Regulation of the exercise-induced expression of the monocarboxylate transporters MCT1 and MCT4 in skeletal muscle

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Abstract Lactate is a dynamic substrate that has great potential as an energy source. During exercise, lactate is transported out of contracting muscle cells and is oxidized by the heart and oxidative types of muscles. The diffusion of monocarboxylates, such as lactate and pyruvate, across the plasma membranes of mammalian cells is facilitated by a family of integral membrane transport proteins called monocarboxylate transporters (MCTs). Currently, 14 unique members of the MCT family have been identified. In skeletal muscle, 2 isoforms with different kinetic properties, MCT1 and MCT4, are expressed and have been identified as key transporters regulating lactate flux across the plasma membrane. MCT1 is primarily expressed in oxidative fibers, in which lactate can be used as fuel for mitochondrial oxidation, and is closely associated with the rate of lactate uptake. MCT4 is highly expressed in glycolytic fibers and may be responsible for the removal of lactate produced by glycolysis. Rapid changes in the expression of MCT1 and MCT4 can occur in response to acute exercise, the regulation of which may involve transcriptional and post-transcriptional mechanisms. Increased MCT expression may be the primary factor enhancing the rate of lactate flux. However, the molecular regulatory mechanisms by which exercise upregulates MCT expression in muscle are not yet fully understood. It is important to understand how exercise regulates MCT expression so that training programs can be designed to increase the muscle oxidative disposal of lactate.

Keywords : exercise, lactate, signaling, MCTs, skeletal muscle

Introduction

Monocarboxylic acids play a major role in the metabolism of all living cells. Lactate, the end product of glucose metabolism from the glycolytic pathway, is a monocarboxylate, and together with pyruvate, plays an important role in cellular metabolism. Glycolysis produces 2 molecules of lactate for every glucose molecule consumed. Lactate must be transported out of glycolytic muscle cells if high rates of glycolysis are to be maintained. When the flux of lactate from muscle cells does not keep pace with its production, intracellular concentrations increase and cause the pH of the cytosol to decrease, which inhibits glycolysis. Other tissues such as the brain, heart, and oxidative fibers readily oxidize lactate, which can be used as a fuel source for ATP production in mitochondria¹⁻³. In these tissues, lactate is rapidly transported into cells. Thus, the production–oxidation cycle of lactate requires the exchange of this substrate between muscle fibers and other muscles^{1,4-6}, as well as other tissues such as the heart⁷⁻¹⁰ where lactate can be metabolized. The extru-

sion of lactate from muscle cells and its uptake by other muscle cells are regulated by a special family of proteins called monocarboxylate transporters (MCTs). In skeletal muscle, 2 isoforms, MCT1 and MCT4, are thought to be key transporters in lactate metabolism^{1,11}. These transporters facilitate the flux of lactate as well as other monocarboxylates across the plasma membrane with protons. MCTs function effectively not only in the transport of lactate in and out of cells, but are also the main factors that hinder the decrease in pH in exercising muscle because they co-transport protons together with lactate¹²⁻¹⁴.

Although lactate is a monocarboxylate whose transport across the plasma membrane is quantitatively the greatest, MCTs are essential for the transport of many other metabolically important monocarboxylates such as pyruvate and the ketone bodies acetoacetate, β -hydroxybutyrate, and acetate¹⁵. Thus, MCT1 and MCT4 play a central role in glycolytic and oxidative metabolism, and are critical for metabolic communication between muscle fibers during exercise. This is illustrated in Fig. 1, which highlights the important roles of MCTs in regulating monocarboxylic acids in skeletal muscle.

