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original article

Effect of carbohydrate intake and physical exercise on glycogen concentration

Efeito de consumo de carboidrato e de exercício físico sobre a concentração de glicogênio

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Abstract – Carbohydrate is an important source of energy which is stored as glycogen. Since this storage may be related to the use of sports drinks, this study evaluated the changes in liver, skeletal muscle and kidney glycogen content of rats supplemented with maltodextrin and submitted to aerobic exercise at maximum lactate steady state or high-intensity anaerobic exercise. Sixty-nine male Wistar rats, 60 days old at the beginning of the experiment, were used. The training protocol consisted of 8 weeks of continuous aerobic (60 min/day) or intermittent swimming (two periods of 30 min/day at an interval of 10 min and an exercise/rest ratio of 15 seconds), with overloads corresponding to 5% and 10% of body weight, respectively. The animals were supplemented for 37 days with a daily dose of 0.48 g/kg maltodextrin dissolved in water or received pure water. Factorial ANOVA or the Kruskal-Wallis test was used for data analysis. Aerobic training resulted in a significant increase in blood glucose ($p<0.02$) and in a decline in hepatic glycogen content ($p<0.02$), while anaerobic exercise promoted a significant increase in lactate concentration ($p<0.001$) and a decrease in skeletal muscle ($p=0.02$) and kidney ($p<0.03$) glycogen content. Maltodextrin supplementation significantly increased muscle ($p=0.008$) and kidney ($p<0.02$) glycogen content in rats submitted to aerobic exercise. Eight weeks of aerobic and anaerobic exercise caused changes in liver, skeletal muscle and kidney glycogen content, as well as in blood glucose and blood lactate. Maltodextrin supplementation was effective in increasing skeletal muscle and kidney glycogen stores in rats submitted to aerobic exercise.

Key words: Energetic metabolism; Fatigue; Glycogen; Wistar rats.

Resumo – *O carboidrato é um importante substrato energético, sendo armazenado na forma de glicogênio. Considerando que este armazenamento pode estar relacionado com o uso de soluções esportivas, este estudo verificou as alterações nos conteúdos de glicogênio hepático, muscular e renal de ratos suplementados com maltodextrina e treinados em exercício aeróbio no estado estável máximo de lactato ou anaeróbio de alta intensidade. Foram utilizados 69 ratos machos da linhagem Wistar, com 60 dias, no início do experimento. O protocolo de treinamento consistiu de oito semanas de natação em padrão aeróbio contínuo (60 min/dia) ou intermitente (dois períodos de 30 min, com intervalo de 10 min e relação esforço/pausa de 15 segundos), com sobrecargas correspondentes a 5% e 10% do peso corporal, respectivamente. Durante 37 dias, os animais foram suplementados com uma dose diária de 0,48 g/kg de maltodextrina dissolvida em água ou receberam água pura. Para a análise dos dados, utilizou-se a Anova fatorial ou o Teste de Kruskal-Wallis. O treinamento aeróbio ocasionou aumento na glicemia ($p<0,02$) e redução de glicogênio hepático ($p<0,02$), enquanto o exercício anaeróbio proporcionou aumento no lactato ($p<0,001$) e diminuição de glicogênio muscular ($p=0,02$) e renal ($p<0,03$). A maltodextrina causou elevação no conteúdo de glicogênio muscular ($p=0,008$) e renal ($p<0,02$) dos ratos exercitados em exercício aeróbio. Oito semanas de exercício aeróbio e anaeróbio proporcionaram importantes alterações nos conteúdos de glicogênio hepático, muscular e renal, assim como na glicemia e no lactato sanguíneo. A suplementação com maltodextrina foi efetiva em elevar o conteúdo de glicogênio muscular e renal de ratos exercitados em exercício aeróbio.*

Palavras-chave: *Fadiga; Glicogênio; Metabolismo energético; Ratos wistar.*

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INTRODUCTION

The energy necessary for muscle contraction during physical exercise can be supplied by the aerobic and anaerobic metabolism[1] and the predominance of one or the other pathway varies according to the intensity and duration of training loads[2]. Moreover, glycogen stores are fundamental to sustain performance during physical exercise of different intensities and durations[2].

