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Induction of amino acid transporters expression by endurance exercise in rat skeletal muscle

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ABSTRACT

We here investigated whether an acute bout of endurance exercise would induce the expression of amino acid transporters that regulate leucine transport across plasma and lysosomal membranes in rat skeletal muscle. Rats ran on a motor-driven treadmill at a speed of 28 m/min for 90 min. Immediately after the exercise, we observed that expression of mRNAs encoding L-type amino acid transporter 1 (LAT1) and CD98 was induced in the gastrocnemius, soleus, and extensor digitorum longus (EDL) muscles. Sodium-coupled neutral amino acid transporter 2 (SNAT2) mRNA was also induced by the exercise in those three muscles. Expression of proton-assisted amino acid transporter 1 (PAT1) mRNA was slightly but not significantly induced by a single bout of exercise in soleus and EDL muscles. Exercise-induced mRNA expression of these amino acid transporters appeared to be attenuated by repeated bouts of the exercise. These results suggested that the expression of amino acid transporters for leucine may be induced in response to an increase in the requirement for this amino acid in the cells of working skeletal muscles.

1. Introduction

Skeletal muscle is a highly plastic tissue that has the ability to alter the amount and composition of its subcellular components (such as contractile machinery, mitochondria, and glycolytic enzyme levels) in response to a variety of chronic perturbations, including, but not limited to, altered states of neural activity, mechanical stress, and physiological activity, as well as hormonal manipulation [1]. These adaptive processes in skeletal muscle should be accompanied by dynamic protein turnover (*i.e.*, new synthesis of required components and degradation of others).

It is now well recognized that protein synthesis is blunted during exercise, regardless of the type of exercise (*e.g.*, resistance exercise, or non-resistance exercise, like endurance exercise), and that, after exercise, muscle protein synthesis is upregulated during the recovery period [2–6]. Leucine, which is one of the branched-chain amino acids, is a good fuel for working skeletal muscle [7,8]. Moreover, leucine is not only one of the building blocks for proteins, but also upregulates protein synthesis through activation of the mammalian target of rapamycin complex (mTORC) 1 pathway [9,10]. These characteristics of leucine suggest that the requirement for leucine may be increased not only in working skeletal muscle, but also in muscle during recovery from exercise.

Plasma membrane transport system L is the pathway responsible for importation of large neutral amino acids, such as leucine [11]. System L transporters are composed of heterodimers of an amino acid permease (SLC7A5 or SLC7A8; or L-type amino acid transporter (LAT) 1 or LAT2) and the 4F2hc (SLC3A2; CD98) glycoprotein [12,13]. System L operates as an obligatory 1:1 heteroexchanger, facilitating uptake of leucine in exchange for certain cytoplasmic amino acids, such as glutamine. System A transporters (*e.g.*, SLC38A2; sodium-coupled neutral amino acid transporter (SNAT) 2) functions as secondary active transporters, coupling the uphill transport of amino acids (such as glutamine) to the inward movement of Na^+ down the electrochemical gradient, which is generated by Na^+-K^+ ATPase [14]. Recently, proton-assisted amino acid transporters (*e.g.*, SLC36; PAT1) have been proposed to activate the mTORC1 pathway in an amino acid-dependent manner on the surface of the lysosome [15,16].

These reports led us to hypothesize that amino acid transporters for leucine (which is utilized for fuel, protein synthesis, and as activator of mTORC1) on the plasma and lysosomal membranes in skeletal muscle would be induced by the exercise. In the present study, we determined whether endurance exercise would induce the expression of LAT1, CD98, SNAT2, and PAT1 in rat skeletal muscles. Furthermore, we determined whether expression of these amino acid transporters would be modified by the repeated bouts of endurance exercise training.

