Histol Histopathol (2001) 16: 29-35

http://www.ehu.es/histol-histopathol

Histology and Histopathology

Cellular and Molecular Biology

Effect of endurance running on cardiac and skeletal muscle in rats

P. Díaz-Herrera[1], A. Torres[2], J.A. Morcuende[3], J.M. García-Castellano[1], J.A.L. Calbet[4] and R. Sarrat[2]

[1]Department of Morphology, School of Medicine, University of Las Palmas de Gran Canaria, Gran Canaria, Spain,

[2]Department of Morphological Sciences, School of Medicine, University of Zaragoza, Zaragoza, Spain,

[3]Department of Orthopedic Surgery, Iowa Hospital & Clinics, Iowa City, IA, USA and [4]Department of Physical Education, School of Sciences of Physical Activity and Sport, University of Las Palmas de Gran Canaria, Gran Canaria, Spain

Summary. We studied the effect of resistance running on left cardiac ventricle size and rectus femoris muscle fiber composition. Ten male Wistar rats were trained on a treadmill 6 days per week for 12 weeks. Ten rats remained sedentary and served as controls. A higher endurance time (40%) and cardiac hypertrophy in the trained animals were indicators of training efficiency. Morphometric analysis of the left ventricle cross-sectional area, left ventricular wall, and left ventricular cavity were evaluated. The endurance-running group demonstrated a hypertrophy of the ventricular wall (22%) and an increase in the ventricular cavity (25%); ($p<0.0001$). Semi-quantitative analysis of rectus femoris fiber-type composition and of the oxidative and glycolytic capacity was histochemically performed. Endurance running demonstrated a significant ($p<0.01$) increase in the relative frequency of Type I (24%), Type IIA (8%) and Type IIX (16%) oxidative fibers, and a decrease in Type IIB (20%) glycolytic fibers. There was a hypertrophy of both oxidative and glycolytic fiber types. The relative cross-sectional area analysis demonstrated an increase in oxidative fibers and a decrease in glycolytic fibers ($p<0.0001$). Changes were especially evident for Type IIX oxidative-glycolytic fibers. The results of this study indicate that the left ventricle adapts to endurance running by increasing wall thickness and enlargement of the ventricular cavity. Skeletal muscle adapts to training by increasing oxidative fiber Type. This increase may be related to fiber transformation from Type IIB glycolytic to Type IIX oxidative fibers. These results open the possibility for the use of this type of exercise to prevent muscular atrophy associated with age or post-immobilization.

Key words: Endurance exercise training, Fiber type composition, Oxidative capacity, Skeletal muscle, Cardiac hypertrophy

Offprint requests to: Pilar Díaz-Herrera, Avd. de Canarias, 18ª, 10B, DP: 35002, Las Palmas de Gran Canaria, Islas Canarias, Spain. e-mail: Pilard@cicei.ulpgc.es

Introduction

The practice of sport activities has increased in the last few decades to the point of becoming a fundamental component of the way of life. Individuals of all ages participate in both leisure and competitive sports. It has been demonstrated that exercise may help to prevent the structural abnormalities of bone (i.e., osteoporosis), and cardiovascular and musculoskeletal systems associated with the ageing process in animals (Agüera et al., 1990; Fitzsimons et al., 1990), including humans (Baumman et al., 1987; Proctor et al., 1995). In addition, exercise as a rehabilitation technique also helps to prevent muscle atrophy associated with disuse. It has been demonstrated that when a muscle is immobilized, there is a rapid atrophy of slow-twitch fibers and progressive atrophy of subtype IIB of fast-twitch fibers (Grimby et al., 1980; Baugher et al., 1984; Halkjaer-Kristensen and Ingermann-Hansen, 1985a; Slager et al., 1985; Kannus et al., 1987). There are also alterations of the glycolytic and oxidative enzymatic activity (Arvidsson et al., 1986; Halkjaer-Kristensen and Ingermann-Hansen, 1985b), mATPase activity, and glycogen concentration (Stanish et al., 1982). These changes may contribute to chronic fatigue and instability of the musculoskeletal segment affected.