Carbohydrate is an important energy substrate for exercise, which is stored as glycogen[2]. Glycogen reserves are closely related to performance and the length of time an effort can be sustained during a given exercise[3]. During the early stages of exercise, most energy produced from carbohydrates is derived from muscle glycogen and its use decreases with increasing load duration. The reduction in muscle glycogen stores is compensated for by a greater participation of blood glucose in the production of energy from carbohydrates[4].

Like skeletal muscle, other tissues can adapt in different manners to sustain exercise. These adaptations result from the response of multiple organs and systems[5]. In this respect, changes in the glycogen stores of the liver and kidney can be the result of different adaptations of these organs to different physical training patterns.

Another factor of interest in studies designed to understand the adaptations of different tissues in response to training is the use of carbohydrate solutions to improve performance. In fact, to effectively maintain the physiological response during exercise, fluids containing variable amounts of electrolytes and carbohydrates are used to improve sports performance[6]. The benefits of supplementation with carbohydrates, such as maltodextrin, can be explained mainly by the maintenance of high levels of blood glucose, probably preventing a decrease in performance associated with hypoglycemia[7].

One of the issues currently discussed is the possibility of saving glycogen stores in different tissues through the ingestion of carbohydrate solutions[8,9], considering the good relationship between glycogen availability and performance during moderate and exhaustive exercise[10]. In this respect, studies investigating liver and kidney responses to the chronic consumption of carbohydrate beverages and its consequences for glycogen stores are sparse.

The objective of the present study was to evaluate changes in liver, muscle and kidney glycogen content, as well as in blood glucose levels, of rats supplemented with maltodextrin and submitted to aerobic exercise at maximum lactate steady state (MLSS) (moderate) or high-intensity anaerobic exercise (exhaustive).

METHODOLOGICAL PROCEDURES

Animals

Sixty-nine male Wistar rats, 60 days old and weighing 199 to 299 g (276.0 ± 22.3 g) at the beginning of the experiment, were used. The animals were obtained from the Animal House of the Federal University of Pelotas (Universidade Federal de Pelotas - UFPel) and were fed balanced standard chow

(Nuvilab® CR1) and water *ad libitum*. The animals were kept in collective cages at a controlled room temperature of 21-25°C under a 12-h light/dark cycle.

The experiments were conducted in accordance with specific Brazilian resolutions on Bioethics in Animal Experimentation (Law No. 6638 from May 8, 1979 and Decree No. 24645 from July 10, 1934). The study was approved by the Ethics Committee on Animal Experimentation of UFPel (Permit No. 23110.005873).

Experimental groups

The animals were transferred to the Laboratory of Biochemistry and Exercise Physiology of UFPel (LABFex/UFPel), weighed, and randomly divided into six groups: sedentary, unsupplemented (sedentary-water, n=12) and supplemented with maltodextrin(sedentary-CHO, n=12); submitted to continuous aerobic exercise with a load corresponding to the MLSS, unsupplemented (aerobic-water, n=11) and supplemented with maltodextrin (aerobic-CHO, n=11); submitted to high-intensity anaerobic exercise, unsupplemented (anaerobic-water, n=12) and supplemented with maltodextrin (anaerobic-CHO, n=11).

Training protocol

The training period comprised 10 weeks. The first two weeks were used for adaptation to the liquid medium (five times per week) in a collective tank at a water temperature of 30±1°C using progressive overloads (the loads were adjusted weekly according to changes in the animal's weight). The subsequent 8 weeks consisted of swimming exercises on 5 consecutive days per week, with each session lasting 60 min. The animals were submitted to continuous exercise at MLSS (60 min/day with a load corresponding to 5% of body weight[11]) or intermittent exercise (two periods of 30 min/day at an interval of 10 min with a load corresponding to 10% of body weight[11] and an exercise/rest ratio of 15 seconds). The experiment was conducted under a reversed light/dark cycle (light between 18:00 and 6:00 h).