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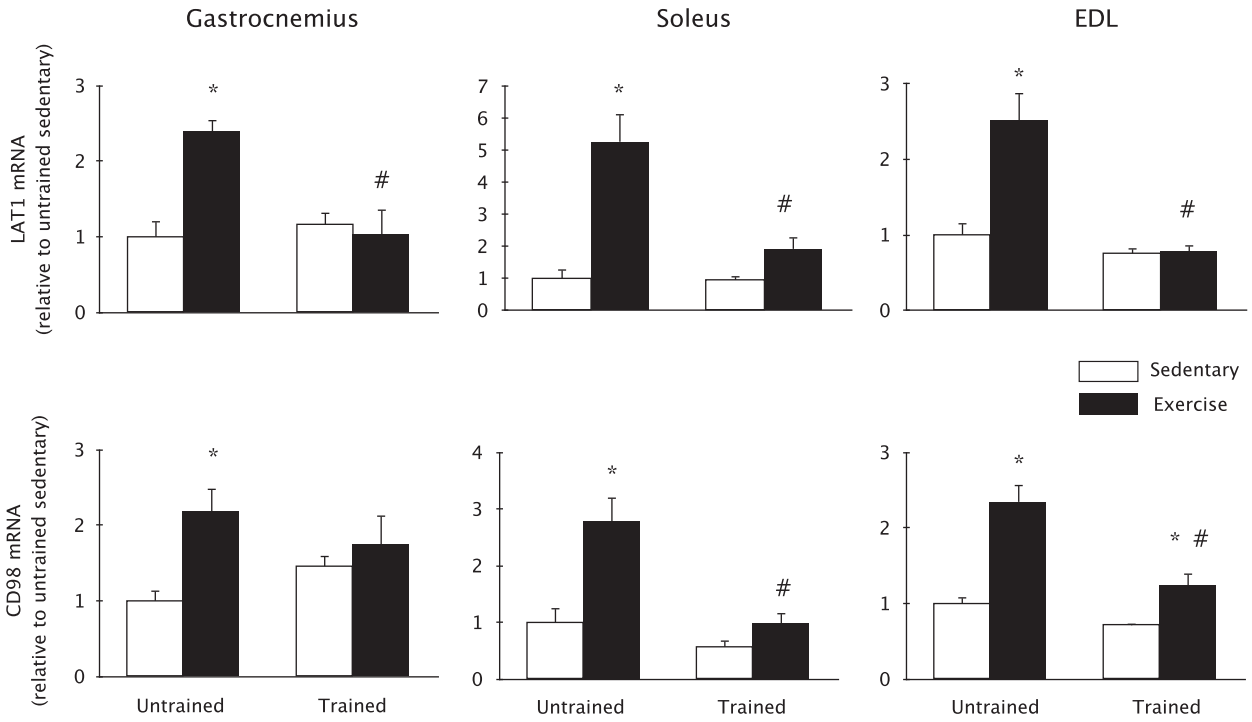

Fig. 1. The expression of mRNAs encoding system L amino acid transporters (LAT1 and CD98) is induced by a bout of endurance exercise in rat skeletal muscles. Values represent means ± SE for 4 rats. $^{*}P \leqslant 0.05$ vs sedentary group. $^{\#}P \leqslant 0.05$ vs untrained group.

2. Materials and methods

Sixteen male Wistar/ST rats aged 5-weeks-old were obtained from Japan SLC Inc., Hamamatsu, Japan. All procedures involving animals were approved by the Experimental Animal Care Committee of Shigakkan University. The rats were divided into untrained and trained ($n = 8$ in each group) groups. Rats in the trained group ran on a motor-driven treadmill 5 times per week (for a total of 12 times). The speed of the treadmill and running time were gradually increased and finally reached 28 m/min for 90 min. Rats in the untrained group were walked on a treadmill at a speed of 12 m/min for 10 min, with the same frequency of training as the trained group.

On the final day, the rats of both untrained and trained groups were subdivided into sedentary and exercise ($n = 4$ in each group) groups. The rats of the exercise group ran on the treadmill at 28 m/min for 90 min, and were anesthetized with sodium pentobarbital (50 mg/kg BW). Gastrocnemius, soleus, and extensor longus digitorum (EDL) muscles were quickly removed and freeze-clamped with an aluminum block that had been pre-cooled in liquid nitrogen. After collecting muscle samples, rats were sacrificed by severing the diaphragm and heart. The rats of the sedentary group were treated in a same manner except that they did not run on the treadmill.