It has been observed that endurance-running produces changes in fiber composition on gluteus medius muscle in horses (Rivero et al., 1993), on the long head of the triceps on miniature swine (McAllister et al., 1997), and on human muscle (Coggan et al., 1992a,b). In addition, the aerobic and anaerobic enzymatic fiber activities are also affected with endurance running (Rivero et al., 1995b; McAllister et al., 1997). However, there was a high degree of variability in fiber composition and enzymatic activity observed by different authors using similar training programs (Ho et al., 1980; Roy et al., 1997). Those differences have been related to several factors: breed (Rivero et al., 1995a), age (Agüera et al., 1990; Coggan et al., 1992b; Proctor et al., 1995), heritage (Simoneau et

al., 1986), muscle type (McAllister et al., 1997), sample technique (Rivero et al., 1993), and the design of training program (Trape et al., 1995; Pincivero et al., 1997). Fiber type transformation (Jansson et al., 1990; Fitts and Wildrick, 1996; McAllister et al., 1997) and longitudinal fiber splitting (Ho et al., 1980; Tamaki et al., 1992; Roy et al., 1997) have been suggested as mechanisms responsible for endurance and strength training in animals. On the other hand, exercise also affects cardiac muscle. Cardiac muscle hypertrophy has been observed after endurance running in different species including miniature swine (McAllister et al., 1997) and humans (Breisch et al., 1986; White et al., 1987). Furthermore, using echocardiograms, it has been observed that different sport activities result in differences in ventricular wall thickness or increase in ventricular cavity size (Maron and Facc, 1986).

Despite the difficulties of transfering data from animals to human, animal models are used to understand the behaviour of cardiac and skeletal muscle subjected to physical exercise in both healthy (McAllister et al., 1997; Roy et al., 1997) and pathological conditions (Kannus et al., 1998a,b). Our purpose was to investigate, by morphometric and histochemical means, the effects of endurance running on muscle trophism. We have paid special attention to Type IIX fibers, present in rodent muscles, which have been recently identified in human muscle (Fitts, 1992; Smerdu et al., 1994; Ennion et al., 1995). At present, there are not many works with clinical orientation which analyze this topic referring to physical training.

Materials and methods

Experimental animals

Twenty male 16-18-week-old Wistar rats, each weighing between 235 and 280 g were randomly divided into two equal groups. One group remained sedentary (Sed) while the other group underwent endurance-running (ER). To keep body weights approximately equal between groups, sedentary rats were slightly food restrained (about 10% less food provided).

Endurance-running program

Training was performed on a motorised treadmill over a 10-week period (6 days/wk). The daily exercise duration was gradually increased to 60 min/day over the first 3 weeks of training.

During the final 4-10 weeks a training session remained at 60 min in duration. In this period of time a training session consisted of a 5-minute warm-up at 11.5 m/min, 50 min at 22 m/min and a 5-minute cool-down at 9.2 m/min. This program corresponds to maximal oxygen consumption (VO_2max) of 65-75% according to Bedford et al. (1979) and Patch and Brooks (1980).

Determination of training effectiveness

Administering a maximal running test, pre and post-training, to Sed and ER rats the efficacy of exercise training was assessed. This test consisted of 4 stages of running: stage 1, 12 m/min (4 min); stage 2, 12 m/min (8 min); stage 3, 15 m/min (8 min); and stage 4, 22 m/min (to exhaustion). Typically, rats ran briefly into stage 4 before training and extended this time post-training. Total running time to exhaustion was recorded. Heart weight to body weight ratio (HW/BW; g/kg) was determined when Sed and ER rats were euthanized. Cross-sectional area of left ventricle, left cross-sectional area of ventricular wall and left cross-sectional area of ventricular cavity were also measured.

Skeletal muscle and heart sampling

The rectus femoris muscle was removed under pentobarbital anaesthesia (30 mg/kg), and a cross-section of the muscle at midbelly was embedded in OTC compound (Tissue-Tek II, Miles Laboratories, Naperville, IL) and oriented so that fibers could be cut transversely. Specimens were quick-frozen in isopentane pre-cooled to approximately -160 ºC with liquid nitrogen. Samples were stored at -80 ºC until used for histochemical analysis. Cross sections of ~1 cm, from the upper part of the left ventricle, at the level of the mitral valve, were fixed in buffered formalin, dehydrated in graded ethanol, and paraffin embedded. Transverse sections of 5 μm were stained with Martin trichrome technique and used for morphometric studies.

