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Original Article

Effects of voluntary wheel running and amino acid supplementation on skeletal muscle of mice

Maria Antonietta Pellegrino · Lorenza Brocca · Francesco Saverio Dioguardi · Roberto Bottinelli · Giuseppe D'Antona (✉)

M. A. Pellegrino · L. Brocca · R. Bottinelli · G. D'Antona

Department of Experimental Medicine, Human Physiology Unit, University of Pavia, Via Forlanini 6, 27100 Pavia, Italy

F. S. Dioguardi

Department of Internal Medicine, University of Milan, 20122 Milan, Italy

✉ G. D'Antona
Phone: +39-382-507252
Fax: +39-382-507664
E-mail: gdantona@unipv.it

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Abstract The aims of the present study were as follows: (1) to examine the adaptational changes to chronic endurance voluntary exercise and (2) to investigate the effects of amino acid supplementation on the adaptational changes induced by endurance training in hindlimb (gastrocnemius, tibialis, soleus) and respiratory (diaphragm) muscles of mice. Male C57Bl6 mice were divided in four groups: control sedentary, sedentary supplemented with amino acid mixture (BigOne, 1.5 mg g day⁻¹ in drinking water for 8 weeks), running (free access to running wheels for 8 weeks), and running supplemented with amino acid mixture. Myosin heavy chain (MHC) isoform distribution was determined in all muscles considered. Fiber cross-sectional area (CSA) was measured in the soleus muscle. In all muscles except the tibialis, endurance training was associated with an overall shift towards the expression of slower MHC isoforms. Amino acid supplementation produced a shift towards the expression of faster MHC isoforms in the soleus and diaphragm muscles, and partially antagonized the effects of training. Immunohistochemical analysis of CSA of individual muscle fibers from the soleus muscle suggests that voluntary running

produced a decrease in the size of type 1 fibers, and amino acid supplementation during training resulted in an increase in size in both type 1 and type 2A fibers. Collectively, these results suggest that the endurance adaptations induced by voluntary running depend on the muscle type, and that amino acid supplementation is able to modulate both fiber size and MHC isoform composition of skeletal muscles in sedentary and exercised mice.

Keywords Endurance training · Myosin heavy chain · Plasticity · Amino acids

Introduction

Exercise-induced skeletal muscle adaptations to voluntary wheel running and involuntary treadmill training have been evaluated in several studies and appear to depend on type and amount of exercise. In rats, these exercise protocols have been associated with a number of endurance-related muscle adaptations, including increased heart mass/body mass ratio (Baldwin et al. 1977; Tibbits et al. 1978; Hickson et al. 1983), increased percentages of slower, more oxidative contracting fibers (Fitzsimons et al. 1990; Ishihara et al. 1991; Sullivan et al. 1995; Demirel et al. 1999; Pansarasa et al. 2002) and higher mitochondrial enzyme levels (Holloszy 1967; Dudley et al. 1982; Rodnick et al. 1989; Kriketos et al. 1995; Pansarasa et al. 2002). Also in mice, voluntary (Dohm et al. 1994; Houle-Leroy et al. 2000; Allen et al. 2001; Harrison et al. 2002) and forced running (Kemi et al. 2002) protocols have been used to evaluate exercise performance and muscle endurance adaptations including cardiac hypertrophy and increased muscle oxidative capacity (Swallow et al. 1998; Houle-Leroy et al. 2000; Allen et al. 2001). Aspects that have received relatively less attention include the effects of voluntary running on size and type distribution of muscle fibers. Few studies (Garland et al. 1995; Allen et al. 2001; Harrison et al. 2002) have analyzed the effects on myosin heavy chain (MHC) isoform expression of skeletal muscles, and single-fiber cross-sectional area (CSA). Allen and co-authors (Allen et al. 2001) demonstrated a significant shift from 2B to 2A MHC expression in both gastrocnemius and tibialis muscles following free wheel running. On the contrary Zhan et al. (1999) detected no appreciable change in the fiber type percentage in the medial gastrocnemius of house mice after voluntary exercise. Uncertainty also exists regarding the effects of voluntary training on fiber CSA. Allen and co-authors detected changes in fiber size of both slow and fast fibers of the gastrocnemius muscle, whereas no significant changes in the mean fiber size were detected in tibialis muscle (Allen et al. 2001). Also, no changes of fiber CSA were observed by Zhan et al. (1999) in the medial gastrocnemius in both random-bred and

genetically selected mice. Furthermore, information regarding the effects of wheel running on postural and respiratory muscle fiber size and phenotype is still lacking. Thus, considering that mice are a species widely used for molecular biology and genetics, and that the free wheel running protocol is an easy and useful tool to highlight exercise adaptations in healthy and pathologic conditions, the first aim of this study was to evaluate the phenotype endurance adaptations of limb muscles versus postural muscles and diaphragm.