Total RNA was extracted from the muscles with a SV Total RNA Isolation System (Promega, Madison, WI). Complementary DNA (cDNA) was generated using the PrimeScript RT reagent kit (Takara Bio, Ohtsu, Japan). Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was performed to measure mRNA levels of genes encoding the amino acid transporters, as described previously [17]. The primers for amino acid transporter genes were as follows: 5′-TCAAGCCGGTCTTCCCCACTTG-3′ (forward) and 5′-CACA CGGGTAGCAGCCTTCACA-3′ (reverse) for LAT1 cDNA, 5′-CCTGAATC CTTATGAGGGCTTG-3′ (forward) and 5′-CAGAATTCCAAAGGCCTG AGAG-3′ (reverse) for CD98 cDNA, 5′-GTGGCGTAGTCGTGATG-ATTG-3′ (forward) and 5′-CAGATGGACCGTTCAGTTTGA-3′ (reverse) for SNAT2 cDNA, 5′-GCGACTCCTCTACCAATTCCAC-3′ (forward) and 5′-CAGAGGCTGAGCCACACCTAA-3′ (reverse) for PAT1 cDNA, 5′-CGAACGTCTGCCCTATCAAC-3′ (forward) and 5′-GCCTTCCTTGGA TGTGGTAG-3′ (reverse) for 18S rRNA cDNA. The relative standard curve method was used to analyze the data, with relative amounts of unknown samples being calculated using linear regression analysis. The expression level for amino acid transporter mRNAs were normalized to the level for 18S rRNA and expressed relative to the level in sedentary rats that had not been trained. Data were expressed as means ± SE.

To evaluate the differences between groups, data were analyzed by 2-way factorial analysis of variance. If a significant difference was found, a Fisher's PLSD test was used for the post hoc test. Values of $P < 0.05$ were defined as statistically significant.

3. Results and discussion

The expression of LAT1 mRNA was induced by an acute bout of endurance exercise in gastrocnemius, soleus, and EDL muscles (Fig. 1). After the repeated bouts of exercise, however, the exercise-induced expression of LAT1 mRNA was no longer observed in those three muscles and the expression levels in trained-exercise groups were significantly lower than that in untrained-exercise groups in those muscles. The expression of CD98 mRNA was also induced by an acute bout of endurance exercise in gastrocnemius, soleus and EDL muscles (Fig. 1). After the repeated bouts of exercise, however, the exercise-induced expression of CD98 mRNA was no longer observed in gastrocnemius and soleus muscles. The expression level of CD98 mRNA in trained-exercise groups were significantly lower than that in untrained-exercise groups in soleus and EDL muscles. The expression of SNAT2 mRNA was induced by an acute bout of exercise in gastrocnemius, soleus and EDL muscles (Fig. 2). After the repeated bouts of exercise, however, the exercise-induced expression of SNAT2 mRNA was no longer observed in gastrocnemius and soleus mus-

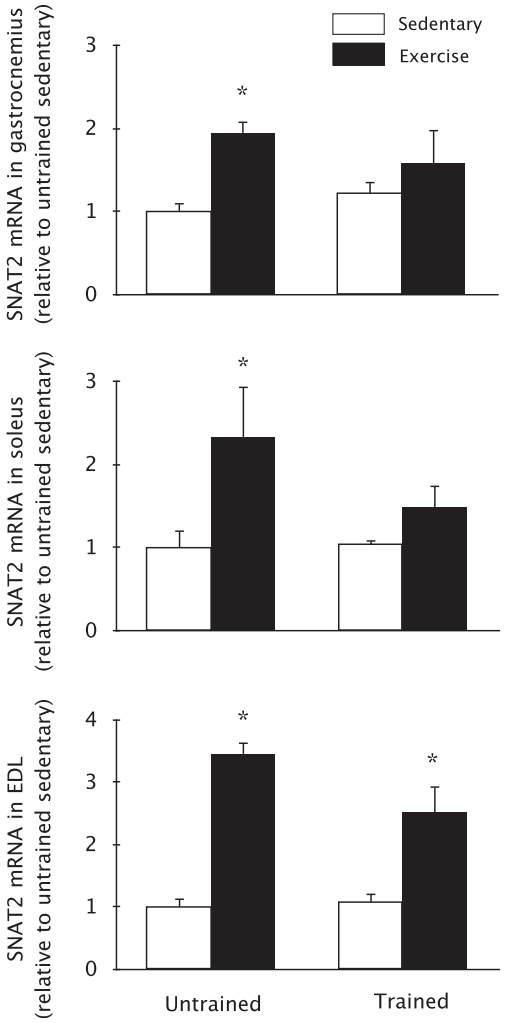

Fig. 2. The expression of mRNA encoding system A amino acid transporter (SNAT2) is induced by a bout of endurance exercise in rat skeletal muscles. Values represent means ± SE for 4 rats. $^{*}P \leqslant 0.05$ vs sedentary group.

