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Histomorphometric analysis of the response of rat skeletal muscle to swimming, immobilization and rehabilitation

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The objective of the present study was to determine to what extent, if any, swimming training applied before immobilization in a cast interferes with the rehabilitation process in rat muscles. Female Wistar rats, mean weight 260.52 ± 16.26 g, were divided into 4 groups of 6 rats each: control, 6 weeks under baseline conditions; trained, swimming training for 6 weeks; trained-immobilized, swimming training for 6 weeks and then immobilized for 1 week; trained-immobilized-rehabilitated, swimming training for 6 weeks, immobilized for 1 week and then remobilized with swimming for 2 weeks. The animals were then sacrificed and the soleus and tibialis anterior muscles were dissected, frozen in liquid nitrogen and processed histochemically (H&E and mATPase). Data were analyzed statistically by the mixed effects linear model ($P < 0.05$). Cytoarchitectural changes such as degenerative characteristics in the immobilized group and regenerative characteristics such as centralized nucleus, fiber size variation and cell fragmentation in the groups submitted to swimming were more significant in the soleus muscle. The diameters of the lesser soleus type 1 and type 2A fibers were significantly reduced in the trained-immobilized group compared to the trained group ($P < 0.001$). In the tibialis anterior, there was an increase in the number of type 2B fibers and a reduction in type 2A fibers when trained-immobilized rats were compared to trained rats ($P < 0.001$). In trained-immobilized-rehabilitated rats, there was a reduction in type 2B fibers and an increase in type 2A fibers compared to trained-immobilized rats ($P < 0.009$). We concluded that swimming training did not minimize the deleterious effects of immobilization on the muscles studied and that remobilization did not favor tissue re-adaptation.

Key words: Swimming training; Female rat skeletal muscles; Immobilization; Morphometry; ATPase

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Introduction

Skeletal muscle has the important property of adapting to different functional demands by means of cellular structural modifications, such as disuse by immobilization to high resistance exercises (1).

Immobilization, a procedure frequently employed in the treatment of the musculo-skeletal system, often has some deleterious effects such as atrophy, intramuscular fibrosis, loss of extensibility, and reduced resistance of ligaments and tendons (2,3). In addition, it causes reductions in size and number of mitochondria, of the quantity of glycogen and resting ATP, of ATP concentration with exercise, of protein synthesis, and of capillary density (4).

There is a discrepancy in the literature regarding the degree of atrophy produced in different fiber types. How-

ever, the most common observation is that all fiber types and subtypes are affected similarly (1,5). In view of the reversibility of atrophy, muscles with a predominance of type 1 fibers, such as the soleus, seem to recover more rapidly than muscles with a predominance of type 2 fibers, such as the tibialis anterior, mainly due to better circulation and protein renovation (6,7).

Physical exercise is the counter-measure most extensively studied in order to relieve or prevent these responses to immobilization. However, the process of musculo-skeletal tissue restructuring seems to require more time than that needed to cause atrophy by immobilization (3). Several studies have focused on physical exercise applied after a period of inactivity, but few studies have emphasized the potential improvement of subjects who exercise before immobilization (8), this topic being the main subject of the present investigation.

It has been reported that structural and mechanical characteristics of skeletal muscle are preserved by long-term strength training (9). The effort in aerobic exercise, regular low-tension and high-repetition physical activity, such as walking or swimming for 30-60 min, improves muscle cell capacity (10,11). This type of training increases the quantity of mitochondria as well as glycogen content, capillary density, capillary relation per fiber, mean number of capillaries in contact with each muscle fiber, and the proportion of muscle cells identified as having high oxidative capacity (4,10,11). Some investigators suggest that endurance training maintains the aerobic capacity of muscles but does not slow down the loss of muscle mass, atrophy of type 2 fibers and muscle force production with aging (12,13). This negative effect on the cross-sectional area was confirmed by another study (14). In contrast to these findings, Klitgaard et al. (15) reported that chronic swimming in rats induces an increase in muscle cross-sectional area.

Experiments in rats, cavies, dogs, rabbits and other mammals attempt to simulate the physical stress conditions observed in humans as a contribution to a more efficient monitoring of the systemic changes due to exercise. Rats are the animals most frequently used because, in addition to being small and easy to manipulate, they present a good response to swimming exercise (16,17). Detailed quantitative information regarding the extent to which the loss of morphological and functional properties of skeletal muscle can be prevented during immobilization is scarce in the literature (3). Thus, the hypothesis that physical training prior to immobilization may cause skeletal muscle to better withstand atrophy has not been well documented.