Recently protein or amino acid supplements have been frequently associated with exercise training in order to promote protein anabolism, muscle size and possibly strength. Most studies have focused on resistance training, and they have demonstrated that amino acid supplementation during the early recovery from resistance exercise may be responsible for a stimulatory effect on protein synthesis and be important for the development of hypertrophy (Biolo et al. 1995, 1997; Rasmussen et al. 2000; Blomstrand and Saltin 2001; Levenhagen et al. 2002). The precise mechanism involved has not been elucidated and appears related to the interactive effect of increased availability of intracellular amino acids, which in part might depend on enhanced blood flow and elevated muscle protein breakdown induced by exercise (Rasmussen et al. 2000). The latter findings suggest that the increased muscle delivery of amino acids co-operates with post-exercise protein synthesis (Tipton et al. 1999). No clear results have been reported for amino acid administration during endurance training. Some studies reported an increase of endurance performance after branched-chain amino acid (BCAA) supplementation in both rats (Calders et al. 1999) and humans (Blomstrand et al. 1995; Antonio et al. 2000). Others have demonstrated that administration of BCAA alone (Blomstrand et al. 1995) or combined with tryptophan (Blomstrand et al. 1991; van Hall et al. 1995) is not able to affect time to exhaustion during sustained exercise in humans and during acute treadmill running in rats (Verger et al. 1994).

Finally, in the elderly, evidence is accumulating that age-related loss in skeletal muscle mass may be partly counteracted by amino acid administration to both untrained (Volpi et al. 1998) and trained (Esmarck et al. 2001) individuals.

Furthermore, no studies have analyzed the impact of amino acid supplementation on muscle phenotype, namely fiber type or MHC isoform distribution, and CSA of muscle fibers.

Thus the second aim of the present study was to evaluate the adaptive response of the muscle phenotype to amino acid overload in both sedentary and trained mice. Together, our results demonstrated that the adaptive changes due to voluntary running depend on the muscle considered, and that amino acid supplementation is able to modulate both fiber size and MHC isoform composition of skeletal muscles.

Some of the results have been previously published in abstract form (D'Antona et al. 2001).

Methods

Animal treatment

The study was carried out on male C57Bl6 mice [average initial body mass 31 (3) g] purchased from Charles River (Calco, Italy). The animals were caged separately and randomly divided in four groups of eight animals: control sedentary group [C, water and standard diet ad libitum (18.8% protein content; Dottori Piccioni, Italy)], sedentary supplemented with amino acids (A, BigOne, Professional Dietetics, Milan; 1.5 mg g day⁻¹ in drinking water for 8 weeks), running (R, free access to running wheels for 8 weeks, water and standard diet ad libitum), and running supplemented with purified amino acids (RA, free access to running wheels for 8 weeks, and BigOne 1.5 mg g day⁻¹ in drinking water). Body mass, water and food consumption of each mouse were monitored weekly.

At the end of the experimental period, the animals were killed by cervical dislocation under ether anesthesia, as approved by the local Animal Ethic Committee. The heart and the following muscles were dissected and accurately weighed: diaphragm, soleus, gastrocnemius and tibialis.

The muscle samples were immediately immersed in fluid nitrogen and stored at -80°C until they were used for the subsequent analysis. The samples used for immunohistochemical analysis were carefully mounted in OCT (Tissue-tek) embedding medium before freezing.

Voluntary cage wheel exercise

Voluntary running was performed in hamster-sized plastic cage spheres with a stand (diameter of 12 cm) (Trixie, Heimtierbedarf, Jarplund-Weding). The exercise balls were fitted with magnetic counters (model mate-1, Techwell, Taiwan) and placed into 42×26×20 cm cages. After calibration for the wheel diameter, the magnetic counters measured the daily distance run and the total distance run. Each morning, daily distance and total distance were recorded for each trained animal, and the magnetic counter was reset.

Whole muscle MHC isoform composition

MHC isoforms were used as a molecular marker to assess the fiber-type composition of each muscle, and their expression was studied with polyacrylamide gel electrophoresis. Briefly, whole

muscle samples were dissolved in Laemmli solution (Laemmli 1970). Small amounts of the samples (2 μ l, corresponding to 500 ng of myosin) were applied onto 8% polyacrylamide gels prepared according to the method described by Talmadge and Roy (1993).

Electrophoresis was run for 2 h at 200 V, and 24 h at 250 V, and gels were stained with Coomassie blue. In the region of MHC isoforms (molecular mass \sim 220 kD), four bands were separated that corresponded, in order of migration from bottom to top, to MHC-1 (slower MHC isoform), MHC-2B (fastest isoform), MHC-2X and MHC-2A (intermediate velocities) (Pellegrino et al. 2003).

