T₃ increases lactate transport and the expression of MCT4, but not MCT1, in rat skeletal muscle

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LACTATE IS TRANSPORTED across the plasma membrane via a pH-dependent monocarboxylate transporter (MCT) system (19, 26). A family of MCT proteins has now been identified (19). Among the most prominent of these are MCT1 and MCT4. MCT1 is expressed ubiquitously in most tissues, including heart and skeletal muscle (5, 34, 35). In contrast, MCT4 is expressed primarily in fast-twitch skeletal muscle fibers (5, 9, 10). The expression of MCT1 and MCT4 is regulated by muscle contraction. When contractile activity is essentially aerobic in nature (chronic electrical stimulation for 7 days), there is an increase in MCT1 but not MCT4 (10). However, when muscle activity (exercise training) is more intense, an increase in both MCT1 and MCT4 is observed (15, 41).

Other factors are also known to alter the expression of MCTs. For example, within the first 84 days of life, soleus muscle MCT1 is increased 38% and remains stably expressed for up to 1 yr. Concomitantly, MCT4 is reduced by 60% within the first 84 days of life and has declined by 90% at the end of a 1-yr period (21). Circulating lactate concentrations may also influence MCT expression, since MCT1 and MCT4 were increased by 37 and 86% in a patient with a mitochondrial myopathy, in whom circulating lactate levels at rest were increased two- to threefold (3). In a rodent model of congestive heart failure, when the heart begins to depend more extensively on carbohydrate metabolism, MCT1 expression is increased (24). Thus it appears that the expression of MCTs is altered when the metabolic demands on the tissue are being altered, whether through an increased postural activity in the developing soleus muscle or when tissue metabolism is altered by disease processes.

Whether MCT1 and MCT4 are hormonally regulated has not yet been established. The administration of triiodothyronine (T₃) can have profound effects on growth and development as well as muscle metabolism. For example, T₃ increases oxygen consumption (i.e., basal metabolic rates) and the activities of selected enzymes (1, 48), as well as genes encoding the sarco(endo)plasmic reticulum Ca²⁺-ATPase isoforms SERCA1 and SERCA2a (51) and the Na⁺-K⁺-ATPase α₂- and β₂-isoforms (2). T₃ has also been shown to alter the expression of the glucose transport proteins GLUT1 and GLUT4 (52, 53) in an isoform-specific and tissue-specific manner. In very young rats (<50 g), 3 days of T₃ ingestion rapidly induced the expression of the glucose transporter GLUT4 in soleus and extensor digitorum longus muscles (52, 53). In contrast, in rat heart, GLUT4 protein content was not altered by T₃, whereas GLUT1 content was reduced (53). Thus the expression of many proteins, including substrate transporters, appears to be influenced by T₃.

The regulation of T₃ target genes is mediated through thyroid hormone response elements (TRE). The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The TRE consensus motif (5′-AGGTTCA-3′) has been found in the 3′-UTR of both MCT1 (5′-2211–2216-3′) (22, 49) and MCT4 (5′-1069–1074-3′) (42) in the rat. Yet it remains to be shown that T₃ treatment alters not only MCT mRNA but also MCT protein expression. We (10, 21) have previously observed that MCT protein expression is regulated in an isoform-specific and tissue-specific manner in heart and muscle. Changes in MCT protein expression in these tissues occur primarily through posttranscriptional mechanisms, since there is little evidence that MCT mRNA and protein levels were associated. Therefore, in the present studies, we examined the tissue-specific (heart and red and white muscle) and isoform-specific effects of T₃ on MCT1 and MCT4 at the mRNA and protein levels. In addition, we also examined the effects of T₃ on lactate transport into giant sarcosomal vesicles and the plasmalemmal MCT1 and MCT4.

**METHODS**

**Animals.** Male Sprague-Dawley rats (∼240 g) were used. Ethical approval was obtained for this work from the committee on animal care at the University of Waterloo. Animals were bred in the University of Waterloo animal care facilities. They were housed in an air-conditioned room (24°C) on a 12:12-h light-dark cycle. They were handled daily and were allowed free access to water and food ad libitum. During the 7-day experimental period, the drinking water was supplemented with T₃ (900 µg/100 g body wt 1 day⁻¹), which is in excess of the T₃ required to saturate the T₃ receptors (38). A lower dosage (360 µg/100 g 1 day⁻¹) has been used in previous studies to upregulate skeletal muscle GLUT4 (52).