The intermittent swimming sessions had a daily duration of 70 min and were performed as follows: the animals (in groups of three and a maximum of four rodents) were placed in a cylinder (80 cm in diameter and 100 cm in depth) with an open bottom. This cylinder was transferred to a tank so that the animals would perform 15 seconds of swimming exercise. After this period, the cylinder was lifted with a steel wire so that the animals would rest for 15 seconds. This procedure comprising 15 seconds of exercise and 15 seconds of rest was performed by the animals over a period of 30 min (first period). The animals then rested passively for 10 min. The second period of 30 min of intermittent swimming exercise was started as described for the first period. This protocol was repeated until the penultimate day of training.

The body weight of the animals was monitored every Monday and the overload was corrected according to changes in body weight. Animals of the sedentary groups were placed in a tank with shallow water (depth

of 10 cm, immersion bath) at a temperature of 30±1°C for 15 min, on 5 consecutive days per week, and were used as controls. After each training session, the animals were dried and kept at room temperature (21-25°C).

On the last day of the experiment, unsupplemented and supplemented animals of the groups trained at high intensity swam until exhaustion to determine the effect of anaerobic exhaustive exercise on the dependent variables studied. After administration of the solutions, the animals were put back into the tank to perform continuous exercise until exhaustion with an overload of 10% of fixed body weight. Exhaustion was defined when the animal remained submersed for more than 30 seconds.

Supplementation protocol

Supplemented animals of the sedentary group and groups trained at MLSS and at high intensity received a 12% solution (m/v) of maltodextrin dissolved in distilled water through a gastric tube (gavage)[12]. The carbohydrate dose administered was 0.48 g/kg weight in a volume of 1 mL per 250 g of animal weight. The volume was increased or reduced by 0.02 mL per 5 g of weight above or below the baseline body weight. Unsupplemented animals of the sedentary group and groups trained at MLSS and at high intensity received only pure water using the same technique as described for the supplemented groups. The animals were supplemented five times per week during the training period, for 40 days until the day of sacrifice. The animals did not receive the carbohydrate supplement during the adaptation period since the duration and intensity of training were lower than those used during the training period. However, the animals were exposed to the gastric tube to permit adaptation to the manipulation. The solutions were administered to animals of the trained groups after they had been submitted to swim warm-up for 2 min.

Tissue samples and analysis

The animals were sacrificed (25 mg/kg thiopental) on the last day of training immediately after the aerobic or exhaustive exercise sessions, or after one hour of rest following the administration of the maltodextrin solution or pure water to animals of the sedentary groups. Blood samples and tissue samples of the liver, kidney and gastrocnemius muscle (white and red portions) were collected. Whole blood (25 μL) was collected without anticoagulant for the determination of lactate concentration. Additionally, about 3 mL of whole blood was collected without anticoagulant and immediately centrifuged at 3,000 rpm for 10 min for the separation of serum. Aliquots of the freshly collected serum were stored at -20°C for subsequent analysis of serum glucose. Duplicate samples of the liver, kidney and gastrocnemius muscle (white and red portions) were collected, weighed and stored at -20°C until the time of extraction and quantification of glycogen.

Serum glucose was analyzed by spectrophotometry using a commercial kit from Labtest Diagnóstica (Lagoa Santa, MG, Brazil). Blood lactate concentration was determined in whole blood (collected by cardiac puncture

from each animal by a professional experienced in this collection technique) using an Accusport® lactate analyzer (Boehringer-Mannheim, Germany). Liver, kidney and muscle glycogen was extracted as described by Peixoto and Pereira[12]. The tubes containing the extracted glycogen were stored in a freezer until the time of quantification (up to 5 days). Glycogen content of the liver, kidney and muscle was determined by the method of Krisman[13] and is expressed as mg glycogen per 100 mg tissue.

Statistical analysis

Statistical analysis was performed using the STATISTICA 8.0 for Windows software (Statsoft). When the variables showed a normal distribution, factorial analysis of variance was used for comparison between means, followed by Fisher's post-hoc test. The Kruskal-Wallis test was used for variables showing a nonparametric behavior. The results are expressed as the mean and standard deviation. A level of significance of $p<0.05$ was adopted.