The relative proportions of these four MHC isoforms were determined by means of a computer-assisted densitometer. The areas under the peaks corresponding to the MHC isoforms on the densitometric readings were measured after background subtraction, and the area of each peak was expressed as a fraction of the total area of the four peaks. No correction for molecular mass was calculated.

Immunohistochemistry and muscle-fiber CSA

Serial transverse sections (10 μ m thick) of the soleus muscle samples mounted in OCT embedding medium were cut in a cryostat at -20°C and collected on coated slides. Cryosections were immunostained using two monoclonal antibodies against MHC isoforms: BA-F8 against MHC-1, SC-71 against MHC-2A. The staining procedure has been described in detail (Bottinelli et al. 1991; Pellegrino et al. 2003).

A secondary rabbit anti-mouse IgG antibody conjugated with peroxidase (DAKO P-0260) was used to reveal the binding of the primary antibodies. Two fiber types, type 1 and type 2A, were identified and CSA values were determined. Images at $\times 100$ magnification of the stained sections were captured using a video camera mounted on a light microscope (Laborlux, Leitz, Germany) and fed to a PC. Fiber CSA was measured for type 1 and type 2A fibers using Scion Image analysis software (NIH, USA). About 200 fibers per muscle were measured. CSA was expressed in micrometers squared.

Drugs and chemicals

All chemicals were purchased from Sigma (St. Louis, Mo.). The single amino acid composition and the relative percentage of the dietary supplement (BigOne) used in this study are reported in Table 1. The daily dose of the supplement was calculated with the purpose of mimicking the recommended daily dose of the mixture in exercising humans ($\sim 0.1 \text{ g kg day}^{-1}$), considering an

average mouse mass of 31 g and applying the Kleiber power function of basal metabolic rate scaling $aM(b)$, where a corresponds to a scaling constant, M is body mass, and b is the scaling exponent ($3/4$ best-fit b value for mammals) (Kleiber 1932; Darveau et al. 2002). Concentration of the amino acid mixture in drinking water was adjusted to the average daily water consumption of sedentary (6 ml) and exercising (12 ml) mice.

[Table 1 will appear here. See end of document.]

Statistical analysis

Data are expressed as means (SD). Statistical significance of the differences between means was assessed by ANOVA followed by Student–Newman–Keuls test. Comparisons have been reported between the following groups: C and A, R and RA, C and R, A and RA. A probability of less than 5% was considered significant ($P<0.05$).

Results

Body parameters

After 8 weeks, R ($n=8$) and RA ($n=8$) mice had run total distances of 337 (165) and 329 (144) km respectively, with average daily distances of 7.3 (3) and 6.4 (2) km, with no statistically significant difference. In both groups, the daily distance run appeared to be significantly lower during the first week, and increased during the second week and the third week, with no statistically significant changes during the following weeks (data not shown). The average values of daily food and water consumption, body mass gain and muscle-mass/body-mass ratio for the four groups of mice are shown in Table 2. Daily water consumption appeared to be significantly increased in both trained groups (R, RA), which did not show any significant variations for food consumption in comparison with the C group. Only amino acid administration to untrained mice (A) induced a significant decrease in food consumption in comparison with all the other groups. At the end of the 8-week experimental period, A mice showed a slight increase in their body mass in comparison with C mice, whereas a body mass loss was induced by training alone, and was partly prevented by amino acid supplementation to trained mice (RA, Table 2).

[Table 2 will appear here. See end of document.]

In all skeletal muscles except the soleus of A mice, muscle-mass/body-mass ratio did not show a statistically significant difference among groups, thus indicating that the mass of the hindlimb

skeletal muscles changed in close proportion with the body mass. On the contrary, the heart-mass/body-mass ratio showed a significant increase after training both in R and RA groups in comparison with the C group. Furthermore, an overall increase of the diaphragm-mass/body-mass ratio was also observed in A, R and RA groups, and appeared to be statistically significant only in R mice in comparison with C mice.

MHC isoform expression

MHC isoform composition was determined in three limb muscles (soleus, tibialis and gastrocnemius) and in one respiratory muscle (diaphragm) as an index of the fiber-type composition.

Average values of the MHC isoform composition of the soleus muscle in C and treated mice are shown in Fig. 1A. No significant difference in MHC-1 content was found among the groups. Amino acid supplementation (A) induced a significant increase in MHC-2B content, with a concomitant decrease in MHC-2A, and no statistically significant change in MHC-2X in comparison with C mice. On the contrary, voluntary wheel running (R) induced an increase of MHC-2A with a reduction of MHC-2X, and the disappearance of MHC-2B in comparison with C. RA was associated with a significant decrease of MHC-2A, a slight and not significant increase in MHC-1 and MHC-2X, and the disappearance of MHC-2B in comparison with C.