Body weights were monitored on a daily basis throughout the study. Heart and red (RG) and white gastrocnemius (WG) muscles, as well as blood samples (cardiac puncture), were obtained from anesthetized animals (Somnotol 60 mg/kg ip) before T₃ treatment (day 0) and after 1, 2, 4, and 7 days of T₃ treatment. Tissues were frozen in liquid N₂ and kept at −80°C until analyzed. Serum samples were analyzed for free T₃ by use of a radioimmunoassay (Diagnostic Products, Los Angeles, CA). In the follow-up studies designed to measure lactate uptake by giant sarcosomal vesicles, we obtained muscle tissue from untreated (day 0) and 7-day-T₃-treated animals.

**Western blotting of MCT1 and MCT4.** Proteins were isolated from RG and WG muscles and the heart, and MCT1 and MCT4 were detected using Western blotting. We have previously described these procedures in detail (9, 10, 34, 35, 54). MCT1 cDNA and MCT4 cDNA were a gift from Dr. A. P. Halestrap, Department of Biochemistry, University of Bristol, Bristol, UK.

A 1.9-kb fragment containing the coding sequence of MCT1 cDNA was isolated from the full-length (3.3-kb) MCT1 cDNA by digestion with the EcoRI restriction enzyme (22) and subcloned into the EcoRI restriction enzyme site of pBlueScript (KS). The orientation was checked by digestion with HindIII restriction endonuclease. Template DNA was linearized with XbaI restriction enzyme, and a digoxigenin (DIG)-labeled antisense MCT1 riboprobe was generated by in vitro transcription with T₃ RNA polymerase. MCT4 cDNA was originally subcloned into BamHII/ApaI restriction enzyme sites of pBlueScript (54). DIG-labeled antisense MCT4 riboprobe was generated by digestion of the template DNA with XbaI restriction enzyme and in vitro transcription with T₇ RNA polymerase.

The ingredients for RNA transcription included 1–2 µg of DNA template plus the NTP mix [2.5 mM CTP, 2.5 mM GTP, 2.5 mM ATP, 1.625 mM UTP (Promega, Madison, WI), and 0.875 mM Dig-11 UTP (Boehringer Mannheim)], 20 mM DTT, 100 U/1 µg of DNA template (Promega), and 1× RNA polymerase buffer (5× buffer: 400 mM Tris-HCl, pH 7.5, 60 mM MgCl₂, and 20 mM spermine-HCl (Promega)) maintained at room temperature. The appropriate RNA polymerase [T3 or T7 RNA polymerases (Boehringer Mannheim)] was added (20 IU/1 µg of DNA template) and incubated for 2 h at 37°C. The DNA template was then digested for 10 min at 37°C with RNase-free DNase (1 IU/1 µg of DNA template; Promega). After precipitation in ethanol and centrifugation at 12,000 rpm for 15 min, the probe was resuspended in 10–20 µl of DIG Easy-hyb hybridization buffer (Boehringer Mannheim) or standard hybridization buffer with 50% formamide (5× SSC, 50% formamide, 0.1% N-lauroylsarcosine sodium, 0.02% SDS, and 2% blocking reagent (Boehringer Mannheim)).

After prehybridization of the membrane for ≥4 h at 68°C, the prehybridization buffer was replaced with the same buffer containing the DIG-labeled antisense RNA probe, and the membrane was incubated with the probe overnight at 68°C. High-stringency washes (65°C, 0.1× SSC) and chemiluminescent detection were performed in accordance with the protocol supplied by the manufacturer (DIG Northern Starter Kit, Boehringer Mannheim), and the membrane was exposed to Kodak BioMax film. After exposure, the film was developed in Kodak developer and fixed in Kodak fixer. mRNA band densities were obtained by scanning the blots on a densitometer connected to a computer with appropriate software. These were normalized to the 28S RNA signal. No further normalization occurred, as all data were loaded onto the same gel.