Enzyme-histochemical methods (Fig. 1)

Serial sections were cut 10 μm thick in a cryostat at -20 ºC, mounted on gelatine-coated glass slides and incubated for Ca^{2+}-activated myofibril adenosine triphosphatase (mATPase, EC 3.6.1.3) active at pH 9.4 after three different levels of pre-incubation at pH 9.4, 4.6, and 4.3 (Fig. 1A-C). Nicotinamide dinucleotide dehydrogenase (tetrazolium oxidoreductase (NADH-TR, EC 1.6.99.3)) was used to show fiber oxidative capacity; and phosphorilase stain was used to show glycolytic activity. mATPase and NADH-TR staining methods were used according to Dubowitz (1985). Serial sections from the same area respectively stained with mATPase, NADH-TR and phosphorilase were used to distinguish different fiber types (Amman et al., 1993; Hamalaine and Pete, 1993).

Quantitative analysis

Rectus femoris muscle

Transverse sections from the rectus femoris were divided into three zones around the central connective-tissue fascia. The deeper one, which is the one with the highest number of Type I fibers, was selected for

morphometry (Medina-Sánchez et al., 1991). Images were captured into the Leika Qwin® computerized image analysis system by using a Leitz light microscope attached to a digital JVC® camera interfaced to a PC computer. The morphometric analysis was based on seven randomly selected artifact-free areas, with distinct cell borders, from each sample. Measurements were made only in those areas where the myofibers had been sectioned mostly transversally. A mean of 400±25 fibers (36±2 Type I fibers, 170±11 Type IIA fibers, 47±4 Type IIX fibers and 100±6 Type IIB) were classified and measured per animal. Type IIC fibers were not present.

For both sedentary and the training group, the relative frequency (RF; %), the mean cross-sectional area (CSA; μm^2) and the relative cross-sectional area (rCSA) of the various fiber types were obtained. The relative cross-sectional area (rCSA; %) fiber type was also calculated. For each group, this parameter was calculated dividing the product of the percentage of that fiber type in the group and the mean fiber area (μm^2) of that type by the sum of these products for the entire fiber types in the group (Sullivan and Armstrong, 1978; Rivero et al., 1995a).

Left cardiac ventricle

Heart analysis was developed on seven randomly selected transverse sections from each sample. Images of all left ventricular transversal sections were captured in the computer, directly from the slide, by a digital Sony® camera and measured by the Image Pro® computerised image analysis system. For both sedentary and endurance-running animals, the total cross sectional area of the left ventricle (lvCSA; mm^2), the cross sectional area of the ventricular cavity (vcCSA; mm^2), and the cross sectional area of the ventricular wall (vwCSA; mm^2) were determined.

Statistical analysis

All data were presented as means ± SE. One-way analysis of variance (ANOVA) was used to compare differences in fiber types, fiber sizes and oxidative capacity between sedentary and training animals. The same treatment was used to test parameters used for training effectiveness determination and to compare differences in cardiac area between Sed and ER animals. The Scheffé test was used for post hoc analysis. In all comparisons, the null hypothesis was rejected at the level of significance of 0.05. Statistical treatment of data was performed using the general linear model procedures developed by Statistical Analysis Systems.

Results

Running performance history

After training, the ER group demonstrated an increase of 40% in time (Pre-training, 35±4 min; Post-training, 83±4 min) and in running distance (Pre-training, 561±70 m/min; Post-training 1287±58 m/min) before exhaustion; $p<0.0001$. In ER the heart hypertrophy, without body weight increase (Sed, 460±21; ER 458±14; NS), was also recorded as a sign of training efficacy (see below).

Effect of endurance running in the left ventricle

Endurance running also resulted in cardiac muscle changes that reflected the efficacy of training. First, cardiac weight increased by 13% ($p<0.0001$) in the ER group. Second, the ratio cardiac weight/body weight increased by 8% ($p<0.0001$) in ER animals. Third, the left ventricle wall area increased by 22% and the area of the left ventricular cavity increased by 25%; $p<0.0001$. Data are itemised in table 1.