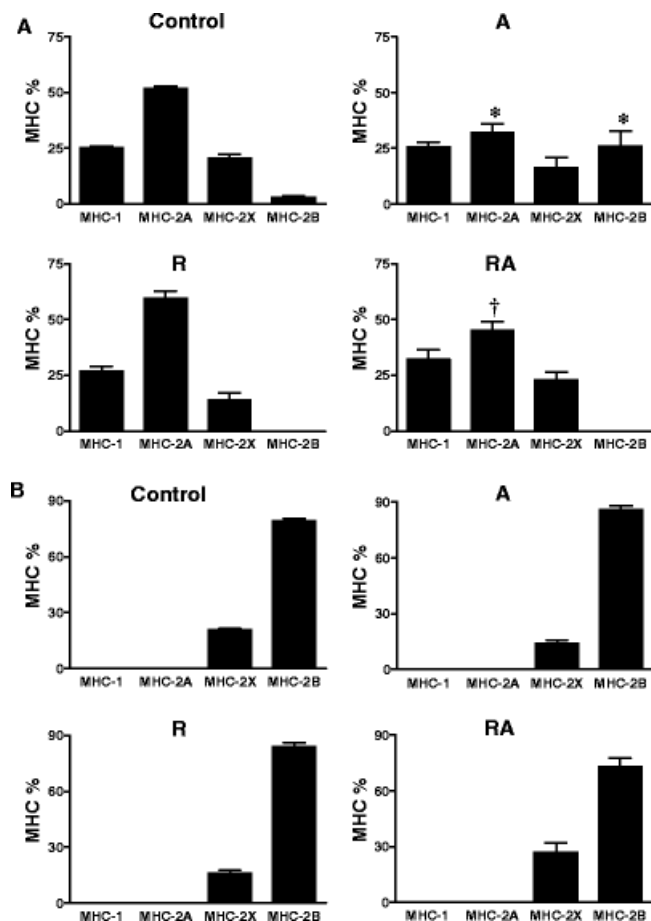


Fig. 1 Effects of treatments on myosin heavy chain (*MHC*) distribution in the soleus (**A**) and tibialis (**B**) muscles. *Significantly different from Control group ($P < 0.05$). †Significantly different from *RA* (running supplemented with amino acid mixture) group ($P < 0.05$). Values are means (SD), $n=8$

The tibialis muscle in C mice showed a clear predominance of MHC-2B content, with a much lower content in MHC-2X, and the absence of MHC-2A and MHC-1 (Fig. 1B). No significant effect of training or amino acid supplementation on MHC isoform content was seen, although a slight, but not significant, reduction in the proportion of MHC-2B isoform and a relative increase of MHC-2X were observed in RA.

As regards the gastrocnemius muscle (Fig. 2A), both C and A mice had a homogeneous MHC-2B isoform content. The gastrocnemius of R and RA showed the appearance of extra MHC isoforms: MHC-2X in R, and MHC-2X and MHC-2A in RA. The diaphragm (Fig. 2B) appeared to be receptive both to training and amino acid supplementation, showing distinct responses to treatments. Amino acid supplementation produced a moderate shift in the MHC composition towards a faster phenotype, with a significant decrease in MHC-2A, and a concomitant increase in MHC-2X. Endurance training was associated with an overall shift towards slower MHC isoforms: a significant

decrease in MHC-2B in R, and a significant increase in MHC-2A in both R and RA in relation to C.