**Preparation of giant sarcosomal vesicles and plasma-lemmal MCT detection.** We also examined lactate uptake by giant sarcosomal vesicles from control and 7-day-T₃-treated animals. For these purposes, RG and WG muscles were pooled, and giant sarcosomal vesicles were obtained as we have previously described (7, 6, 50). Briefly, muscles were scissors lengthwise into thin slices and incubated in 140 mM KCl, 5 mM MOPS, pH 7.4, 150 µM collagenase (type VII, Sigma), and 0.01 mg/ml protease inhibitor aprotinin (A1153, Sigma-Aldrich, St. Louis, MO) for 1 h at 34°C. Fifteen milliliters of the medium were then mixed with 3.7 ml of Porell-KCl-apidoptin solution and allowed to be incubated at room temperature for 30 min to form vesicle suspension. A three-layer step-density gradient was used to isolate the vesicles. The upper layer was composed of KCl-MOPS (3 ml); the middle layer was composed of 4% Niconed in KCl-MOPS.
Lactate uptake by giant sarcolemmal vesicles. Lactate uptake measurements were performed under zero-trans conditions in giant vesicles (50–80 μg protein) at a concentration of 1 mM lactate (0.1 μCi l-[U-14C]lactate/tube), as we recently reported (50). For these purposes, lactate was added to the vesicle suspension and vortexed. A 10-s uptake period was used. Uptake was terminated by the addition of an ice-cold stop solution (3 mM HgCl in 0.1% BSA, and KCl-MOPS). The vesicles were then centrifuged (12,000 g for 4 min), and the supernatant fraction was discarded. To determine the nonspecific activity associated with vesicles, the stop solution was added to the vesicles before the lactate solution was loaded. Radioactivity was determined using a liquid scintillation counter. Standard calculations were used to ascertain the uptake of lactate into the giant vesicles.

Data analyses. The data were analyzed with analyses of variance. Significance was determined with Fischer’s least squares differences post hoc test. All data are expressed as means ± SE.

RESULTS

Body weights at the start of the study did not differ in control (237 ± 3.3 g) and T3-treated animals (239 ± 5 g, P > 0.05). The body weight gain (+50 ± 3.3 g) of control animals during the 7-day experimental period was greater than that of the T3-treated animals (+10 ± 2.3 g, P < 0.05; Fig. 1A). T3 increased the circulating free T3 concentrations markedly, from 0.28 ± 0.05 pg/ml before T3 treatment to 85.5 ± 5.6 pg/ml after 1 day. After 7 days, circulating free T3 concentrations were 134 ± 12.5 pg/ml (Fig. 1B). Circulating and muscle lactate concentrations did not differ in the control and T3 groups (data not shown).

MCT proteins and mRNA: MCT1. In control animals, MCT1 protein content of the heart was about threefold greater than that of RG muscle, whereas MCT1 content of RG was 2–2.5-fold greater than the MCT1 protein content of the WG (Fig. 2A). In the heart and in the RG and WG muscles, there were no changes in MCT1 protein during the 7 days of the T3 treatment period (Fig. 2A).

MCT1 mRNA was more abundant in heart than in either skeletal muscle type in control animals (P < 0.05, Fig. 2B). In marked contrast to the lack of change in MCT1 proteins, T3 increased MCT1 mRNA in the heart (+114%) and in the WG (+49%) and RG (+77%) muscles (Fig. 2B).

MCT proteins and mRNA: MCT4. No changes in muscle MCT4 protein were observed after 2 days of T3 treatment (Fig. 3A). However, after 4 days of T3 treatment, MCT4 protein in both RG (+43%, P < 0.05) and WG (+49%, P < 0.05) muscles was increased and remained stable until day 7 (Fig. 3A). These MCT4 protein increments were similar in the RG and WG muscles (P > 0.05). In agreement with other studies (9, 21), MCT4 protein was not detected in the hearts.

MCT4 mRNA in control WG muscle was greater than in the control RG muscle (Fig. 3B). MCT4 mRNA was not detected in the heart, and this confirms our observations in other studies (9, 21). With T3 treatment, MCT4 mRNA was increased in both RG and WG, although the relative increase was much greater in RG (+300%, P < 0.05) than in WG (+40%, P < 0.05; Fig. 4B).