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Fig. 1 Metabolic pathways involved in monocarboxylate transport across the plasma membrane and mitochondria. Dotted lines indicate the oxidative pathway demonstrated in rat skeletal muscle¹⁶⁾. Dashed lines indicate the oxidative pathway demonstrated in L6 muscle cells¹⁷⁾. Abbreviations: G-1-P and G-6-P, glucose 1-phosphate and glucose 6-phosphate; AcβHB, acetoacetate plus β-hydroxybutyrate; LDH, lactate dehydrogenase; mt LDH, mitochondrial lactate dehydrogenase; PDH, pyruvate dehydrogenase; ATP syn, ATP synthase; TCA cycle, tricarboxylic acid cycle; ETC, electron transport chain; Cyt c, cytochrome c; Cox, cytochrome c oxidase.

Tissue distribution and lactate transport by MCT1 and MCT4

The MCT family of proteins was first identified a decade ago. At present, 14 MCT isoforms have been identified¹⁵⁾. In skeletal muscle, MCT1 was first detected in mitochondria-rich oxidative fibers by Garcia et al.¹⁸⁾. Later, McCullagh et al.¹⁹⁾ compared several rat hindlimb muscles and found a positive correlation between the distribution of oxidative fibers and the MCT1 protein; they also found that muscles rich in oxidative fibers, such as the red gastrocnemius and red tibialis anterior, could take up more lactate from circulation. These findings indicated that the role of MCT1 in the cell membrane may be to transport lactate into muscle cells where it can be used in oxidation^{20,21)}. Several subsequent studies confirmed that the MCT1 protein is correlated with the distribution of oxidative fibers in rat and human muscle^{6,7,11)}. Meanwhile, MCT4 was detected by Wilson et al.⁶⁾. The role of MCT4 may be to remove the lactate produced by glycolysis, which suggests that MCT4 has a different function in the cell membrane than that of MCT1^{6,22)}. Although the quantity of MCTs in the plasma membrane regulating the rate of lactate transport does not necessarily correspond to their expression^{23,24)}, the MCT1 and MCT4 proteins seem to be closely associated with their different functions in the cell membrane^{1,4,19,25)}.

A specific picture of the fiber type distribution of MCTs has also been obtained from immunohistochemical studies of human and rat muscles^{6,11,26,27)}. These studies confirmed at the cellular level that MCT1 was more highly expressed in the sarcolemma of oxidative muscle fibers, especially type I and IIA fibers, than in type IIB fibers. In contrast, immunohistochemical studies have shown that MCT4 is mainly expressed in non-oxidative type IIB fibers as well as type IIA fibers^{11,26,27)}. Several studies have shown that MCTs are also present in the mitochondrial membrane where they may be associated with either pyruvate or lactate transport or both^{2,17,28,29)}. Butz et al.²⁹⁾ showed that MCT1 was present in both subsarcolemmal and intermyofibrillar mitochondria. However, according to Benton et al.²⁸⁾, the expression of MCT1 is limited to subsarcolemmal mitochondria where it is co-expressed with MCT4. MCT2, a high-affinity pyruvate transporter, was also shown to be present in both subsarcolemmal (SS) and intermyofibrillar (IMF) mitochondria¹⁷⁾.

Structure of the transporter complex

Unlike the vast majority of membrane proteins, MCTs are not glycosylated³⁰⁾. Therefore, both MCT1 and MCT4 require an ancillary protein, CD147, a member of the immunoglobulin superfamily with a single transmembrane helix^{31,32)}. This interaction appears to assist MCT expres-

sion in the membrane. In the absence of CD147, these MCT proteins accumulate in the endoplasmic reticulum (ER) or Golgi apparatus³². For example, MCT1 has 12 membrane-spanning domains as well as intracellular N- and C-termini³³ (Fig. 2). Topology predictions have suggested a similar structure for MCT4¹³. Wilson et al.³⁴ reported that the active transporter complex includes two MCT1 and CD147 molecules each.