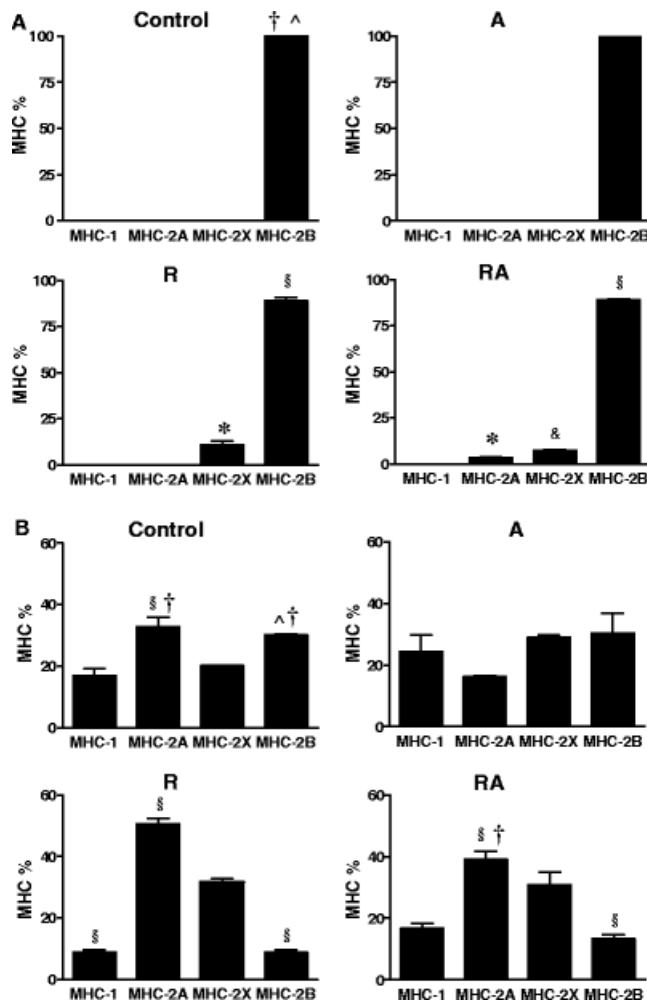


Fig. 2 Effects of treatments on MHC distribution in the gastrocnemius (A) and diaphragm (B) muscles. *Significantly different from Control group ($P < 0.05$). §Significantly different from A (sedentary supplemented with amino acid mixture) group ($P < 0.05$). ^Significantly different from R (wheel running) group ($P < 0.05$). †Significantly different from RA group ($P < 0.05$). Values are means (SD), $n=8$

CSA of individual muscle fibers

CSA of skeletal muscle fibers was analyzed in detail in the soleus muscle, which was taken as an example of a muscle highly responsive to both training and amino acid supplementation (Fig. 1). Examples of consecutive sections stained with antibodies specific for MHC-1 and MHC-2A are shown in Fig. 3. Changes in the number of fibers stained with each antibody are clearly visible, and confirm the trends already observed by the electrophoretic separation of the MHC isoforms. CSA of type 1 and type 2A fibers was measured as described in Methods. In the soleus, CSA

values of both type 1 and type 2A fibers were significantly higher in A than in C (Table 3). On the contrary, CSA of slow fibers was lower, and CSA of type 2A was unchanged in R in comparison with C. Amino acid supplementation to trained mice (RA) was associated with an overall increase of fiber CSA, which appeared more pronounced for type 2A fibers than for type 1 fibers in comparison with C (Table 3).