RESULTS

Table 1 shows the results regarding the effects of training and carbohydrate supplementation on blood lactate concentration in Wistar rats. A significant increase ($p<0.001$) in blood lactate concentrations was observed in animals submitted to high-intensity anaerobic exercise when compared to those submitted to aerobic exercise at MLSS. Wistar rats submitted to high-intensity anaerobic exercise also exhibited significantly higher blood lactate concentrations than sedentary animals ($p<0.001$). No difference in blood lactate concentration was observed between the groups receiving water and maltodextrin supplementation.

A significant increase in serum glucose concentration was observed in animals of the aerobic-water group compared to the sedentary-water group ($p<0.02$) (Table 1).

Table 1. Serum glucose and blood lactate concentrations in Wistar rats of the different experimental groups.

	Sedentary		Aerobic		Anaerobic	
	Water (n=12)	CHO (n=12)	Water (n=11)	CHO (n=11)	Water (n=12)	CHO (n=11)
Serum glucose (mg/dL)	116.3±24.6	130.5±15.5	195.9±81.9*	214.5±92.7	129.1±29.4	134.3±50.4
Blood lactate (mmol/L)	5.1±1.3	4.6±1.2	6.8±4.6	7.8±4.1	17.3±3.2[a,b]	16.1±2.3

Values are the mean ± standard deviation. Water: animals receiving pure water. CHO: animals supplemented with maltodextrin. Sedentary: sedentary animals. Aerobic: animals submitted to continuous aerobic exercise at maximum lactate steady state. Anaerobic: animals submitted to high-intensity anaerobic exercise. *$p<0.02$ versus sedentary-water group. [a]$p<0.001$ versus sedentary-water group. [b]$p<0.001$ versus aerobic-water group. The Kruskal-Wallis test was used for statistical analysis.

Wistar rats of the aerobic-water and aerobic-CHO groups exhibited significantly lower liver glycogen concentrations ($p<0.02$) than sedentary-CHO animals. No significant differences in liver glycogen content were observed between the group submitted to high-intensity anaerobic exercise and the sedentary group and there was no effect of maltodextrin supplementation when these groups were compared (Figure 1).

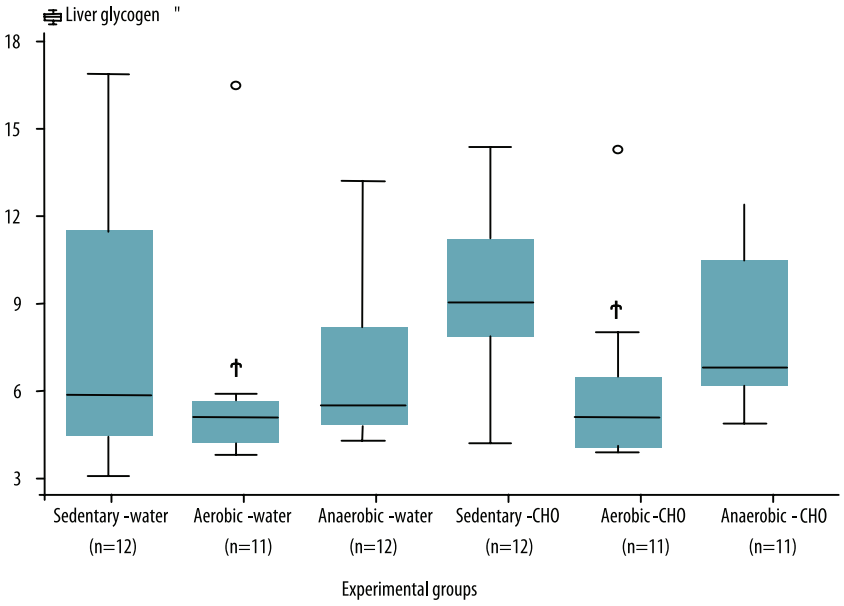

Figure 1. Liver glycogen content (mg/100 mg tissue) in Wistar rats.
Values are expressed as the mean and standard deviation. Water: animals receiving pure water. CHO: animals supplemented with maltodextrin. Sedentary: sedentary animals. Aerobic: animals submitted to continuous aerobic exercise at maximum lactate steady state. Anaerobic: animals submitted to high-intensity anaerobic exercise.↑p<0.02 versus sedentary-CHO group. Factorial ANOVA followed by Fisher's post-hoc test were used for statistical analysis.

