The Effect of the Aerobic Training on Morphological and Histochemical Characteristics of Tibial Anterior Muscle in Rats Fed with High Fat Diet

Efecto de un Programa de Entrenamiento Aeróbico sobre las Características Histológicas y Morfológicas del Músculo Tibial Anterior de Ratas Alimentadas con Dietas Elevadas en Grasa

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SUMMARY: The aim of this study was to investigate the effect of high fat diet and different frequencies of swimming programs in the tibial anterior muscle in male Wistar rats. In conclusion, the aerobic training during two days/week and five days/week caused injuries in muscle fibers and the high fat diet did not cause statically significant results compared to normal diet.

KEY WORDS: Aerobic training; Morphology; Skeletal muscle; Rats; Fat diet.

INTRODUCTION

Obesity, considered a worldwide disease in 1997 by the World Health Organization (Ross *et al.*, 2000), has risen enormously over the past few decades and has reached epidemic proportions in both developed and developing countries, affecting not only adults but also children and adolescents. In some parts of North America, Canada, Eastern Europe, Middle East, Pacific Islands, Australia and China obesity rates have increased 3-fold, since 1980 (Stieger & Cunliffe, 2006). In Brazil, obesity reaches 45% of the population (Guerra *et al.*, 2002). Although the sedentary lifestyle had supplanted physical labor and regular physical activity, some researchers suggest that, in recent decades, changes in diet habits had a dramatic effect on this increasing and alarming worldwide epidemic. In the USA, the intake of chips/crackers/popcorn/pretzels roughly tripled from the mid-1970s to the mid-1990s (Sturm, 2005).

People who are overweight or obese have substantially increased risk for morbidity and mortality from numerous chronic disorders, such as diabetes, hypertension and cardiovascular disease, dislipidemia, gallbladder disease, sleep apnea, infertility, osteoarthritis and some forms of

cancer (Ross *et al.*; Stiegler & Cunliffe; Pollock *et al.*, 1998; Anderson & Wadden, 2000; Stevens *et al.*, 2002; Pedersen, 2007). In recent years, some research has shown that one of the most effective methods which can lead to obesity control is physical activity.

The aim of this study was to investigate the effect of different frequencies of swimming exercise in rats fed with high fat diet on morphology and histochemical characteristics in tibial anterior skeletal muscle.

MATERIAL AND METHOD

Twenty four male Wistar rats (*Rattus norvegicus albinus*), aged 90 days, from Central Biotério of São Paulo State University (UNESP), Campus of Botucatu, were used in this study. The animals were maintained in the Biotério of UNESP, Campus of Presidente Prudente, place where the experiment was made, housed four per cage in a temperature-controlled room (24° C) and a 12-h light/dark cycle. The

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rats were fed with one of two diets as follows: One group (n=12) received standard chow produced by Nuvital Nutrients Ltda (NUVILAB-CR1) (Colombo, PR, Brazil). The other group (n=12), received a high fat diet (cafeteria diet) (Rotchell & Stock, 1981; Prats *et al.*, 1989) formulated by Nutrition and Metabolism Laboratory of Federal University of Sao Carlos (DEFMH/CCBS/UFSCAR). Food and water were available ad libitum.

The rats were assigned in six sub-groups (4 animals each) and remained sedentary, until their weight reached at least 350g. After the period the groups were distributed as follows: Two control sub-groups remained sedentary, one ingested standard chow pellet (SS), and the other ingested a high fat diet (SH) throughout the experimental period;

Four sub-groups were exercised: two groups, two times per week in an intermittent way, and two groups were exercised five times per week on continuous way. One of each group ingested a standard chow pellet (CS) and the other ingested a high fat diet (CH). The sequence of intermittent group and continuous group had been based on Duarte's protocol (Duarte, 2001). The training consisted of continuous swimming for 60 min/day, on consecutive days, during 10 weeks. This exercise was performed in tanks of water with the capacity for 10 animals, measuring 40x60x80cm. The water temperature was maintained between 32°C and 36°C and was changed daily.

The surgical procedure began twenty-four hours after the last training, with intraperitoneal anaesthesia of sodium Thiopental (20mg/100g corporal weight). After this the animals were weighed in the electronic scale (g) and the tibial anterior muscle was removed and stained with Hematoxylin and Eosin (HE) and Sudan Black (Dubowitz, 1985). The morphology was analyzed and the degree of fiber growth evaluated using the lesser fiber diameter method (Dubowitz). This procedure allowed the identification of three different

types of staple fibres: Fast-twitch-glycolytic (FG); Fast-twitch-oxidative-glycolytic (FOG) and Slow-twitch-oxidative (SO). The results are expressed as means $\pm$ standard deviation. The comparisons of the absolute data among groups were performed by ANOVA, followed by Least Significant Difference post-hoc test for multiple comparisons. The level of significance was set at $P < 0.05$. All analysis was performed using the SPSS software, version 13.0 (SPSS Inc, Chicago, IL). The ethical committee of Universidade do Oeste Paulista approved all procedures of this research.