Effect of endurance running on rectus femoris muscle

Muscle fiber type identification

In the deeper muscle samples, both Sed and ER animals demonstrated slow-twitch Type I fibers and fast-switch Type IIA, IIX and IIB fibers (Fig. 1A,B). NADH-TR demonstrated that Types I, IIA and IIX showed a high oxidative enzymatic capacity, while Type IIB did not (Fig. 1C). Type IIB and IIX fibers showed a high glycolytic activity while type IIA and Type I fibers exhibited either a moderate glycolytic activity or no activity (Fig. 1D).

Muscle fiber composition

In trained animals, there was an increase in the relative frequency of fiber Type I, IIA and IIX (24%, 8% and 16% respectively); $p<0.01$. On the other hand, the relative frequency of Type IIB fibers decreased by 20% ($p<0.01$). Data for fiber type composition are illustrated in Table 2 and Figure 2.

Muscle fiber area

The CSA of the four fiber types significantly increased ($p<0.0001$) in the ER group: Type I (22%), Type IIA (14%), Type IIX (12%) y Type IIB (4%). Findings for fiber area are shown in Table 2 and Figure

Table 1. Effect of endurance training on left ventricle.

ANIMALS	HW (g)	HW/BW (g/kg)	lvCSA (mm^2)	vwCSA (mm^2)	vcCSA (mm^2)
Sedentary	13.32±1	29.37±2	21.79±3	18.77±2	3.01±0.5
Endurance	17.21±1*	35.12±3*	34.59±4*	29.46±3*	
5.13±0.7* Running					

HW: heart weight; HW/BW: heart weight to body weight ratio; CSA:

3.

Relative cross-sectional area

The percentage of cross-sectional area of Type I, Type IIA, and Type IIX fibers was significantly increased. ($p<0.0001$). On the other hand, the rCSA of Type IIB was decreased ($p<0.0001$). Data for rCSA is shown in Table 2 and Figure 4.

Oxidative and glycolytic capacity

When fiber Type I, IIA and IIX were considered,

Table 2. Effect of endurance training on skeletal fibers from rectus femoris muscle.

FIBER	SEDENTARY			ENDURANCE RUNNING		
	RF %	CSA$^{\xi}$ μm^2	rCSA %	RF %	CSA$^{\xi}$ μm^2	rCSA %
I	6	1750±335	2	9**	2787±674*	6*
IIA	43	2075±499	23	47*	2770±627*	31*
IIX	20	3785±924	2	26***	4784±162*	30*
IIB	30	6824±2090	53	18***	7292±1673*	31*

RF: relative frecuency; CSA: cross-sectional area; rCSA: relative cross-sectional area. n=10. $^{\xi}$: values are mean±SE. p Value: *p<0.0001, **p<0.001, ***p<0.01