Changes in MCT1 and MCT4 mRNA and protein levels in muscle after a single acute bout of exercise

Previous studies have shown that acute exercise has a rapid effect on MCT1 and MCT4 expression in the skeletal muscles of humans and rats (summarized in Table 1). Although studies have been limited, it was shown that MCT1 and MCT4 expression at mRNA and protein levels was transiently upregulated by a single bout of prolonged exercise (>2 h) at a moderate intensity in rats³⁵ and humans³⁶. However, the expression of the MCT1 and MCT4 proteins may not always be concomitantly upregulated after acute exercise. Green et al.³⁸ showed that 16 h of heavy intermittent cycling exercise (6 min of exercise at 90% $\dot{V}O_2$ peak per hour for 16 h) in untrained subjects rapidly increased MCT4 protein levels (up 24%), but that MCT1 protein levels remained unchanged. However, whether or not changes in MCT protein expression occur after an acute bout of high-intensity muscle activity is more controversial. When MCT1 and MCT4 were measured immediately after an acute period of high-intensity interval training (HIT) in human subjects, their expression was observed to decrease acutely by approximately 20%³⁹. While this finding is concordant with the results of Tonouchi et al.²⁴ who reported a downregulation in the

plasma membrane protein contents of both MCT1 (10%) and MCT4 (25%) immediately after a 10-min high-intensity electrical stimulation in rats, other studies have shown that high-intensity exercise training can increase MCT1 and/or MCT4 protein levels in muscle^{40,41}. We recently examined the effects of an acute bout of high-intensity intermittent swimming exercise, a model of sprint interval exercise, on MCT1 and MCT4 protein expression in rat fast-twitch skeletal muscle, and compared them with the effects of 6 h of low-intensity prolonged swimming exercise, a model of endurance exercise. Our results demonstrated that MCT4 protein levels increased 18 h after acute, brief, high-intensity intermittent swimming with no change in MCT1 levels⁴², while the expression of MCT1 and its accessory protein, CD147 immediately increased at the end of a low-intensity prolonged swimming exercise, and remained elevated for 18 and 24 h, respectively⁴³.

Previous studies on humans have shown that MCT1 protein expression, but not MCT4 protein expression, is responsive to endurance training⁴⁴, whereas the protein expression of both these isoforms is responsive to high-intensity intermittent⁴¹, strength⁴⁵, and sprint⁴⁶ training. Thus, it has previously been suggested that MCT1 protein regulation in the skeletal muscle may occur irrespective of the type of training or exercise intensity⁴⁵. MCT4 protein expression is less easily perturbed by muscle activity, and apparently requires a more intense exercise^{38,42,47}. Our recent results have demonstrated the differential regulation of MCT1 and/or MCT4 protein expression after a single bout of swimming exercise at different intensities^{42,43}, which suggests that exercise intensity may be a regulating factor of the expression of MCT1, MCT4, and CD147 in fast-twitch muscle.

Fig. 2 The hypothetical structure of the MCT1/CD147 complex on the cell membrane modified from Wilson et al.³⁴. The CD147 is considered to be the chaperon protein for MCT1.

Table 1. Evidence for acute exercise-induced MCT1 and 4 upregulation in skeletal muscle of human and rat.

Acute exercise and electrical stimulation	Change	Muscle	Sex	Species	Reference
30 min treadmill exercise	↔MCT1 protein	soleus, red tibialis anterior, white gastrocnemius	♂	Rat	23
Exhaustive treadmill exercise	↔MCT1 protein	soleus	♂	Rat	37
5-6 h prolonged cycle exercise	↑ MCT1 protein ↑ MCT4 protein	vastus lateralis	♂	Human	36
10 min high-frequency electrical stimulation	↓ MCT1 protein ↓ MCT4 protein	hindlimb muscle	♂	Rat	24
2 h moderate-intensity treadmill exercise	↑ MCT1 protein ↑ MCT4 protein ↑ MCT1 mRNA ↑ MCT4 mRNA	soleus, red and white gastrocnemius soleus, red gastrocnemius red and white gastrocnemius red gastrocnemius	♀	Rat	35
high-intensity treadmill exercise to exhaustion	↑ MCT1 protein ↔MCT4 protein ↔MCT1 mRNA ↔MCT4 mRNA	vastus lateralis	♂	Human	48
45 s high-intensity cycle exercise	↓ MCT1 protein ↓ MCT4 protein	vastus lateralis	♀	Human	39
16 h heavy intermittent cycle exercise	↔MCT1 protein ↑ MCT4 protein	vastus lateralis		Human	38
5 min high-intensity intermittent swimming	↔MCT1 protein ↑ MCT4 protein ↑ MCT1 mRNA ↑ MCT4 mRNA	epitrochlearis epitrochlearis Triceps branchii muscle Triceps branchii muscle	♂	Rat	42
6 h low-intensity prolonged swimming	↑ MCT1 protein ↔MCT4 protein ↑ MCT1 mRNA ↑ MCT4 mRNA	epitrochlearis epitrochlearis Triceps branchii muscle Triceps branchii muscle	♂	Rat	43