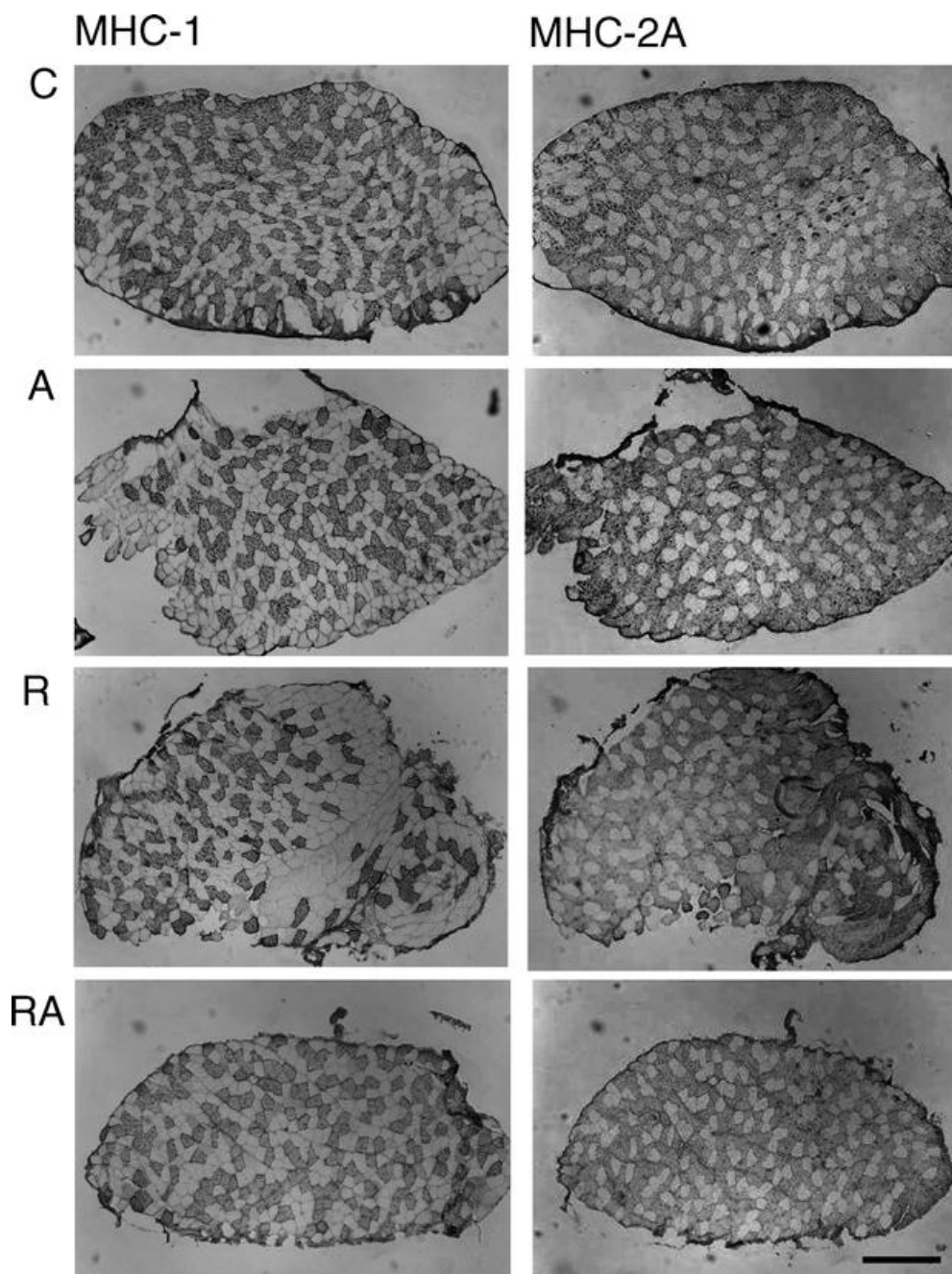


Fig. 3 Examples of immunostaining of the soleus muscle with anti-slow-MHC and anti-MHC-2A antibodies. Bar, 300 μ m. C Control group

[Table 3 will appear here. See end of document.]

Discussion

Adaptational responses to wheel running

The daily distance run per day reported in the present study is similar to that reported by Allen et al. (2001) for inbred C57BL/6 and Houle-Leroy et al. (2000) for outbred Hsd:ICR mice. The results obtained show that the intensity and duration of the exercise protocol were able to induce adaptational responses to exercise, including body mass loss, cardiac and diaphragm hypertrophy, and changes in MHC composition and fiber size.

Following long-term voluntary running, the muscle-mass/body-mass ratios indicated no change in the mass of the soleus, gastrocnemius and tibialis, which is consistent with the observation that endurance training does not determine hypertrophy in limb muscles (Booth and Thomason 1991). The selective hypertrophy observed in the heart, and a general fast-to-slow transition of MHC isoform composition of skeletal muscles, suggest that the exercise protocol actually determined endurance training (Pette 1998; Allen et al. 2001). The latter finding is in general agreement with previous studies in which the voluntary running protocol was used (Wernig et al. 1990; Allen et al. 2001), although it is at variance with another finding (Zhan et al. 1999). Such a discrepancy is likely due to the variable response of skeletal muscles to the exercise protocol. Indeed, we observed a larger fast-to-slow shift in soleus and diaphragm muscles in comparison with the gastrocnemius muscle, and no shift in the tibialis. The greater response of the soleus and diaphragm might be due to the higher MHC-1 and MHC-2A content in comparison with other muscles of the mice, the former being a postural muscle, and the latter a chronically active respiratory muscle. Moreover, such a chronic exercise at low intensity as voluntary running is bound to determine a more intense recruitment of the soleus (Baldwin and Haddad 2001) and the diaphragm than of gastrocnemius and tibialis, as the soleus is the slowest muscle of the limb (Pellegrino et al. 2003) with the highest oxidative metabolism, and the diaphragm is a respiratory muscle whose activity must increase to match the respiratory requirements of a chronic aerobic activity. The observation of diaphragm hypertrophy after training supports the latter hypothesis.

Interestingly, in the work by Allen et al. (2001), both the gastrocnemius and tibialis had a higher percentages of MHC-1, 2A and 2X pre-training than in present study, and both responded to training. Moreover, the response of the tibialis that had a significantly higher MHC-1 and 2A content than gastrocnemius was more pronounced. Collectively these observations suggest that voluntary running determines endurance training, and that such training produces larger effects

on muscles. These effects are slower pre-training, and they are likely to be more intensively recruited during training.

Surprisingly, neither in the soleus nor in the diaphragm did MHC-1 content significantly increase. However, as previously suggested (Sugiura et al. 1990, 1992; Wernig et al. 1990), an increase in MHC-2A content and a decrease in MHC-2B content is a clear indication of a shift towards a slower phenotype. Moreover, it is well established that contractile and energetic properties of types 2A, 2X and 2B vary greatly in small mammals (Schiaffino and Reggiani 1996) and in humans (Bottinelli and Reggiani 2000), with type 2A fibers being twofold slower than type 2B fibers.