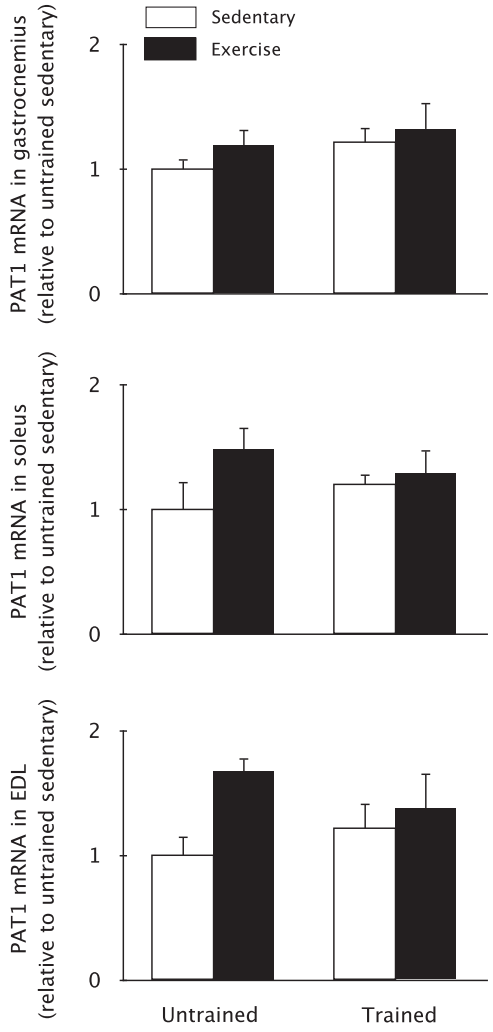

Fig. 3. The expression of the mRNA encoding proton-assisted amino acid transporter (PAT1) in rat skeletal muscles. Values represent means ± SE for 4 rats.

cles. Expression of PAT1 mRNA was slightly but not significantly induced by an acute bout of exercise in soleus and EDL muscles (the *P* values of main effect (Exercise) of 2-way ANOVA in soleus and EDL muscles were 0.138 and 0.063, respectively, Fig. 3). After the repeated bouts of exercise, no difference of the expression was observed between sedentary and exercise groups. These results suggested that expression of both system L and system A amino acid transporters, also presumably PAT1, were induced by an acute bout of exercise and their exercise-induced expression was then attenuated by repeated exercise training.

In the present study, the exercise-induced expression of amino acid transporters was attenuated by repeated exercise training. Although we could not measure the protein contents of amino acid transporters due to the unavailability of suitable antibodies for detecting endogenous amino acid transporters in muscle, it could not be excluded that the protein levels of amino acid transporters were increased as an adaptive response to the repeated bouts of exercise. The increase in the amino acid transporter protein levels may attenuate the exercise-induced stimuli to bring about gene expression. Another possibility is that the repeated exercise training attenuated expression of amino acid transporters by changing fueling in the trained muscle. A decrease in the relative intensity of the exercise or an increase in the ability to use free fatty acids for making ATP, which is often seen in muscle as an adaptive response to endurance training [1], may lead to a decreased requirement for leucine in the trained muscle.

It seemed to be reasonable that the expression of amino acid transporters for leucine was induced by exercise. Leucine has been reported to be used as a fuel in working muscle [7,8]. Furthermore, leucine upregulates protein synthesis by activating the mTORC1 pathway [9,10]. Our results suggested that the expression of amino acid transporters for leucine would be induced by exercise, because the requirement for leucine as a fuel, as a building block for proteins, and/or as a stimulator of mTORC1 would be increased in the muscle. Drummonds et al. [18] have reported that the expression of amino acid transporters were induced by a bout of resistance exercise in human skeletal muscle, suggesting that amino acid transporters are induced not only by exercise that induces muscle hypertrophy, but also by exercise that upregulates the oxidative capacity, for increasing the amino acid sensitivity of the muscle. This also suggests that common mechanisms may operate in induction of the expression of amino acid transporters in both types of exercise. Rapamycin has been reported to downregulate the expression of some amino acid transporters, including LAT1 [19,20], suggesting that mTORC1 regulates the expression of LAT1. Our previous study showed that mTORC1 signaling was downregulated by an acute bout of endurance exercise in the muscle [17]. These results suggested that expression of amino acid transporters for leucine would be regulated not only by mTORC1, but also by other mechanisms.

In the present study, we showed that expression of amino acid transporters for leucine was induced by an acute bout of endurance exercise. Furthermore, the exercise-induced expression of genes encoding amino acid transporters was attenuated by repeated exercise training. These results suggested that expression of genes encoding amino acid transporters for leucine may be induced in response to an increased requirement for this amino acid in the cells of working skeletal muscles.

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