Gastrocnemius muscle glycogen concentrations were significantly lower (p=0.02) in the anaerobic-water group when compared to the aerobic-CHO group. Animals of the aerobic-CHO group exhibited significantly higher gastrocnemius muscle glycogen concentrations than animals of the sedentary-water (p=0.007) and sedentary-CHO group (p=0.008) (Figure 2).

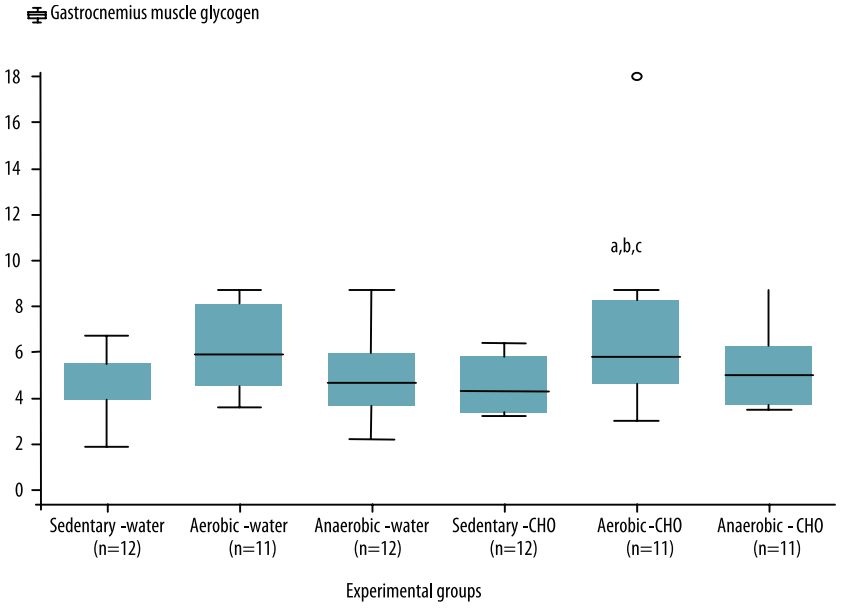

Figure 2. Gastrocnemius muscle (white and red portions) glycogen content (mg/100 mg tissue) in Wistar rats.
Values are expressed as the mean and standard deviation. Water: animals receiving pure water. CHO: animals supplemented with maltodextrin. Sedentary: sedentary animals. Aerobic: animals submitted to continuous aerobic exercise at maximum lactate steady state. Anaerobic: animals submitted to high-intensity anaerobic exercise. [a]p=0.007 versus sedentary-water group. [b]p=0.008 versus sedentary-CHO group. [c]p=0.02 versus anaerobic-water group. Factorial ANOVA followed by Fisher's post-hoc test were used for statistical analysis.

Animals of the aerobic-CHO group exhibited significant increases in kidney glycogen content when compared to the sedentary-water ($p<0.04$), aerobic-water ($p<0.05$), anaerobic-water ($p<0.003$), and anaerobic-CHO groups ($p<0.02$) (Figure 3). A significant reduction in kidney glycogen content was observed in the anaerobic-water group compared to animals of the sedentary-CHO group ($p<0.03$) (Figure 3).