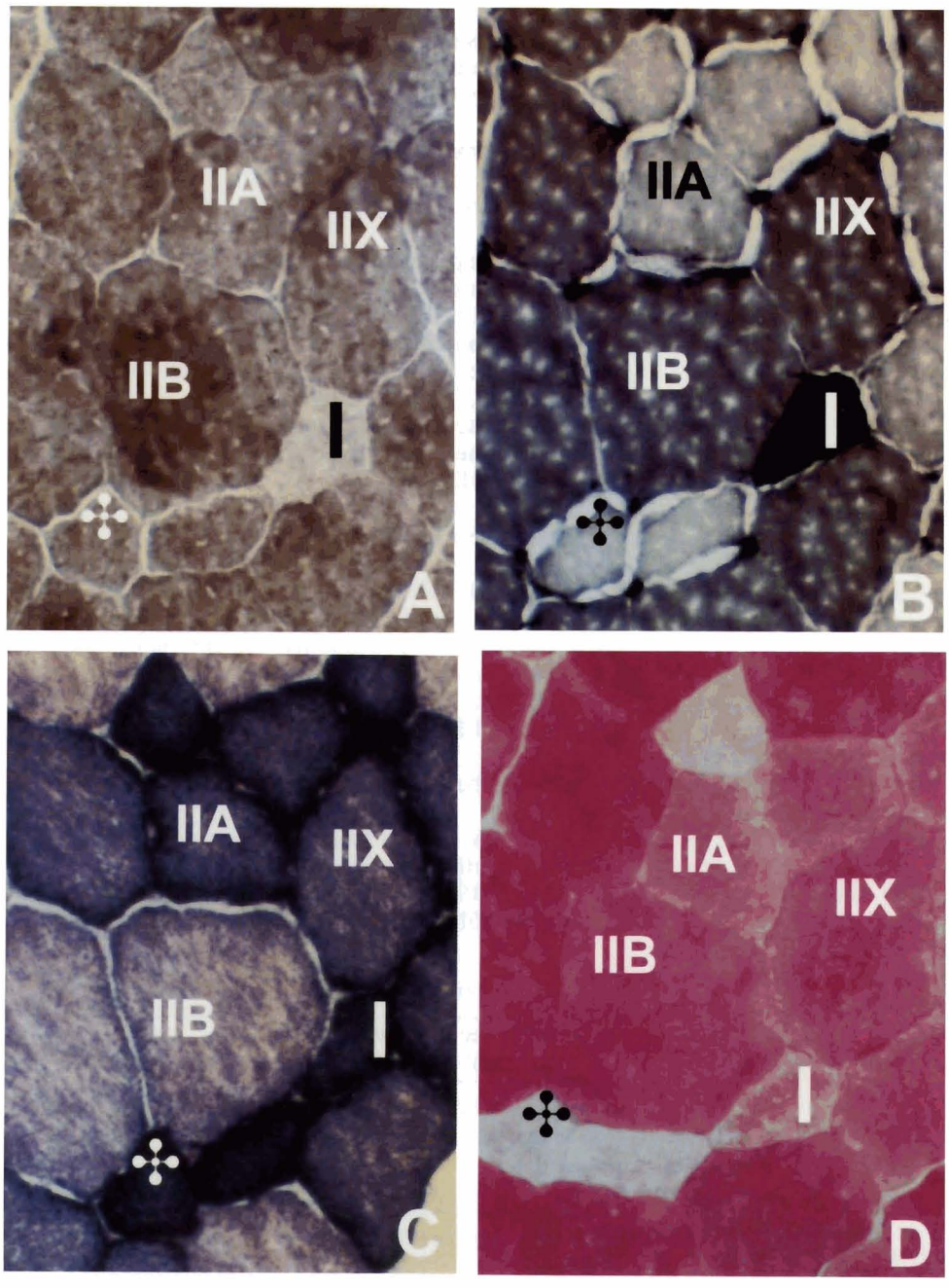

Fig. 1. Identification of Types IIB, IIX, IIA and I fibers in serial cross-sections from rectus femoris muscle of rat stained for: activated myofibrillar adenosine triphosphatase (mATPase) at pH 9.4 **(A)**, mATPase at pH 9.4 pre-incubated at pH 4.6 **(B)**, NADH-tetrazolium reductase **(C)**, and phosphorilase **(D)**. Asterisk: no-glycolytic Type IIA fibers. x 1000.

there was an increase (12%) in number of fibers and an increase in cross-sectional area (16%) in the ER group ($p<0.01$). On the other hand, there was a decrease in number of glycolytic (Type IIB) fibers (20%) and the cross-sectional area also decreased (4%) ($p<0.001$). The rCSA oxidative fibers (ER, 68%; Sed, 28%; $p<0,001$) was larger than the glycolytic fibers (ER, 68%; Sed, 31%; $p<0.001$). Overall, the higher the oxidative activity the lower the glycolytic activity.

Discussion

Reduction in muscle body mass is a process that happens under different conditions, such as ageing or immobilization. With this phenomenon underlie alterations in size and relative proportion of the different fiber types and in the oxidative capacity of the fibers (Baumman et al., 1987). It has been reported that ageing results in a decrease in the size and oxidative capacity of Type II fibers in healthy individuals, but does not change Type I fibers (Proctor et al., 1995). On the other hand, other authors have reported an increase in Type I fibers (Fitzsimons et al., 1990). Muscle atrophy associated with immobilization results from an immediate size decrease in Type I fibers, and a slow decrease in Type IIB fibers (Grimby et al., 1980; Baugher et al., 1984; Halkjaer-Kristensen and Ingermann-Hansen, 1985a; Slager et al., 1985; Kannus et al., 1987). Other changes have been described, such as enzymatic alterations and glycogen concentration activity (Stanish et al., 1982; Halkjaer-Kristensen and Ingermann-Hansen, 1985; Arvidsson et al., 1986).