↑, increase; ↓, decrease; ↔, no change.

The regulation of MCT1 and MCT4 protein expression appears to be more complex after acute exercise because the increase in MCT protein levels has not always been accompanied by a concurrent increase in corresponding mRNA levels. Bickham et al.⁴⁸⁾ reported increased MCT1 protein levels after a single exercise bout in humans without a corresponding increase in MCT1 mRNA levels, which suggests the post-transcriptional regulation of MCT1 expression. Similarly, Coles et al.³⁵⁾ showed that increases in MCT1 and MCT4 protein levels occurred with or without a concomitant increase in corresponding mRNA levels after a 2-h treadmill session in rats. The major regulating mechanism may be transcriptional; however, MCT1 may also allow the translational control of expression. Alternatively, it may be that pools of MCT1 mRNA are stored in mitochondrial ribonucleoproteins, which allow for rapid translation when more MCT1 is needed¹²⁾.

Potential regulatory mechanism of MCT1 and MCT4 expression

Although numerous studies have confirmed that the transport of lactate across rat skeletal muscle plasma membranes and MCTs can undergo adaptive changes, the molecular mechanisms involved in this regulation have

not yet been fully understood. At present, it has been suggested that MCT1 and/or MCT4 expression may be regulated via different mechanisms. In recent years, the identification of a lactate-sensitive transcription factor network in cultured L6 rat myocytes suggests that lactate may act as a signaling molecule to regulate MCT1, but not MCT4 expression⁴⁹⁾. Hashimoto et al.⁴⁹⁾ showed that lactate-induced upregulation of MCT1 was accompanied by a concurrent increase in PGC-1 α mRNA. Indeed, PGC-1 α has been identified as a key coactivator of some transcription factors that rapidly increase in muscles after exercise⁵⁰⁻⁵²⁾ and is involved in oxidative phenotypes and mitochondrial biogenesis⁵³⁾. Thus, MCT1 upregulation seems to share several similarities with mitochondrial biogenesis via lactate-PGC-1 α signaling. Benton et al.⁵⁴⁾ demonstrated that MCT1 was closely regulated by PGC-1 α . Increased PGC-1 α protein levels, obtained by transfecting PGC1 α -pcDNA into rat muscles, induced an increase in the protein expression of MCT1 and its ancillary protein CD147, but did not increase the expression of MCT2 or MCT4⁵⁴⁾. These findings strongly suggest that PGC-1 α is one of the key regulators of contraction-induced MCT1 expression.