The effect of voluntary running on CSA of individual fibers was studied in the soleus, as this appeared to be the most responsive muscle to voluntary training that has never been studied before in this respect. Interestingly, CSA of type 1 fibers decreased, and CSA of type 2A fibers was unchanged following training. The latter results are consistent with the observation that, with the exception of the diaphragm, endurance training does not represent a stimulus for muscle hypertrophy (Table 2). Moreover, the CSA data are in agreement with previous observations on endurance training in humans that showed no effect on fiber size (Fitts et al. 1989; Schluter and Fitts 1994) or even a decrease (Widrick et al. 1996). Previous analyses of CSA of individual fibers following voluntary running have shown either no change in the gastrocnemius (Zhan et al. 1999), or an increase in the gastrocnemius but not in the tibialis (Allen et al. 2001). Such lack of consistency might find an explanation in the different muscles examined, and in the variable responses of postural/slow muscles and of fast muscles to the exercise protocol, as suggested above.

Amino acid supplementation

It is widely accepted that hyperaminoacidemia at rest moves the balance between protein synthesis and degradation towards synthesis (Bennet et al. 1989; Smith et al. 1998).

Furthermore, to date, several studies have focused on the stimulatory effect of amino acid supplementation on post-resistance-exercise repair and synthesis of muscle proteins (Gibala 2000). It has been demonstrated that hyperaminoacidemia and strength training have an additive effect on net muscle protein balance, and both oral (Tipton et al. 1999) and intravenous (Biolo et al. 1997) amino acid supplementation during the early recovery from resistance exercise promotes muscle hypertrophy (Biolo et al. 1995; Rasmussen et al. 2000; Blomstrand and Saltin 2001; Esmarck et al. 2001; Levenhagen et al. 2002).

Amino acid supplementation during endurance training has been less studied. Previous data have suggested that amino acid supplementation in combination with carbohydrates may serve to

minimize lean tissue mass loss through stimulation of insulin secretion (Hutton et al. 1980; Castellino et al. 1987; Rasmussen et al. 2000) and alterations in the ratio of free tryptophan to BCAA, thus diminishing the elevation of 5-hydroxytryptamine and improving the physiological and psychological responses to endurance (Blomstrand et al. 1989, 1991). However, no definitive data have emerged on the effects of amino acid supplementation on the endurance capacity in small mammals and humans (Blomstrand et al. 1991, 1995; Verger et al. 1994; van Hall et al. 1995; Calders et al. 1999; Antonio et al. 2000; Blomstrand and Saltin 2001), and no data are available on fiber-type composition and single-fiber CSA after long-term endurance training and supplementation in rodents and humans.

The amino acid supplement used in the present study was a balanced mixture of all the essential amino acids and non-essential tyrosine. Among the amino acids in the mixture, leucine (30%) and tyrosine (0.4%) may play a key role in determining the observed muscular effects. In fact leucine supplementation is known to participate greatly in protein metabolism regulation (Nair et al. 1992), whereas tyrosine, as the rate-limiting dopaminergic precursor, may relieve the adverse effects of physical stress (Deijen and Orlebeke 1994; Avraham et al. 1996, 2001; Deijen et al. 1999; Georgiades et al. 2003).

Amino acid supplementation per se decreased food consumption, as expected, on the basis of the extra caloric intake represented by the amino acid mixture in the drinking water, and determined hypertrophy of the soleus muscle, with no change in the other muscles examined or the heart. Amino acid supplementation determined a significant shift towards faster MHC isoforms in the soleus and diaphragm, and no shift in the tibialis and gastrocnemius. The lack of an effect on the MHC isoform distribution of the latter two muscles is very likely due to their homogenous MHC-2B isoform composition that actually prevents any further shift towards a faster phenotype. Interestingly, notwithstanding the clear slow to fast shift among fast MHC isoforms, the percentage of MHC-1 did not change, suggesting no effect of the amino acid mixture on fiber-type transformation from the slow to the fast types 2A, 2X and 2B.

On the contrary, amino acid supplementation had similar effects on the size of both type 1 and type 2A fibers, as shown by the analysis of CSA of individual fibers in soleus. The clear difference in the fiber size between slow and fast fibers observed in C mice appeared to be maintained after amino acid supplementation (Table 3).

Amino acid supplementation during voluntary running had significant effects on body mass, muscle mass, fiber size, and MHC isoform distribution. Indeed endurance training and amino acid supplementation showed opposite effects.

