 activity on TRβ1 DNA binding. Using EMSA, we demonstrate that short-term T3 treatment fails to alter the DNA binding activity of TR or CREB in any tissue. These results are consistent with previous studies showing that phosphorylation of CREB on Ser133 does not alter CREB DNA-binding activity and instead increases the association of CRE-bound CREB with its coactivator CREB-binding protein (26). The effect of phosphorylation on TR DNA binding is not fully characterized, but it has been shown that TR can bind DNA in the presence and absence of T3, and thus alterations in DNA-binding activity are not crucial in modulating TR-dependent transcription (34). Since we did not ascertain the identity of the proteins bound to the TRE in this study, we cannot make any conclusions about specific TR isoforms. Nevertheless, none of the TRE-protein complexes showed a change in binding with T3 treatment in any tissue. Therefore, although the EMSAs failed to show an effect of T3 treatment, this result does not preclude the activation of CREB or TR by T3-activated kinases. In light of this, it will be necessary to use additional techniques, such as T3 treatment of cultured skeletal muscle cells and reporter gene assays, to identify the events downstream of kinase activation.

Our laboratory has previously shown that 5 days of T3 treatment are sufficient to increase PGC-1α protein expression in skeletal muscle (17). However, it is not known whether this increase is mediated transcriptionally or posttranscriptionally. PGC-1α mRNA was increased 12-fold by 6 h of T3 treatment in LIV of hypothyroid animals (32). Our results add to this study by showing that 12 h (but not 6 h) of T3 treatment in euthyroid rats also increase PGC-1α mRNA expression in skeletal muscle. Clearly, the effect of T3 on PGC-1α mRNA is considerably delayed relative to the more rapid effects of the hormone on kinase activation, as reported here. This result supports the hypothesis that T3 may act through the activation of kinases to induce the expression of PGC-1α. However, the possibility also remains that the effects of T3 were secondary to T3-induced alterations in physical activity levels, tissue metabolic rate, temperature, or neural input. While we observed no changes in the activity patterns of the animals following T3 injection, the other possibilities remain to be investigated.

Our results are also unique in demonstrating that the alteration of PGC-1α mRNA by short-term T3 treatment is fiber type specific, with the effect observed in SOL, but not in PL muscle. This finding is consistent with the relatively higher sensitivity for T3 displayed by slow-twitch vs. fast-twitch muscle (2, 16, 33). It will now be important to determine whether this elevated PGC-1α mRNA expression is a consequence of enhanced mRNA stability or augmented transcription. In the case of transcription, three possibilities exist (Fig. 7): 1) transcriptional upregulation via the presence of a TRE in the PGC-1α promoter (pathway 1); 2) the nongenomic activation of a kinase (or other signaling protein) that activates a transcription factor that directly increases PGC-1α promoter activity (pathway 2); or 3) the genomic upregulation of a transcription factor (via a TRE), which then binds to the PGC-1α promoter and increases PGC-1α transcription (pathway 3). Supporting the second possibility, two of the kinases that were activated by T3 in the present study, p38 and AMPK, are both capable of increasing PGC-1α promoter activity through the activation of the downstream transcription factors, activating transcription factor 2 and GATA/upstream stimulatory factor 1, respectively (Ref. 1; Irrcher et al., unpublished observations). Studies employing reporter gene assays to measure PGC-1α promoter activity, as well as mRNA stability assays, will aid in the elucidation of the mechanism through which T3 increases PGC-1α mRNA expression.

In summary, T3 alters the phosphorylation status of several kinases in a tissue-specific manner in vivo. Specifically, both p38 and AMPK are phosphorylated by T3 in slow- and fast-twitch skeletal muscle. These kinases are important in several physiological processes in skeletal muscle, including mitochondrial biogenesis (1, 35). Since T3 is a potent regulator of mitochondrial content in this tissue (31), these kinases may play a role in T3-induced mitochondrial biogenesis. Furthermore, although T3 regulates the expression of ~400 skeletal muscle genes (9), it has been suggested that the majority of these genes lack a TRE in their promoter (31), suggesting that T3 may activate a subset of these genes nongenomically. We hypothesize that the activation of p38 and AMPK represents important downstream effects of nongenomic T3 signaling in skeletal muscle. Studies on the necessity of these kinases for T3-induced phenotypic alterations will shed light on the in vivo consequences of this emerging class of TH actions.
REFERENCES