Exercise-induced changes in MCT4 expression may not be regulated by PGC-1 α signaling. Other molecular factors may contribute to the regulation of MCT4 expression in fast-twitch muscle fibers. Ullah et al.⁵⁵⁾ showed that

MCT4 was upregulated by a hypoxia inducible factor 1 α (HIF-1 α) transcription factor-mediated mechanism, which suggests that HIF-1 α may be a regulating factor of MCT4 expression. Because MCT4 is inversely related to the oxidative capacity of muscle, the transcriptional corepressor RIP140 protein, which contributes to glycolytic fast-twitch fibers and downregulates mitochondrial biogenesis, has been proposed as a possible candidate regulator of MCT4 expression^{54,56}. Because β_2 -adrenergic stimulation of skeletal muscle is a signal that may be associated with intensive exercise, previous studies showed that clenbuterol, a β_2 -adrenergic agonist, increased RIP140 and MCT4 protein levels in the rat gastrocnemius⁵⁶⁻⁵⁸. Other studies have also shown that RIP140 behaves as a transcriptional coactivator of a number of transcription factors in various cellular responses^{59,60}. RIP140 may act as a coactivator by direct interaction with the NF- κ B subunit Rel A as well as the transcriptional factor CREB-binding protein (CBP) via its own NH₂-terminal domain^{60,61}. Further studies are needed to clarify the role of RIP140 and its transcriptional factors in the regulation of acute intensive exercise-associated MCT4 expression in muscle.

Another candidate molecular factor regulating MCT1 and/or MCT4 expression may be associated with changes in metabolic demands or changes in the energy charge of cells. A potential link between energy charge and gene transcription regulation may be the enzyme 5'AMP-activated protein kinase (AMPK). AMPK is activated primarily when the cellular energy charge decreases^{62,63}. A previous study suggested that exercise-inducible AMPK activation may be one of the key modulators of transcriptional regulation in skeletal muscle⁶⁴. To mimic the effects of exercise-induced AMPK activation in muscle, many studies have used the pharmacological agent aminoimidazole-4-carboxamide ribonucleoside (AICAR), an activator of AMPK, because the pharmacological activation of AMPK by AICAR is a key stimulus for increasing the expression of mRNA and protein in skeletal muscle. A recent study showed that AICAR treatment increased both MCT1 and MCT4 mRNA in a muscle fiber-type-specific manner⁶⁵. This indicates the possible involvement of an AMPK-mediated pathway in increased MCT1 and MCT4 transcription. Moreover, it is interesting to note that AICAR-induced MCT4 mRNA expression in prototypic embryonal rhabdomyosarcoma (RD) cells was blocked by compound C, an AMPK inhibitor, which suggests that AMPK activation may modulate the promoter activity of the MCT4 gene⁶⁵. Recently, we showed that 1mM caffeine, which increased AMPK phosphorylation without increasing calcium/calmodulin-dependent kinase II, up-regulated both MCT1 and MCT4 mRNA expression in rat epitrochlearis muscle⁶⁶. When AMPK phosphorylation was inhibited by dantrolene, neither MCT1 nor MCT4 mRNA expression was increased⁶⁶. This suggests that cytosolic Ca²⁺ and AMPK activation may be important mediators of MCT1 and MCT4 mRNA levels in skeletal

muscle. The hypotheses regarding the mechanisms that regulate MCT1 and MCT4 expression in response to exercise can be seen in Fig. 3. Further studies are needed to investigate the detailed mechanisms of the AMPK downstream pathway in the regulation of MCT4 expression.

Fig. 3 Hypotheses for the mechanisms responsible for increased transcriptional expression of MCT1 (Top) and MCT4 (bottom) in response to exercise. Exercise-induced expression of the *MCT1* and *MCT4* genes may be regulated by different mechanisms. MCT1 is closely regulated by PGC-1 α , which is associated with the AMPK or lactate signaling pathway. AMPK and p38MAPK also directly phosphorylate PGC-1 α , which leads to an increase in the transcriptional activation of the PGC-1 α promoter through an auto-regulatory mechanism^{50,67}. It has been suggested that MCT4 may be regulated by AMPK signaling or RIP140, which behaves as a coactivator of various transcription factors in response to exercise. HIF-1 α is a transcription factor necessary for the induction of the *MCT4* gene by hypoxia. Solid lines indicate the effects on muscles. Dashed lines indicate effects that have yet to be substantiated in muscles.

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