The objective of the present study was to determine by means of morphometric analyses to what extent swimming training prior to immobilization with a cast facilitates or not the process of morphologic recovery induced by rehabilitation.

Material and Methods

Animals and experimental groups

Female Wistar rats weighing 235-285 g (mean, 260.52 ± 16.26 g) were obtained from the Central Animal House of the Ribeirão Preto Campus, University of São Paulo. The animals were housed 3 to a standard cage, with a total area of 1162.5 cm^2, at room temperature in the Laboratory of Bioengineering, Faculty of Medicine of Ribeirão Preto, University of São Paulo, with free access to water and standard chow. The animals were divided into four experimental groups of 6 rats each: control (CG), trained (TG), trained-immobilized (TIG), and trained-immobilized-rehabilitated (TIRG).

All experimental protocols were approved by the Ethics Committee for the Use of Animals of the Ribeirão Preto Campus (protocol #04.1.374.53.4).

Experimental protocols

CG animals were housed in standard cages (total floor area of 1162.5 cm^2) for 42 days. The animals in the trained groups (TG, TIG, and TIRG) were submitted to swimming for 6 weeks, 5 days per week, for a total of 30 sessions. Two days of rest were allowed between weeks for muscle recovery. During the first week, the animals were allowed to adapt by undergoing training sessions of increasing duration from 10 to 20, 30, 40, and 50 min. In the second week, they trained for 2 days of 50-min sessions followed by 3 days of 60-min sessions, with no body overload. At the third week, training for progressive adaptation to effort was started with the introduction of a load of 5% of body mass and swimming for 60 min/day for both weeks 3 and 4. In the last 2 weeks, the animals swam for 60 min/day with an 8% work overload. Peijie et al. (18) reported that swimming with external weight equal to 6% of body mass corresponded to high-intensity activity. As other studies indicate, 6 weeks represent a sufficient time for the induction of structural and functional changes in the organism of rats (19).

For the groups submitted to cast immobilization (TIG and TIRG), casts were prepared according to the protocol described by Carvalho et al. (20) and left in place for 7 consecutive days. Animals were anesthetized with an intramuscular injection of 7 mg/kg hyaline combined with 60 mg ketamine and the right pelvic limb was immobilized with the hip and knee in extension and the ankle in plantar flexion.

TIRG animals were remobilized by swimming training (5 consecutive days for 2 weeks, for a total of 10 sessions) in a rehabilitation program that was the same as the first 2 weeks of swimming training.

After the experimental period, the animals were sacrificed with excess sodium thiopental anesthesia, the right posterior limb submitted to trichotomy and the soleus and tibialis anterior muscles exposed by a skin incision. Fragments of the center of the ventral portion of the dissected muscles were recovered, coated in talc and frozen in liquid nitrogen. The material was stored at -80°C until use.

Morphological analysis

Transverse sections (5 µm thick) of the muscle fragments were obtained with a Tissue-Tek® $Cryo_3$® cryotome (Sakura, USA), stored in a freezer at -80°C and later processed by the following staining and histochemical procedures: hematoxylin and eosin (H&E), Gomori trichrome (modified), and myofibrillar adenosine triphosphatase (mATPase, pH 9.4, 4.6, and 4.3) (21).

Morphometric analysis

Morphologic analysis was carried out in all 6 rats of each group using a Leica DM 2500 light microscope (UK). For morphometry, a digital Leica DC 300FX camera and the QualiView-Atonus software were used and the images were obtained from slides processed with mATPase at acid pH. Considering the lesser diameter (the greatest distance between the opposite sides of the narrowest aspect of the fiber) (22), 100 fibers with light and dark staining and 50 fibers with intermediate/hybrid staining were measured. The proportion of fibers (relative distribution of fibers in the field) was assessed by counting fibers in 5 random fields in preparations of both muscles. Type 1 fibers (T1F), type 2A fibers (T2AF), and intermediate/hybrid-type fibers (Int/HTF) were counted for the soleus muscle, and T1F, T2AF, Int/HTF, and type 2B fibers (T2BF) were counted for the tibialis anterior muscle in its deep portion.