Lactate transport and plasma membrane MCT1 and 4. Lactate uptake and plasma membrane MCT1 and MCT4 proteins were examined in giant sarcolemmal vesicles obtained from control and 7-day-T3-treated animals. T3 treatment did not increase the plasma membrane MCT1 protein, whereas the plasmalemmal MCT4 protein was increased slightly, +13% (Fig. 4A, P < 0.05). At an external lactate concentration of 1 mM lactate, the uptake of this monocarboxylate into the giant vesicles was doubled (Fig 4B, P < 0.05).
DISCUSSION

This is the first study that has examined the regulation of MCT isoform expression by T3, a hormone that can have profound effects on gene expression in muscle. There are a number of novel observations. After T3 administration, 1) both MCT1 mRNA and MCT4 mRNA were upregulated, but 2) this was accompanied only by an increase in MCT4 protein, not MCT1 protein. Seven days of T3 treatment also 3) increased the rate of lactate transport into giant vesicles, but 4) there was only a slight increase in plasmalemmal MCT4. Thus T3 treatment upregulated MCT protein expression in an isoform-specific manner and increased the rate of lactate transport, although this may not be attributable to the changes in sarcolemmal MCT4 alone.

T3 consumed in the drinking water raised the circulating concentration of free T3. In addition, a characteristic feature of T3 treatment, namely a reduced rate...
of weight gain (47, 52), was also observed in our studies. T3 is known to alter the metabolic capacities of muscle cells by increasing the activities of enzymes in the oxidative and glycolytic pathways (1, 47, 48) and the expression of the glucose transporter GLUT4 in skeletal muscle (52). An increase in GLUT4 in the skeletal muscles of T3-treated animals was also observed in this study (data not shown). Importantly, not all metabolic parameters change in unison in response to T3 in muscle tissue. For example, in response to T3, uncoupling protein-2 and -3 mRNAs are increased in plantaris muscle, whereas there is no change in citrate synthase activity (47), and SERCA1 mRNA is increased, whereas this occurs only transiently in SERCA2A mRNA (51). Similarly, we observed T3-induced changes in GLUT4 and MCT4 proteins, but not in MCT1 protein or in selected enzymes (citrate synthase, phosphofructokinase; data not shown). Thus, in general, our observations of T3 treatment parallel observations in previous studies with this hormone (i.e., reduced weight gain, protein-specific responses to T3).

T3 treatment upregulated MCT1 mRNA in RG and WG and in the heart, and MCT4 mRNA in RG and WG muscles. We have previously shown (9, 21) that MCT4 is not expressed in adult rat hearts, although it is present in the neonatal heart [10 days of age (21)]. Despite the T3-induced increase in both MCT1 and MCT4 mRNAs, only MCT4 expression in skeletal muscle was increased, whereas MCT1 protein expression was not altered either in RG and WG muscle or in the heart. Thus it appears that, with T3 treatments, MCT1 protein expression is regulated by posttranscriptional mechanisms. Previously, it has also been shown that MCT1 protein expression is regulated by posttranscriptional mechanisms in chronically contracting skeletal muscles (10). However, pretranslational processes appear to regulate MCT1 expression in aging rats (21) and in rats with chronic heart failure (24). It appears that T3 regulates MCT4 protein expression by both pretranslational and posttranscriptional processes, since the MCT4 protein was increased similarly by T3 treatment in RG and WG muscles, but the relative increase in MCT4 mRNA was far greater in RG than in WG muscles. Over the course of a 1-yr period (days 10–365), MCT4 protein expression in soleus muscle appeared to be regulated by posttranscriptional mechanisms (21). Collectively, the present studies and others indicate that the expression of MCT1 and MCT4 can be regulated by either posttranscriptional or pretranslational mechanisms or by both mechanisms at the same time.