RESULTS

The comparison of body weight of the rats in the beginning and ending of the study are presented in the Table I. In the Table II are presented the amount and percentage of fibers according to their diameters. The three groups high fat fed presented higher proportion of hypertrophic fibers. Table III is indicated fiber's hypertrophy in groups. The IN, IH and IS showed hypertrophy statistically significant.

In the Fig. 1 fibers are stained with Hematoxylin and Eosin (HE). The SS and SH groups showed fibers with different diameter, polygonal shape, myonuclei (M) and some hypertrophic fibers (H), this last is also observed in some cells of IS group. The CS and CH muscle samples showed more damaged cells, with basophilic (B), some round cells, atrophic cells and splitting (longitudinal segmentation of the cytoplasm) with internal nuclei. In the IH group there were also observed atrophic fibers and splitting.

In the Fig. 2 the transversal section of tibial anterior muscle are stained with Sudan Black. The SS and SH groups, present SO fibers with a high lipids concentration, more evident in the subsarcolemmal region; FOG fibers with

Table I. Mean and standard deviation of body weight (g) of the rats and comparisons among different groups, and comparisons of the initial and final weight of each group.

Groups	Initial weight	Final weight	P value
SS	450.0 $\pm$ 58.63	489.4 $\pm$ 52.16	0.05
SH	435.0 $\pm$ 37.91 ^b	523.5 $\pm$ 52.36 ^{abc}	0.03
IS	425.0 $\pm$ 30.62 ^b	518.1 $\pm$ 14.78 ^{abc}	0.002
IH	443.8 $\pm$ 37.50 ^{ab}	551.0 $\pm$ 50.74 ^c	0.005
CS	368.8 $\pm$ 12.50 ^{ab}	469.6 $\pm$ 14.06 ^{ab}	0.001
CH	430.0 $\pm$ 41.08 ^b	545.1 $\pm$ 37.32 ^c	0.005

SS= Sedentary normal diet fed; SH= Sedentary hypercaloric diet fed; IS = Intermittent trained normal diet fed; IH= Intermittent trained hypercaloric diet fed; CS= Continuous trained normal diet fed; CH= Continuous trained and hypercaloric fed; Superscript letters means statistical differences among groups in the beginning and in the final of experiment.

Table II. Distribution of fibers of tibial anterior muscle, according to their diameters.

Groups	Fibers diameters (µm)				
	N	< 20	21 - 40	41 - 60	> 60
SS	400	174 (40.7%)	214 (50.1%)	39 (9.1%)	
SH	400	3 (0.7%)	146 (35.7%)	204 (49.8%)	57 (13.9%)
IS	400	151 (37.5%)	150 (37.5%)	99 (24.7%)	
IH	400	1 (0.2%)	139 (34.7%)	217 (54.2%)	43 (10.7%)
IS	400	118 (29.5%)	221 (55.2%)	60 (15.0%)	
CH	400	1 (0.2%)	116 (29.0%)	189 (47.2%)	95 (23.7%)

SS= Sedentary normal diet fed; SH= Sedentary hypercaloric diet fed; IS = Intermittent trained normal diet fed; IH= Intermittent trained hypercaloric diet fed; CS= Continuous trained normal diet fed; CH= Continuous trained and hypercaloric fed

Table III - Comparison of fiber size (µm) of the different experimental groups.

	N	mean	sd	Confidence interval 95%	
				Lower bound	Upper bound
SS	427	45.13 ^a	10.99	44.09	46.18
SH	409	45.64 ^a	12.61	44.42	46.87
IN	400	49.26 ^b	16.40	47.64	50.87
IH	400	46.60 ^a	11.39	45.48	47.72
IS	400	46.87 ^a	13.08	45.59	48.16
CH	400	50.37 ^b	14.46	48.91	51.75

SS = Sedentary normal fed; SH= Sedentary High fat fed; IN= Intermittent normal fed; IH= Intermittent high fat fed; IS= continuous normal fed; CH= continuous high fat fed. Different subscript letters, means statistical difference.

moderate lipids concentration and weak lipid concentration in FG fibers. The IN group presents high lipids concentration on SO and FOG fibers and weak reaction in FG fibers. In the IH group, lipids concentration is higher in FG compared to SO and FOG fibers. The CS group presented greater lipids concentration in SO, FOG and FG fiber, compared to the other groups. In the CH group, SO and FOG fibers presented high lipids concentration compared to FG fiber, which showed weak reaction.

DISCUSSION

The data reported in the present study indicate that the tibial anterior muscle showed different diameter fibers between 45.1 µm in SS group to 53.03 µm in CH group and the mosaic pattern of fibers did not present alterations between sedentary and trained groups. The protocol used in this study was in concordance with the position stand of American College of Sports Medicine (Pollock *et al.*).