Amino acid supplementation to trained mice appeared to more than counteract the loss of body mass observed following training. The observation that the muscle-mass/body-mass ratio of all limb muscles was not different in C and RA, suggests a relative equal effect of the amino acid treatment on muscle mass and body mass. That CSA values of both type 1 and type 2A fibers of the soleus muscle were higher in RA than in R and C, suggesting that amino acid supplementation maintains muscle mass through a relative hypertrophy of individual muscle fibers, and that such effect more than counteracts the effect of training itself. Finally, the larger hypertrophic effect of amino acid supplementation on type 2A than on type 1 fibers suggests that the shift towards a faster phenotype in RA could be at least partially due to a preferential increase in size of fast fibers.

The reason for the distinct adaptational response to the chronic administration of amino acids in trained and untrained mice in terms of fiber size is unknown, and it might depend on the combination of a selective stimulatory effect of the amino acid mixture on fast MHC synthesis and the absence of effects of prolonged endurance exercise on slow-fiber size (Fitts et al. 1991; Fitts and Widrick 1996).

Finally, no effects of the chronic supplementation were observed on daily run distance and entire performance, thus suggesting, in agreement with previous studies (Verger et al. 1994; Blomstrand et al. 1995; Vukovich et al. 1997), the inefficacy of amino acid supplementation in enhancing endurance capacity.

In conclusion, our findings confirm that the voluntary wheel running protocol may be considered as a powerful tool to analyze the adaptational muscle changes caused by endurance exercise and pharmacological treatments in the mouse. We demonstrated, for the first time, that chronic voluntary running is associated with selective muscle adaptation in both postural and respiratory muscles, including changes in fiber size and fast-to-slow MHC transition. Furthermore, this study reports the first description of the differential effects of chronic amino acid supplementation in untrained and endurance-trained conditions. Notwithstanding, it can be hypothesized that amino acid administration may reduce the net protein degradation (Carli et al. 1992), possibly as a result of increased MHC synthesis. The molecular mechanisms underlying such changes and the basis of the fiber-type selectivity need to be further investigated.

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Table 1 Composition and relative percentage of the amino acid dietary supplement (BigOne)

Amino acid	Molecular mass (g mol ⁻¹)	Percentage (%)
Leucine	131.2	30.5
Lysine	146.2	13.2
Isoleucine	131.2	15.6
Valine	117.1	19.6
Threonine	119.1	10.8
Cysteine	121.2	4.4
Histidine	155.2	2.7
Phenylalanine	165.2	1.6
Methionine	149.2	1.0
Tyrosine	181.2	0.4
Tryptophan	204.2	0.2

Table 2 Body parameters and muscle-mass/body-mass ratio. Data are presented as means (SD). *A* Amino acid supplementation, *R* free wheel running, *RA* free wheel running and amino acid supplementation, *n* no. of mice, m_g body mass gain, m_b body mass, FC daily food consumption, WC daily water consumption, m_m muscle mass

	<i>n</i>	Control	<i>A</i>	<i>R</i>	<i>RA</i>
m_g (g)	8	3.66 (2.42)	4.39 (3.98)	-0.75 (2.72)*	0.33 (1.81)
FC	8	5.32 (0.82)	2.10 (0.65)*	6.61 (1.22)	6.16 (0.65)
WC	8	7.24 (1.36)	6.32 (0.82)	12.33 (3.23)**	12.86 (1.97)**
Heart $_{m/m}$ (%)	8	0.40 (0.05)	0.50 (0.04)	0.58 (0.03)***	0.56 (0.04)***
Soleus $_{m/m}$ (%)	8	0.034 (0.01)	0.048 (0.01)*	0.032 (0.01)	0.037 (0.01)
Tibialis $_{m/m}$ (%)	8	0.21 (0.08)	0.19 (0.03)	0.19 (0.06)	0.17 (0.04)
Gastrocnemius $_{m/m}$ (%)	8	0.50 (0.08)	0.56 (0.06)	0.49 (0.13)	0.49 (0.10)
Diaphragm $_{m/m}$ (%)	8	0.31 (0.05)	0.36 (0.03)	0.41 (0.06)***	0.34 (0.05)

*Significantly different from the other groups ($P<0.05$)

**Significantly different from Control and A ($P<0.05$)

***Significantly different from Control ($P<0.05$)

Table 3 Effects of treatments on cross-sectional area (micrometers squared) of muscle fibers in the soleus muscle. Values are means (SD), $n=4$

Group	MHC-1	MHC-2A
Control	2,309 (619)	2,101 (487)
A	2,581 (619)*	2,328 (406)*
R	2,004 (670)*	2,160 (504)
RA	2,485 (696)***	2,684 (614)*****

*Significantly different from Control ($P<0.05$)

**Significantly different from A ($P<0.05$)

***Significantly different from R ($P<0.05$)