We also examined the effects of T3 on lactate transport into skeletal muscle. For these purposes, we used the giant sarcolemmal vesicle preparation, which has been used by us (33, 50) and others (25, 27) to examine the rates of lactate uptake across the sarcolemma, in the absence of any lactate metabolism. However, because rodent skeletal muscles express both MCT1 and MCT4 (9, 10, 54), it is difficult to examine lactate uptake in relation to either MCT1 or MCT4 alone. Therefore, we examined lactate uptake at a low concentration (1 mM lactate in the assay medium). This concentration is well below the $K_m$ for lactate uptake for both MCT1 and MCT4 [MCT1 ($K_m = 3–5$ mM (12, 32)] and MCT4 [MCT4 ($K_m = 20–30$ mM (11, 14, 32)]. Low substrate concentrations, below the $K_m$, provide the most sensitive indication of changes in transporter-mediated uptake. In addition, this low concentration also minimizes the rate of lactate diffusion (25, 36, 37, 44).

Changes in lactate uptake are difficult to attribute to either MCT1 or MCT4 alone, unless only one of these is altered and if it is assumed that the experimental treatments have not altered the intrinsic activity of the MCTs. Despite the large change in homogenate MCT4
protein in RG (+43%) and WG (+49%) muscles, there was a surprisingly small change in plasmalemmal MCT4 (+13%), suggesting that the MCT4 increase was likely confined to other subcellular compartments, such as the intracellular MCT4 depot and/or the T tubules (9). In the present study, it would seem difficult to account for a doubling of lactate uptake in the T3-treated animals by the modest change in plasmalemmal MCT4 (+13%) and no change in the plasmalemmal MCT1. In a recent study (50), we also observed that lactate uptake was increased by 10 min of intense muscle contraction, but plasma membrane MCT1 was not altered and plasma membrane MCT4 was reduced by 20–25%. Thus these studies (present study and Ref. 50) suggest that we cannot easily attribute changes in lactate uptake solely to the quantity of MCT1 and/or MCT4 in muscle homogenates or at the sarcolemma. Therefore, some other possibilities need to be considered.

It is possible that the intrinsic activity of MCT1 or MCT4 is altered. Such regulation has now been amply demonstrated for the glucose transporters GLUT4 and GLUT1 (4, 8, 13, 16, 20, 29, 43, 46) as well as for the fatty acid transporter FAT/CD36 (31). Alternatively, skeletal muscle may express additional MCTs, which might account for these substantial increases in lactate uptake despite modest increases (present study) or even decreases in sarcolemmal MCT4 (50), whereas sarcolemmal MCT1 remains unaltered (present study and Ref. 50). A possible candidate is MCT2. This protein has been detected in hamster heart and skeletal muscle (17) and in human heart and muscle (30), as well as in other tissues (17, 18, 23, 28, 30, 39, 40). However, some have favored the idea that MCT2 is a high-affinity pyruvate transporter \( K_m = 25–80 \mu M \) (11, 30). But MCT2 also has an affinity for lactate \( [K_m = 0.74 \mu M (11), 6.5 \mu M (30)] \) that is comparable to the \( K_m \) for lactate by MCT1 \( 3.5–6.5 \mu M (12, 30, 32) \). Thus it would seem important to examine MCT2 in skeletal muscle. Unfortunately, this work has been hampered previously by the lack of a suitable antibody (Bonen A, unpublished data), although a suitable MCT2 antibody is now commercially available. In recent work, we have also found evidence that several other MCTs are also coexpressed in muscle (Bonen A, unpublished data), but whether these are also regulated by T3 or other physiological stimuli remains to be determined. Clearly, accounting for the flux of lactate in biologically important tissues has become difficult because of the apparent coexpression of a number of MCTs in the same tissue.

In summary, we have shown that T3 treatment increases MCT1 and -4 mRNAs. However, only MCT4 protein is upregulated, not the MCT1 protein. Interestingly, the increase in muscle homogenate MCT4 was far greater than the increase in sarcolemmal MCT4, suggesting that the increase in MCT4 protein was confined to other subcellular compartments in muscle. T3 treatment also doubled vesicular lactate transport, although the increase in sarcolemmal MCT4 protein would seem to be too modest to account for this, suggesting that other mechanisms and/or additional MCTs may have been involved in the increased rate of trafficking of lactate across the plasma membrane.

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DISCLOSURES

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