EFFECTS OF CARBOHYDRATE SUPPLEMENTATION AND DIFFERENT TYPES OF EXERCISE TRAINING ON BLOOD CELLS CONCENTRATIONS

EXERCISE AND SPORTS SCIENCES

ORIGINAL ARTICLE

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ABSTRACT

Introduction: The participation of athletes in sessions of intense and prolonged exercise can lower the number and functional capacity of circulating leukocytes. On the other hand, the intake of a carbohydrate solution can attenuate the immunosuppressive effects of exercise. Objective: To evaluate the effects of aerobic and anaerobic exercises, and carbohydrate supplementation on blood concentrations of total and differential counts of leukocytes, hemoglobin and serum glucose in Wistar rats. Methods: 69 Male Wistar rats (60 days) were divided into six groups: sedentary non-supplemented (n = 12) and supplemented (n = 12); trained in Maximum Lactate Steady State (MLSS) not supplemented (n = 11) and supplemented (n = 11); trained at high intensity non-supplemented (n = 12) and supplemented (n = 11). The training protocol consisted of eight weeks of continuous swimming pattern MLSS (60min.day^{-1}) or intermittent (two periods of 30 minutes, for exercise with 10 minutes rest), with overloads corresponding to 5% and 10% of body weight, respectively. For 37 days the animals were supplemented with a daily dose of 0.48 g.kg-1 maltodextrin dissolved in water or pure water. Results: There was no effect of carbohydrate supplementation and the two types of training on blood concentrations of leukocytes. The anaerobic exercise ($p = 0.04$) and the use of maltodextrin ($p = 0.003$) resulted in increases in blood hemoglobin concentrations, while aerobic exercise caused an increase in the concentration of serum glucose ($p < 0.02$). Conclusion: The different types of exercises were not involved with leukopenia, anemia, or hypoglycemia that could lead to early muscle fatigue and decreased performance.

Keywords: leukocytes, hemoglobins, maltodextrin, athletic performance.

INTRODUCTION

Immune system monitoring of athletes has become an important part of physical preparation[1]. In order to improve training programs in the long run, the concentration and function of the leucocytes have become relevant[2]. A special reason is the interruptions the elite athletes impose to their training when sick and this fact may influence on the physical preparation and performance during competition days[1]. Additionally, the participation in repeated intense and prolonged exercise sessions may decrease the circulating number and functional capacity of leucocytes[3]. Hypoglycemia caused during exercise also results in high response to stress and aassociated immunosuppression[4].

The circulation of white cells in the blood rapidly increases with exercise[5]. However, the training effect on the immune function depends on the intensity and the kind of exercise practiced[6]. Different kinds of training may cause varied immunological responses. Thus, the emphasis on endurance exercises has become especially interesting, since the high training volume may increase the risk to diseases[1].

On the other hand, the use of sports supplements presents potential to improve performance[7] the same way the consumption of a carbohydrate solution during training is recommended to attenuate some immunosuppressive effects of prolonged exercise[8]. Thus, the aim of this study was to verify the effects of aerobic exercise on the amount of maximal lactate steady state (MLSS), anaerobic of high intensity and of the supplementation with maltodextrin on the total and differential count of leucocytes, the content of hemoglobin and concentration of serum glucose of Wistar rats.

METHODS

Animals

Sixty nine male Wistar rats with 60 days and weighing at the beginning of the experiment between 199-409 grams were used. The animals came from the Animal Facility of the Federal University of Pelotas RS, Brazil (UFPel) and were fed with balanced standard chow (Nuvilab® CR1), given water *ad libitum* and distributed in collective cages. Room temperature was controlled between 21-25°C and photo period was of 12h light and 12h dark.

Experimental groups

The animals were transferred to the Laboratory of Biochemistry and Exercise Physiology from UFPel (LABFex/UFPel), weighed an randomly distributed in six groups: sedentary non-supplemented (n = 12) and supplemented (n = 12); trained in MLSS non-supplemented (n=11) and supplemented (n=11); trained in high intensity non-supplemented (n=12) and supplemented (n=11).