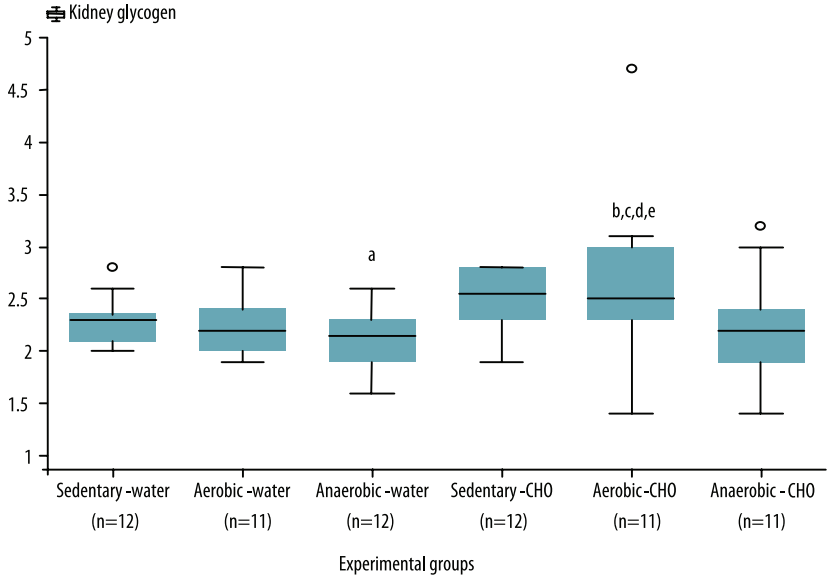

Figure 3. Kidney glycogen content (mg/100 mg tissue) in Wistar rats.
Values are expressed as the mean and standard deviation. Water: animals receiving pure water. CHO: animals supplemented with maltodextrin. Sedentary: sedentary animals. Aerobic: animals submitted to continuous aerobic exercise at maximum lactate steady state. Anaerobic: animals submitted to high-intensity anaerobic exercise. [a]$p<0.03$ versus sedentary-CHO group. [b]$p<0.04$ versus sedentary-water group. [c]$p<0.05$ versus aerobic-water group. [d]$p<0.003$ versus anaerobic-water group. [e]$p<0.02$ versus anaerobic-CHO group. Factorial ANOVA followed by Fisher's post-hoc test were used for statistical analysis.

DISCUSSION

Animal models provide appropriate conditions for the study of cell metabolism events involved in the metabolic adaptations to exercise, which would not be feasible otherwise. Studies investigating the metabolism of different tissues at different exercise intensities and during the chronic administration of liquid macronutrient supplements are sparse in the literature. The present study evaluated the changes in liver, muscle and kidney glycogen content, as well as in glucose and lactate levels, of rats supplemented with maltodextrin and submitted to aerobic exercise at MLSS or high-intensity anaerobic exercise.

Blood lactate is an important marker of physical performance[11]. Like the increase in lactate concentration, increases in glucose levels represent adaptations in the ability to sustain effort[14]. Therefore, increases in blood lactate and glucose concentrations are influenced by the increase in load intensities, as demonstrated in the present study and widely reported in the literature[11,14].

In contrast to the present results, another study demonstrated a reduction in lactate production in the soleus muscle of Wistar rats at the end of

8 weeks of exercise on a treadmill at an intensity of 25% above the aerobic/anaerobic metabolic transition, compared to a group of animals trained at a velocity corresponding to the metabolic transition[15]. In a study involving adult male Wistar rats submitted to low-intensity and exhaustive swimming exercise, lactate concentrations were found to be lower in trained animals than in sedentary animals[16]. After 8 weeks of swimming exercise at 80% of maximum load, a reduction in blood lactate concentration was identified when compared to the group of sedentary animals[17]. The divergences between these studies and the present study are probably due to differences in the experimental protocols. In those studies, the animals were sacrificed after maximum exercise, while in the present study the animals were sacrificed under conditions of rest, MLSS and exhaustion.