Statistical analysis

Data were analyzed using mixed-effect linear models, considering the diameter of the fibers or the proportion of fibers as response variables. The models were fitted to the data by using the animals as the random factor. Multiple comparisons were performed by contrasts. For each of the linear models, residual normalities were checked using normal probability plots. All statistical analyses were performed using the SAS software, version 9, with the level of significance set at ≤ 0.05.

Results

Morphologic analysis of the soleus muscle

As shown in Table 1, TG induced changes of a degenerative-regenerative nature, observed as lobulated fibers, necrosis, cell fragmentation, fiber size variation and centralized nucleus and a slight increase in connective tissue, in the soleus muscle fibers (compare CG, Figure 1A, with TG, Figure 1B). These structural changes were proportionally intensified after immobilization (TIG, Figure 1C), with the presence of necrotic fibers, an inflammatory infiltrate, cellular lobulation and variation in fiber size. The remobilization process (TIRG, Figure 1D) caused modifications of a predominantly regenerative nature. A moderate increase of the perimysial connective tissue is shown in the inserts of Figure 1C and D.

Morphologic analysis of the tibialis anterior muscle

As shown in Table 1, structural changes of the tibialis anterior muscle fibers were minor except for variation in size. Among the variables analyzed, fiber size showed an intense variation in TG (data not shown) and TIG (Figure 1E) and mild variation in TIRG (Figure 1F). Slight cellular lobulation was observed in TIG and TIRG and a slight increase in the expression of connective tissue was observed in TG and TIG.

Morphometric analysis

Lesser fiber diameter of the soleus and tibialis anterior muscles. Table 2 presents the mean lesser fiber diameters

Table 1. Morphological changes of soleus and tibialis anterior muscle fibers.

	Soleus				Tibialis anterior			
	CG	TG	TIG	TIRG	CG	TG	TIG	TIRG
Centralized nucleus	0	++	+	++	0	0	0	0
Variation in size	++	+++	+++	+++	0	+++	+++	+
Lobulated fibers	+	++	++	+	0	0	+	+
Increased connective tissue	0	+	++	++	0	+	+	0
Fragmentation of nucleus or cell	0	+	+	+				
Inflammatory infiltrate	0	0	+	+				
Necrosis	0	+	+++	0				

Data are reported for 6 rats in each group. CG = control group; TG = swimming trained group; TIG = swimming trained and immobilized leg group; TIRG = swimming trained, immobilized leg and rehabilitated group. 0 = no change found, + = mild incidence, ++ = moderate incidence, +++ = intense incidence.

obtained for the groups studied. Swimming training (CG x TG) did not affect the lesser diameter of T1F or T2AF, but did have a negative effect on Int/HTF size (CG x TG, $P < 0.001$) in the soleus muscle. Immobilization after training caused atrophy of only T1F and T2AF (TG x TIG, $P < 0.001$). Rehabilitation after swimming training and immobilization, in turn, did not cause significant changes in the lesser diameter of T1F, T2AF or Int/HTF (TIRG x TIG; TIRG x TG, NS). No significant changes in the lesser fiber diameter were observed in the tibialis anterior muscle.

Fiber proportion in the soleus and tibialis anterior muscles. The results obtained for the soleus muscle indicated that only swimming training caused an increase in T2AF and a decrease in T1F compared to CG (TG x CG, $P < 0.001$; Table 3). For the tibialis anterior muscle, immobilization after training caused a reduction in T2AF and an increase in T2BF compared to swimming training alone (TG x TIG, $P < 0.001$). However, rehabilitation after swimming and immobilization (TIRG) caused an increase in T2AF and a reduction in T2BF compared to TIG (TIG x