Training protocol

The training period was of ten weeks, being the two first ones of adaptation to the water medium (five times per week) with pro-

gressive load and in a collective tank with water at the temperature of 30 ± 1°C. The eight subsequent weeks were of swimming exercises, five consecutive days for a week and 60 minutes per session, in a continuous or intermittent way (two periods of 30 minutes, with 10 minutes of recovery; exercise and recovery duration was of 15 seconds). The experiment was performed in the light cycle between 6pm and 6am. The loads used corresponded to 5% of body weight for the continuous standard exercise in MLSS (aerobic) or of 10% of body weight for the intermittent exercise, being considered each training load of high intensity[9]. The body weight of the animals was monitored every Monday and the load was corrected from the weight alteration. The animals from the sedentary group were placed in a tank with shallow water, 10 cm deep (immersion bath) at the temperature of 30 ± 1°C, for 15 minutes, five consecutive days per week and they were used as controls. After each swimming session, the rodents were dried and placed in environment with between 21 and 25ºC to avoid physiological complications derived from the cold and humidity. On the last day of the experiment, the animals from the group trained at high intensity non-supplemented and supplemented swam until exhaustion. Exhaustion was determined when the animals remained submersed for a period longer than 30 seconds[10]. The exercise until exhaustion was performed with the aim to verify the effect of the anaerobic exercise until exhaustion on the dependent variables of this study.

Supplementation protocol

The supplemented animals from the sedentary group, trained at MLSS and trained at high intensity were supplemented through a gastric tube (gavage) with liquid carbohydrate solution at 12% (m/v) of maltodextrin dissolved in pure water[10]. The carbohydrate dose administered was of 0.48 $g.kg^{-1}$ of weight, in 1 ml volume for 250 g of animal weight, and at each 5 g of weight above or below the baseline body weight the volume increased or decreased in 0.02 ml. The non-supplemented animals from the sedentary group, trained at MLSS and trained at high intensity received only pure water using the same technique of the supplemented groups. The rats were supplemented five times per week, during the training period, for 37 days. The solutions were administered to the animals from the trained groups after the rodents were submitted to previous swimming warm-up for two minutes.

Blood samples and analyses

The animal sacrifice occurred on the last day of training, immediately after the aerobic exercise sessions, or exhaustion exercise, or after one hour recovery, after the maltodextrin or water solution was administered to the animals from the sedentary groups, when blood samples were collected. About 2 ml of total blood were collected with EDTA for performance of total and differential count of leukocytes as well as the hemoglobin content and 3 ml of total blood without anticoagulant. The serum was separated by centrifuging at 3,000 rpm for ten minutes. Aliquots of this recently collected material were stored at -20ºC for subsequent analysis of the glucose concentration.

Total and differential count of leukocytes was carried out according to the adapted technique by Dantas *et al.*[11] in blood samples diluted in the 1:20 proportion in Turk's fluid; and in blood smears fixed and tinted by the Giemsa method. The hemoglobin content and the serum glucose were determined by spectrophotometry and followed the guidelines in the commercial kits by Labtest (Lagoa Santa/MG/Brazil), references Ref.: 43 and Ref.: 84, respectively.

Statistical analysis

The statistical analysis was conducted with the statistical package *STATISTICA* for *Windows*, version 8, by *Statsoft*. When the variables followed the normal curve, factorial analysis of variance was applied for comparison between means. Concerning the variables which presented non-parametric behavior, the *Kruskal-Wallis*.test was used. The values were expressed as mean and standard deviation, and the significance level adopted was of $p < 0.05$.

Ethics procedures

The experiments with the animals were performed according to the Brazilian specific resolutions about Bioethics in Animal Experimentation (Law # 6638, May 8,1979 and Decree # 24645, July 10,1934); and they were approved by the Ethics Committee in Animal Experimentation (CEEA) from UFPel (law file number 5873/2009).

RESULTS

Table 1 present data about the white cells concentrations. Significant statistical differences have not been observed in the total and differential count of leukocytes among the six experimental groups.

The animals from the high-intensity anaerobic group and which received pure water presented significant increase in the hemoglobin content compared with the animals from the sedentary group and which received pure water = 0.04) and sedentary supplemented with maltodextrin ($p < 0.002$) (figure 1). The high intensity anaerobic training associated with the carbohydrate supplementation demonstrated significant increase in the hemoglobin content compared with the control group of sedentary animals supplemented with carbohydrate ($p = 0.003$). However, the animals trained with continuous aerobic exercise under MLSS load and which received pure water represented significant decrease in the hemoglobin content compared with the animals submitted to high intensity anaerobic exercise and which received pure water ($p < 0.007$) or with the animals supplemented with maltodextrin ($p = 0.01$) (figure 1).

The animals trained with aerobic exercise under MLSS load and which received pure water presented significant increase in the serum glucose concentration compared with the group with sedentary animals which received pure water ($p < 0.02$). There was not significant difference in the glycemic values between the group of rats trained in high intensity anaerobic exercise and which received pure water compared with the group of sedentary animals which received pure water. There was not significant effect either of the supplementation with maltodextrin in the serum glucose concentration between the different experimental groups of the present study (figure 2).