Weight training alone without diet had little effect on body fat mass but resulted in a 1 kg gain in lean body mass in obese young women (Zachwieja, 1996). Dall Pai *et al.* (1983, 1984) presented in the postnatal growth of rats that fibers is in a very active process, with extend from 12th to the 45th live day. The fibers show a linear enlargement, presenting at the same time the highest rates of growth. Extending from 45th to 180th, muscle fibers present a markedly fall in the growth rates, except for white fibers (FG).

The trained groups, especially CS and CH showed several morphological alterations: splitting, basophilia, hypertrophy and muscle fiber with small diameter, like denervation condition; muscle fibers in phagocytosis process, accentually in both continuous groups (normal and hypercaloric) were observed. The presence of 0.2 to 0.7% of muscle fibers with diameter less than 20µm between control and trained hypercaloric groups, suggest that the protocol did not showed significant effect on the number of the fibers; the occurrence of muscle fiber with small diameter can be resulted of atrophic process drawn out and/or new fiber formation

Fig. 1. Cross section of tibial anterior muscle of Wistar rats. SS= Sedentary normal diet fed; SH= Sedentary hypercaloric diet fed; IS = Intermittent trained normal diet fed; IH= Intermittent trained hypercaloric diet fed; CS= Continuous trained normal diet fed; CH= Continuous trained and hypercaloric fed. M=Myonuclei; H=Hypertrophic fibers; B=Basophils. Stained with haematoxylin-eosin (original x128).

Fig. 2. Transversal section of tibial anterior muscle. SS= Sedentary normal diet fed; SH= Sedentary hypercaloric diet fed; IS = Intermittent trained normal diet fed; IH= Intermittent trained hypercaloric diet fed; CS= Continuous trained normal diet fed; CH= Continuous trained and hypercaloric fed. Sudan Black 128X

(Mastaglia & Walton, 1982). Similar results were seen by Camargo *et al.* (2003) which showed atrophic and phagocytized fibers, round shape fibers and internal nuclei accentually in rats trained 5 days/week, 60 min, during 60 days.

On the other hand, exercise can be an excellent way to prevent the complications associated with obesity with a preferential loss of fat from abdominal region (Zachwieja). In particular, the fat is an abundant energy reserve. Fat utilization is under coordinated metabolic control and can only occur under aerobic conditions (Stefanick, 1993). Helge *et al.* (1996) investigated the fat utilization during exercise in male untrained subjects and demonstrated that VLDL-TG made a significant contribution to fuel utilization during exercise after adaptation to a fat-rich diet. Lee *et al.* (2001) studied interaction of different exercises (low and high intensity) and diet (high-fat vs. high-carbohydrate) in rats. The results showed that the regular aerobic training evoked a multitude of biochemical adaptations in skeletal muscle and liver with decreased utilization of carbohydrate and increase in the oxidative lipid in untrained animals fed a high-fat diet.

Further a biochemical adaptation in skeletal muscle, Cameron-Smith *et al.* (2003) reported a short-term, high-fat diet up-regulates lipid metabolism and gene expression in well-trained male cyclists and triathletes muscle and the result showed that fatty acids, in addition to their important role as energy-yielding nutrients, may exert a significant influence on the regulation of gene expression. The impact of peripheral

signals, such as ghrelin and leptin were studied by Christi *et al.* (2006) in the effect of increase lipid intake while maintaining carbohydrate intake constant on hormonal responses during aerobic exercise in endurance-trained men. The results showed that the hormones considered being involved in energy homeostasis (ghrelin, GH, leptin and insulin) respond differently during state of acute negative energy balance with an increase in ghrelin and GH and a tendency for a decrease in leptin and insulin. It is possible that the same similar effects can be happened in this present study that demonstrated a significant effect of the diets (normal and high fat) on the initial and post intervention body weight of the trained groups.

Zachwieja, studied exercise as treatment for obesity and related that the mode of activity may affect the success of an exercise intervention and comparing swim training with treadmill exercise at a similar intensity (heart rate equivalent of 75% of treadmill VO₂max) produced equal improvements in cardiovascular fitness and body composition. This study points to the possibility that swimming exercise could be included as one of several modes of physical activity available to treat or prevent obesity.

In conclusion the swimming training caused morphological alterations, some of them similar to that observed in muscle denervation conditions. Hypertrophy was statistically significant in IN, IH and IS groups and it might be benefiting in body composition adjustment to improve obesity control in who fed with high fat diet.

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RESUMEN: El objetivo de este estudio fue investigar el efecto de dietas ricas en grasas y diferentes frecuencias de natación, en el músculo tibial anterior, de ratas machos Wistar. El entrenamiento aeróbico, durante dos y cinco días por semana, causó lesiones en las fibras musculares y la dieta alta en grasa, no produjo resultados estadísticamente significativos, en comparación con la dieta normal.

PALABRAS CLAVE: Entrenamiento aeróbico; Morfología; Dietas ricas en grasas; Tejido muscular.

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