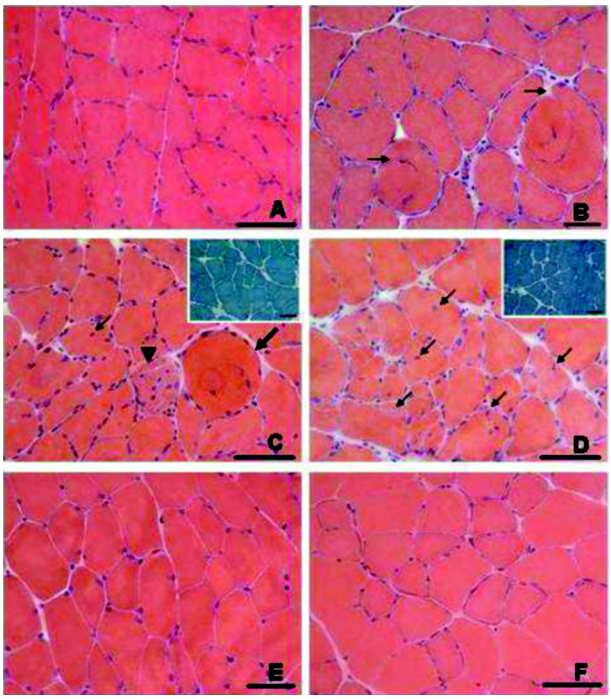

Figure 1. Muscle transverse sections stained with H&E reaction. *A*, Soleus muscle. Micrograph of control rat (CG) showing fibers of uniform size with peripheral nuclei (Bar = 41 µm). *B*, Soleus muscle. Micrograph of swimming trained rats (TG) presenting cellular lobulation, cell splitting (arrow) and a discrete increase in the connective tissue (Bar = 41 µm). *C*, Soleus muscle. Micrograph of swimming trained and immobilized leg rats (TIG) showing cellular lobulation, cell splitting (thick arrow), centralized nuclei (arrow), small cells grouping (arrowhead), and irregularity of fiber size (Bar = 41 µm). *D*, Soleus muscle. Micrograph of swimming trained, immobilized leg and rehabilitated rats (TIRG) presenting irregular fiber sizes and centralized nuclei (arrows; Bar = 41 µm). The insets in C and D indicate a moderate increase of the perimisial connective tissue compared to *A* (modified Gomori stain) (Bars = 29 µm). *E*, Tibialis anterior muscle. Micrograph of TIG showing fibers with peripheral nuclei and irregular fiber sizes (Bar = 41 µm). *F*, Tibialis anterior muscle. Micrograph of TIRG presenting irregularity in fiber size (Bar: 41 µm).

Table 2. Mean lesser diameter of fibers of the soleus and tibialis anterior muscles of rats as the result of swimming training, leg immobilization and rehabilitation.

	Mean lesser diameter (µm)							
	Soleus				Tibialis anterior			
	CG	TG	TIG	TIRG	CG	TG	TIG	TIRG
T1F	49 ± 4.6	46.1 ± 3	38.8 ± 5*+	39.7 ± 2+	32.76 ± 2	33.42 ± 1	37.39 ± 6.1	35.84 ± 4.3
T2AF	40.22 ± 6.8	42.22 ± 1.9	32.11 ± 2.2+	35.16 ± 2.7	30.58 ± 1.4	33.63 ± 1.7	35.59 ± 5	34.54 ± 4.3
Int/HTF	48.03 ± 15.5	35.52 ± 3	31.67 ± 3.5+	31.32 ± 1.5+				32.44 ± 4
T2BF					38.89 ± 3.4	38.56 ± 3.7	39.43 ± 5.1	41.90 ± 5.3

Data are reported as means ± SD for 6 rats in each group. CG = control group; TG = swimming trained group; TIG = swimming trained and immobilized leg group; TIRG = swimming trained, immobilized leg and rehabilitated group; T1F = type 1 fibers; T2AF = type 2A fibers; Int/HTF = intermediate/hybrid-type fibers; T2BF = type 2B fibers.
*$P < 0.001$ compared to TG. +$P < 0.001$ compared to CG (mixed-effects linear models).

TIRG, P < 0.009; Table 3). Rehabilitation (TIRG) caused the appearance of Int/HTF.

Discussion

The objective of the present study was to document whether swimming training prior to immobilization would minimize the deleterious effects of disuse and favor the results of skeletal muscle rehabilitation. Few reports are available about prevention of tissue function deterioration related to disuse and its influence on the rehabilitation process (3), including reports concerning histomorphometric alterations of the skeletal muscle. Most reports involve forms of rehabilitation treatment. In summary, the results of the present study suggest that a 2-week rehabilitation of skeletal muscle tissue submitted to immobilization is not favored by previous swimming training.