DISCUSSION

Participation in training programs of high intensity may increase the risk for higher disorders in its immunocompetence[12]. High intensity or prolonged duration may produce an open window indicating higher risk for infections[13]. However, carbohydrate sup-

Table 1. Comparison between the total and differential count of leukocytes between the experimental groups.

Concentration of circulating leukocytes (n of cells/mm^3)			
	Sed	Aer	Anaer
Total leukocytes	12.133.3 ± 9.832.6* 10.154.2 ± 7.256.0**	14.886.4 ± 11570.5* 14.963.6 ± 10879.6**	14.870.8 ± 7.690.6* 14.840.9 ± 10.477.3**
Neutrophils	1.087.9 ± 893.0* 795.9 ± 574.4**	1.362.3 ± 1.105.2* 1.591.5 ± 1.113.7**	1.087.3 ± 559.7* 1.360.8 ± 1.454.6**
Eosinophils	174.2 ± 162.4* 163.0 ± 162.4**	224.2 ± 209.1* 283.7 ± 350.7**	274.0 ± 243.0* 234.4 ± 148.6**
Basophils	132.7 ± 107.7* 101.5 ± 72.6**	148.9 ± 115.7* 234.5 ± 290.4**	176.2 ± 101.1* 190.5 ± 163.3**
Limphocytes	10.350.2 ± 8.486.9* 8.848.7 ± 6.479.0**	12.867.3 ± 10.435.2* 12.585.4 ± 9.399.7**	12.957.3 ± 7.207.2* 12.483.2 ± 9.184.1**
Monocytes	388.4 ± 320.9* 247.1 ± 210.5**	277.7 ± 243.8* 269.6 ± 168.1**	375.9 ± 222.2* 572.0 ± 539.0**

Values are expressed as mean and standard deviation. *Corresponding to the animals which received pure water. **Corresponding to the animals supplemented with maltodextrin. Sed: sedentary animals. Aer: animals trained in continuous aerobic exercise under Maximal Lactate Steady State load. Anaer: animals trained in high intensity anaerobic exercise. The Kruskal Wallis statistical test was used for the variable Monocytes. The ANOVA factorial statistical test was used for the remaining variables.

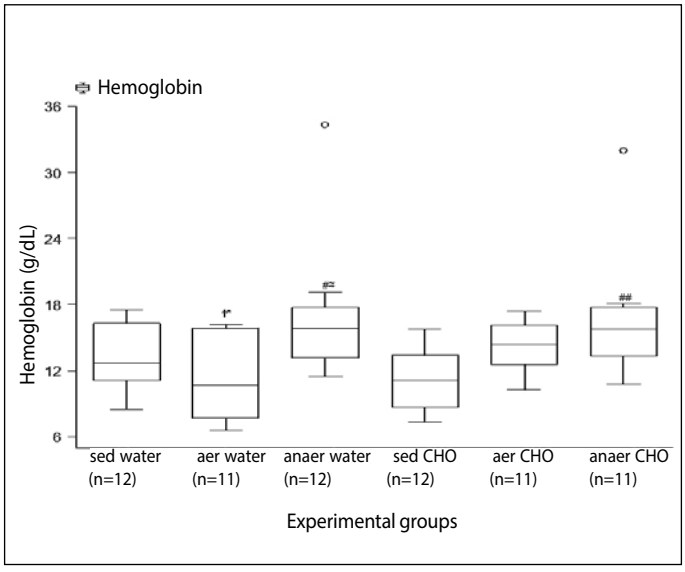

Figure 1. Blood hemoglobin concentration (g/dL) of Wistar rats. Values are expressed as mean and standard deviation.
Water: Received pure water. CHO: Supplemented with maltodextrin. Sed: sedentary animals. Aer: animals trained in continuous aerobic exercise under MLSS load. Anaer: animals trained in high intensity anaerobic exercise. †p < 0.007 versus anaer water. *p = 0.01 versus anaer CHO. *p = 0.04 versus sed water. ‾p < 0.002 versus sed CHO. ##p = 0.003 versus sed CHO. The ANOVA factorial statistical test followed by Fisher's test was used.

plementation may improve the immune function in response to exercise since it preserves glutamine and maintains the glucose availability to the leukocytes[14].