In the present study, continuous aerobic exercise promoted an increase in serum glucose concentration. An increase in serum glucose concentration as a result of the training protocol has also been reported in other studies on animals submitted to aerobic exercise at loads corresponding to the MLSS and supplemented with maltodextrin[18,19]. In a study on male Wistar rats fed a high-carbohydrate or high-fat diet in which blood samples were collected pre- and post-swimming exercise, serum glucose concentration only differed between the pre- and post-exercise experimental groups[20], i.e., between rested animals and animals submitted to 1 h of swimming. When male Wistar rats fed a normal diet and submitted to swimming training of different durations were studied, the groups swimming for 2 and 4 h exhibited higher glucose levels than untrained animals[21]. In another study, no difference in glucose levels was observed between adult male Wistar rats submitted to 4 weeks of swimming exercise with an overload of 5% of body weight and the sedentary group[22]. The present results agree with the studies of Leite[18], Rombaldi[19] and Ochiai and Matsuo[20,21] who reported higher glucose levels in rats submitted to moderate exercise compared to sedentary animals. Different results have been reported by Figueira et al.[22], probably because the authors used a different experimental protocol.

An adequate deposition of glycogen in tissues is essential to sustain performance during exercise. Although kidney glycogen content during exercise has been little studied, this organ also seems to be affected by the adaptive influences of training. In the present study, aerobic training promoted a reduction in liver glycogen levels; a decrease in muscle glycogen content in rats submitted to anaerobic exercise receiving water, and an increase in muscle and kidney glycogen content in rats submitted to aerobic exercise and supplemented with maltodextrin, as well as a reduction in kidney glycogen content in the group of animals submitted to high-intensity anaerobic exercise.

One study showed that the liver glycogen content of rats submitted to aerobic training was influenced by the type of diet consumed. Trained animals consuming a carbohydrate-rich diet had higher liver glycogen concentrations post-exercise than animals receiving a high-fat diet. However, the glycogen content of gastrocnemius muscle was significantly

lower post-exercise[20]. Significant reductions in liver glycogen content were observed in male Wistar rats submitted to exhaustive exercise[23]. On the other hand, no significant difference in liver or muscle glycogen content was observed between adult male Wistar rats submitted to 4 weeks of swimming exercise with an overload of 5% of body weight and animals of the control group[22]. In a study involving rats submitted to aerobic training for 12 weeks, significant increases in gastrocnemius muscle glycogen content were found when compared to the sedentary group[24]. In rats submitted to exhaustive exercise which received a high- or low-carbohydrate diet, muscle glycogen content decreased significantly from pre-test to post-test; however, muscle glycogen content was significantly higher in the group consuming the high-carbohydrate diet[25]. In another study, liver and muscle glycogen content was found to be higher in trained animals than in the group of untrained animals[26]. As mentioned earlier, the differences between these studies and the present study are probably due to differences in the experimental protocols, with the animals of the previous studies being sacrificed after maximum exercise and those of the present study under conditions of rest, MLSS and exhaustion. Furthermore, the type of diet consumed by the animals of the different studies may have also contributed to the results obtained.

The decline in liver glycogen content observed here in animals submitted to aerobic training might be related to the greater supply of glucose to the contracting muscle, a fact also demonstrated by the higher availability of muscle glycogen during aerobic training. These results can be explained by the fact that carbohydrate requirements differ according to training intensity. In this respect, when the intensity of exercise is low, the total glucose oxidation rate is also low. On the other hand, when the exercise intensity increases, the active muscle mass becomes increasingly dependent on carbohydrate as an energy source, exhausting its endogenous stores[27].

We found no additional studies evaluating the changes in kidney glycogen content in rats submitted to physical training and without metabolic anomalies. However, one possible explanation for the alterations in kidney glycogen content observed here in rats submitted to different physical training patterns and receiving a sports drink containing maltodextrin may be the greater supply and utilization of this energy substrate by this organ. The kidney and liver seem to play an important role in the maintenance of glucose levels for physical exercise.

The limitations of this study are related to the need to evaluate the levels of adrenocorticotropic hormone, cortisol, epinephrine and insulin in the different tissues in order to better understand the true utilization of the energy substrates addressed in this study.

CONCLUSIONS

The present results permit us to conclude that high-intensity exercise promoted an increase in blood lactate concentration in rats, while continuous

training at a load corresponding to the MLSS increased glucose levels. Furthermore, both physical training patterns exerted important effects on liver, muscle and kidney glycogen content. Supplementation with maltodextrin was effective in sparing muscle and kidney glycogen stores after continuous aerobic exercise at a load corresponding to the MLSS.

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