Histopathological changes

Muscle overload, especially in sedentary animals, is known to induce muscle injury. Some of the classical signs in the cyclic process of tissue injury and regeneration are cellular basophilia, split fibers resulting from stress on hypertrophied fibers and the presence of a centralized nucleus (23). Appell (24) suggested that muscle fiber degeneration may be caused by the inability of cells to undergo longitudinal deformation during the occurrence of a simple contraction rather than by tissue hypoactivity itself. In the present study, more significant structural alterations were observed in the soleus muscle, especially in the animals of the TIG and TIGR groups, as explained by the references cited above. Alterations in the tibialis anterior muscle observed in TG, TIG, and TIRG rats, although discrete, accompanied morphologic changes such as variation in size and cell lobulation.

Changes in transverse sections

Immobilization after swimming training caused atrophy of the three fiber types evaluated compared to control (CG) or to the swimming only group (TG). These findings agree with those reported by Appell (24), who studied the effect of treadmill training before immobilization in rats and observed a smaller reduction in fiber size in the trained group (5%) than in the group not submitted to previous training (35%). In the present study, the index of atrophy suffered by TIG animals compared to CG values was higher for Int/HTF (about 35%) and lower for T1F and T2AF (about 20%). The procedure of immobilization with a cast used by our research group has reached values of 40% for T1F and T2F (25). In addition, the rehabilitation protocol adopted after training did not favor fiber hypertrophy since the mean values obtained for the TIRG animals were similar to those obtained for the TIG animals but still lower than the controls in contrast to the percentages for different fiber types. Earlier studies by Sandri et al. (26) showed that ubiquitin protein expression in mice increased following one night of voluntary wheel running. Ubiquitin ligase proteins are up-regulated during skeletal muscle atrophy and there is evidence supporting an increase in gene expression of several ubiquitin proteins systematically induced by cachexia, denervation, disuse atrophy, etc. (for a review, see Ref. 4).

Cell volume changes in the tibialis anterior muscle were subtle, even though this muscle is more active in swimming training than the soleus muscle (27), a fact possibly explained by the intensity and/or duration of the present protocol. On the other hand, lengthened immobilization of skeletal muscle either attenuates or prevents muscle atrophy, and in some cases produces hypertrophy (28).

The mechanisms by which skeletal muscle senses and responds to changes in load and exercise are not completely understood. Following short-term swimming exercise (one-daily swimming session in a 3-day swimming training) Matsakas et al. (16) observed reduced myostatin mRNA levels, a negative regulator of muscle mass (29), only in rat gastrocnemius but not in the soleus muscle showing that myostatin expression was more depressed in white than in red fibers in endurance exercises.

Changes in percentages of the different fiber types

The data in the present study demonstrate an increase of T2AF and a reduction of T1F number in the soleus muscle after swimming training (TG). The consecutive

Table 3. Effect of swimming training, leg immobilization and rehabilitation on distribution of muscle fiber types in rat soleus and tibialis anterior muscles.

	Fiber proportion (%)							
	Soleus				Tibialis anterior			
	CG	TG	TIG	TIRG	CG	TG	TIG	TIRG
T1F	69	59	62	60	6	5	3	8
T2AF	28	38*	34	33	26	24	12*	21^{+}
Int/HTF	3	3	3	7				5
T2BF					68	72	85*	67^{+}

Data are reported as percent of fiber types. See Table 2 for explanation of abbreviations.
*P < 0.05 compared to CG. $^{+}$P < 0.01 compared to TIG (mixed effects linear models).

protocols of immobilization (TIG) and rehabilitation (TIRG) did not significantly modify these results. Roy et al. (27) reported a low activity of this muscle during swimming exercise and Pette and Staron indicated a transitional direction of the fibers for situations of disuse (30). In addition, aerobic treadmill training repeated daily for several weeks can affect the MHC gene expression, increasing expression of type I MHC (31). These findings agree with those obtained by us, suggesting a fiber transition from T1F to T2F.

Since the sum of training and immobilization (TIG) and rehabilitation (TIRG) did not cause additional modifications in this study, the model of fiber transition induced by physical exercise suggested by Pette and Staron (30) seems to be reserved for conditions suffered by the soleus muscle during swimming. The short rehabilitation period employed here may not favor an overload on this skeletal muscle and thus, would justify the absence of results described.