Both endurance and high intensity exercises, seem to affect the biological behaviour of the skeletal muscle fiber with structural, metabolic, and biochemical changes. However, there is a great variability in observed response to exercise, and this has been attributed to phenotypic and genotypic differences (Simoneau et al., 1986; Agüera et al., 1990; Coggan et al., 1992b; Hamalaine and Pete, 1993; Rivero et al., 1995a; Trappe et al., 1995).

In this study, an exercise program of endurance running with a VO_2 max between 65-75% in healthy animals was developed to evaluate changes in fiber type composition and oxidative capacity of the rectus femoris muscle. In addition, we also evaluated the changes in cardiac muscle to determine the efficacy of training and to compare the response observed in humans (Breish et al., 1986; Maron and Facc, 1986; White et al., 1987).

It was observed that animals in the endurance running group demonstrated significant differences in rectus femoris fiber composition. The increase in the relative frequency of Type I, IIA, and IIX fibers is in agreement with the data of Mcallister et al. (1997) using

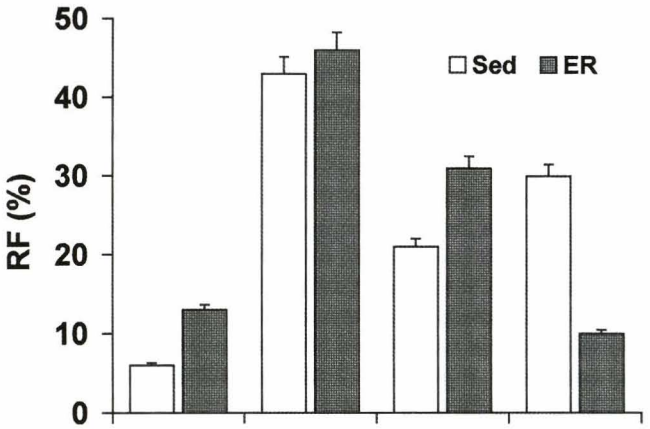

Fig. 2. Increase in the number of Type I, IIA, and IIX fibers and decrease in Type IIB fibers in ER animals as compared with Sedentary rats.

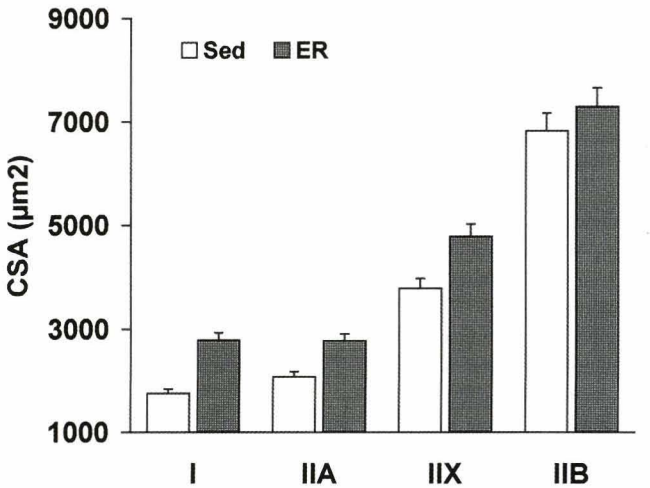

Fig. 3. Increase in cross-sectional area of all Type fibers in ER animals.

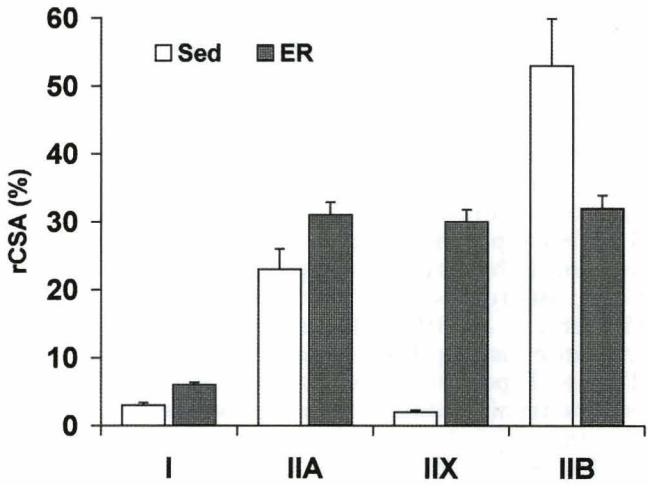

Fig. 4. Increase in the percentage of transversal section of the deeper part of rectus femoris muscle occupied by Type I, IIA and IIX fibers in contrast with the decrease in Type IIB fibers in ER rats.