The swimming protocol did not appear to affect the transition of fibers in the tibialis anterior muscle, but caused a reduction in T2AF number, with a consequent increase in T2BF number in TIG animals compared to control. In the group that underwent rehabilitation, TIRG, the proportion of fibers returned to the levels of control conditions, although with the appearance of a fiber subtype. Sugiura et al. (32) observed that animals submitted to swimming presented a reduction of 2B fibers and an increase of 2D fibers in the extensor digitorum longus muscle. Since the tibialis anterior and extensor digitorum longus muscles execute similar functions in the rat ankle, this disparity of findings can only be justified by the extent of training, 4 as opposed to 6 weeks, used by the authors compared to that used in the present study. The appearance of Int/HTF in TIRG in the present study seemed to be the result of a mechanism of sensitization that was triggered, but not concluded, during the first phase of stimulation (represented by the findings for TG). As already described, several studies indicate that longitudinal immobilization may cause hypertrophy or attenuate muscle fiber atrophy (28). Carlson et al. (33) reported a positive correlation between MHC IIb content and myostatin mRNA abundance in rats during hindlimb unloading confirming the pathways that influence muscle fiber size as hypertrophy. As suggested by Parry (34), a limited range of fiber transitions in response to endurance training, inducing alterations in the MHC pattern, is species- and dose-dependent.

The present data indicated that the swimming protocol before segmental immobilization contributed little to the quantitative or qualitative mechanisms of muscle readaptation in the short period of time used.

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References

1. Michael K. Relationship of skeletal muscle atrophy to functional status: a systematic research review. *Biol Res Nurs* 2000; 2: 117-131.
2. Kannus P, Jozsa L, Renström P, Järvinen M, Kvist M, Lehto M, et al. The effects of training, immobilization and remobilization on musculoskeletal tissue. Training and immobilization. *Scand J Med Sci Sports* 1992; 2: 100-118.
3. Kannus P, Jozsa L, Renström P, Järvinen M, Kvist M, Lehto M, et al. The effects of training, immobilization and remobilization on musculoskeletal tissue. Remobilization and prevention of immobilization atrophy. *Scand J Med Sci Sports* 1992; 2: 164-176.
4. Coffey VG, Hawley JA. The molecular bases of training adaptation. *Sports Med* 2007; 37: 737-763.
5. Ishihara A, Kawano F, Ishioka N, Oishi H, Higashibata A, Shimazu T, et al. Effects of running exercise during recovery from hindlimb unloading on soleus muscle fibers and their spinal motoneurons in rats. *Neurosci Res* 2004; 48: 119-127.
6. Booth FW. Regrowth of atrophied skeletal muscle in adult rats after ending immobilization. *J Appl Physiol* 1978; 44: 225-230.
7. Desplanches D, Mayet MH, Sempore B, Frutoso J, Flandrois R. Effect of spontaneous recovery or retraining after hindlimb suspension on aerobic capacity. *J Appl Physiol* 1987; 63: 1739-1743.
8. Topp R, Ditmyer M, King K, Doherty K, Hornyak J III. The effect of bed rest and potential of prehabilitation on patients in the intensive care unit. *AACN Clin Issues* 2002; 13: 263-276.
9. Pearson SJ, Young A, Macaluso A, Devito G, Nimmo MA, Cobbold M, et al. Muscle function in elite master weightlifters. *Med Sci Sports Exerc* 2002; 34: 1199-1206.
10. Buckwalter JA, Grodzinsky AJ. Loading of healing bone, fibrous tissue, and muscle: implications for orthopaedic practice. *J Am Acad Orthop Surg* 1999; 7: 291-299.
11. Adolfsson J. The time dependence of training-induced increase in skeletal muscle capillarization and the spatial capillary to fibre relationship in normal and neovascularized skeletal muscle of rats. *Acta Physiol Scand* 1986; 128: 259-266.
12. Widrick JJ, Trappe SW, Blaser CA, Costill DL, Fitts RH.

Isometric force and maximal shortening velocity of single muscle fibers from elite master runners. *Am J Physiol* 1996; 271: C666-C675.
13. Harridge S, Magnusson G, Saltin B. Life-long endurance-trained elderly men have high aerobic power, but have similar muscle strength to non-active elderly men. *Aging* 1997; 9: 80-87.
14. DiMauro J, Balnave RJ, Shorey CD. Effects of anabolic steroids and high intensity exercise on rat skeletal muscle fibres and capillarization. A morphometric study. *Eur J Appl Physiol Occup Physiol* 1992; 64: 204-212.
15. Klitgaard H, Brunet A, Maton B, Lamaziere C, Lesty C, Monod H. Morphological and biochemical changes in old rat muscles: effect of increased use. *J Appl Physiol* 1989; 67: 1409-1417.
16. Matsakas A, Friedel A, Hertrampf T, Diel P. Short-term endurance training results in a muscle-specific decrease of myostatin mRNA content in the rat. *Acta Physiol Scand* 2005; 183: 299-307.
17. Matsakas A, Bozzo C, Cacciani N, Caliaro F, Reggiani C, Mascarello F, et al. Effect of swimming on myostatin expression in white and red gastrocnemius muscle and in cardiac muscle of rats. *Exp Physiol* 2006; 91: 983-994.
18. Peijie C, Zicai D, Haowen X, Renbao X. Effects of chronic and acute training on glucocorticoid receptors concentrations in rats. *Life Sci* 2004; 75: 1303-1311.
19. Luciano E, Carneiro EM, Carvalho CR, Carvalheira JB, Peres SB, Reis MA, et al. Endurance training improves responsiveness to insulin and modulates insulin signal transduction through the phosphatidylinositol 3-kinase/Akt-1 pathway. *Eur J Endocrinol* 2002; 147: 149-157.
20. Carvalho CMM, Shimano AC, Volpon JB. Efeitos da imobilização e do exercício físico em algumas propriedades mecânicas do músculo esquelético. *Rev Bras Eng Biom* 2002; 18: 65-73.
21. Bancroft JD, Stevens A. *Theory and practice of histological techniques*. 4th edn. New York: Churchill Livingstone; 1996.
22. Aherne WA, Dunnill MS. *Methods of estimating myofibre size*. London: Arnold; 1982. p 103-118.
23. Mattiello-Sverzut AC, Chimelli L. O papel das células satélites nas respostas adaptativas do tecido muscular esquelético. *Rev Fisioter Univ São Paulo* 1999; 6: 132-139.
24. Appell HJ. Morphology of immobilized skeletal muscle and the effects of a pre- and postimmobilization training program. *Int J Sports Med* 1986; 7: 6-12.
25. Mattiello-Sverzut AC, Carvalho LC, Cornachione A, Nagashima M, Neder L, Shimano AC. Morphological effects of electrical stimulation and intermittent muscle stretch after immobilization in soleus muscle. *Histol Histopathol* 2006; 21: 957-964.
26. Sandri M, Podhorska-Okolow M, Geromel V, Rizzi C, Arslan P, Franceschi C, et al. Exercise induces myonuclear ubiquitination and apoptosis in dystrophin-deficient muscle of mice. *J Neuropathol Exp Neurol* 1997; 56: 45-57.
27. Roy RR, Hirota WK, Kuehl M, Edgerton VR. Recruitment patterns in the rat hindlimb muscle during swimming. *Brain Res* 1985; 337: 175-178.
28. Spector SA, Simard CP, Fournier M, Sternlicht E, Edgerton VR. Architectural alterations of rat hind-limb skeletal muscles immobilized at different lengths. *Exp Neurol* 1982; 76: 94-110.
29. McPherron AC, Lawler AM, Lee SJ. Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. *Nature* 1997; 387: 83-90.
30. Pette D, Staron RS. Mammalian skeletal muscle fiber type transitions. *Int Rev Cytol* 1997; 170: 143-223.
31. Koulmann N, Bigard AX. Interaction between signalling pathways involved in skeletal muscle responses to endurance exercise. *Pflugers Arch* 2006; 452: 125-139.
32. Sugiura T, Morimoto A, Sakata Y, Watanabe T, Murakami N. Myosin heavy chain isoform changes in rat diaphragm are induced by endurance training. *Jpn J Physiol* 1990; 40: 759-763.
33. Carlson CJ, Booth FW, Gorden SE. Skeletal muscle myostatin mRNA expression is fiber-type specific and increases during hindlimb unloading. *Am J Physiol* 1999; 277: R601-R606.
34. Parry DJ. Myosin heavy chain expression and plasticity: role of myoblast diversity. *Exerc Sport Sci Rev* 2001; 29: 175-179.