endurance training in miniature swine, and similar to that obtained by Rivero et al. (1993) in race horses. Moreover, an increase in the relative frequency of Type IIA fibers and a decrease of Type IIB has been reported in ageing adults after endurance training (Coggan et al., 1992a). Different mechanisms have been proposed to explain the variations in fiber composition after exercise training. Fiber transformation has been observed in endurance training in animals (Mcallister et al., 1997), and after high-intensity training (Jansson et al., 1990) and endurance training (Fitts and Wildrick, 1996) in humans. On the other hand, it has been proposed that longitudinal fiber splitting may underlie fiber composition changes. Signs of fiber splitting have been observed in several rat muscles after different weight-lifting programs in horses (Rivero et al., 1995b) and rats (Roy et al., 1997; Tamaki et al., 1992). The design of our study did not allow teh evaluation of fiber splitting.

An increase in the CSA demonstrated an increase in size of the four evaluated fiber types. Fiber hypertrophy was more evident in fiber Type I than IIB. Coggan et al. (1992a) also observed an increase in CSA of fiber I and IIA in elderly human adults after endurance running as did Rivero et al. (1993) in race horses. However, there is a decrease in Type IIB fiber size when compared to our study. This muscle hypertrophy may be related to an increase in muscle use secondary to training since the same effect has been observed after immobilization where fibers that showed decreased size recover their pre-immobilization dimensions (Kannus et al., 1998a,b).

When fiber size and fiber number were related by the rCSA parameter, the area of glycolytic fibers (IIB) was significantly decreased. This was due to a decrease in the number but not in the size of type IIB fibers. Alternatively, there was an increase in the area occupied by oxidative fibers (I, IIA, IIX). This increment was due to both fiber size and fiber number. Since changes in contractility, specifically ATPase activity, have been associated with changes in oxidative capacity, our results suggest that in the rat rectus femoris under endurance running there is an increase in rCSA due to fiber hypertrophy, and hyperplasia of the oxidative fibers. These results are in agreement with those of Mcallister et al. (1997). The increase of rCSA was especially significant for Type IIX fibers. This fiber type is a fast-twitch oxidative-glycolytic fiber, with isometric twitch duration intermediate between the Type IIA and Type IIB fiber type. Moreover, Type IIX fibers are more resistant to the fatigue than Type IIB fibers (Fitts et al., 1992; Smerdu et al., 1994; Ennion et al., 1995). Mcallister et al. (1997) showed in miniature swine that endurance running determines a transformation of Type IIB into Type IIX fibers. Consequently, this type of exercise training could help to improve the resistance to the fatigue of skeletal muscles.

The adaptation of the skeletal muscle to endurance training was also associated with changes in the left ventricular mass. The hypertrophy observed in trained animals was similar to that observed on miniature swine and humans (Breisch et al., 1986; White el al., 1987; McAllister et al., 1997). The increase in the ventricular wall area and ventricular cavity size correspond to the changes known as "athlete heart", characteristic of endurance training. This increase of the left ventricular dimension observed by echocardiography in athletes has been considered a direct response to training (Maron and Facc, 1986).

In conclusion, endurance running in experimental animals results in cardiac changes similar to those observed with this type of exercise program in humans. Endurance running resulted in an increase in rat rectus femoris muscle mass with a hypertrophy of muscle fibers and an increase in the number of oxidative fibers. These data suggests that endurance training could be used to prevent muscle atrophy associated with ageing or after immobilization. In addition, animal models may be used to help in the understanding of muscle physiology and mechanism of adaptation to exercise.

Acknowledgements. Diputación General de Aragón has supported this work. We would like to thank Dr. Adam Levy and Mrs Bethanne Mazza (Senior Research Technician and BA in Writing English) for their help in the translation of the manuscript to English.

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Accepted June 26, 2000