In the present study, no alterations have been observed in the total and differential count of leukocytes with performance of aerobic exercise under MLSS load and with high intensity anaerobic exercise. Likewise, the use of sports solution with maltodextrin did not cause alterations in the blood levels of white cells. Such scenario corroborates that these two training patterns did not cause leucopenia which could be involved with immunosuppression.

Diverse responses in the concentrations of these blood cells were identified in animal models. In male Wistar rats submitted to

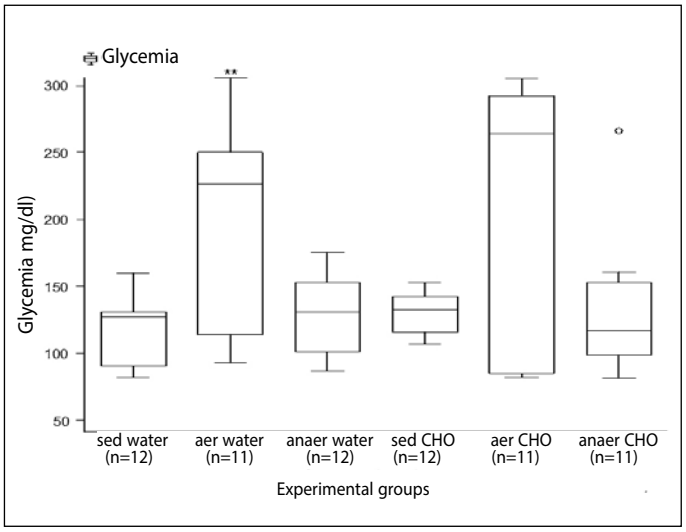

Figure 2. Glycemia (mg/dL) of Wistar rats. Values are expressed as mean and standard deviation.
Water: corresponds to animals which received pure water. CHO: corresponds to the animals supplemented with maltodextrin. Sed: sedentary animals. Aer: animals trained in continuous aerobic exercise under Maximal Lactate Steady State load. Anaer: animals trained in high intensity anaerobic exercise. **p < 0.02 versus sed water. The Kruskal Wallis statistical test was used.

acute exercise sessions of low and moderate intensity significant increase in the total count of leukocytes was detected, as well as in the circulating levels of neutrophils, lymphocytes and monocytes, compared with the group sedentary control[15]. After 36 hours from the end of the training sessions under exercise load to induce overtraining in male Wistar rats, it was observed that apoptosis of neutrophils and lymphocytes was higher when compared with the control group[16]. In another study, it was observed that exhaustion exercise caused leukocytosis after the end of the session compared with the group of control animals[17]. Significant increase was also identified in the total leukocytes count after the animals completed at exhaustion test in swimming compared with the animals from the control group[18]. Trained individuals tend to present low hemoglobin concentrations due to increase in the plasma volume. This sports anemia can also be observed in exercisedindividuals[19]. In the present study, increase in the hemoglobin content has been identified with performance of high intensity anaerobic exercise and with use of sports solution with maltodextrin. However, aerobic exercise provided reduction in the hemoglobin concentration when compared with anaerobic exercise.

The literature demonstrates that in male rats submitted to different kinds of physical exercise, exhaustion led to increase in the levels of hemoglobin concentration[19]. In female rats, effect of exercise on the hemoglobin concentration with decrease in the levels in 36 hours after exercise was observed[20].

In the present study, further studies which associated the use of sports carbohydrate solution and physical exercise on the leukocytes and hemoglobin concentrations in animal models have not been found. Thus, future studies are needed to confirm a possible benefit of maltodextrin supplementation in these blood cells.

Concerning glycemia, the present study demonstrated that continuous aerobic exercise provided increase in serum glucose concentration. In male Wistar rats fed with normal diet

and submitted to swimming training with different duration, it was identified that glycemia was higher in the groups which swam for two to four hours, compared with the animals from the group which did not exercise[21]. In male Wistar rats fed with carbohydrate-rich diet or fat-rich diet and whose blood collections were performed pre and post-swimming exercise, it was observed that the serum glucose concentration was not different among the experimental groups in the post-exercise[22].

Based on the results presented, the limitations of the present study are concerned about the need for evaluation of other cellular markers of the immune system, such as interleukins (IL-6, IL-8, etc.), immunoglobulins (IgA, IgM, IgG) and lymphocyte subpopulations (natural killer cells, lymphocytes-B and lymphocytes-T). There is also need for evaluations of maximal aerobic capacity and erythropoietin concentration.

CONCLUSION

The present study demonstrated that with eight weeks of continuous aerobic training under MLSS load it was possible to observe increase in the glycemic level, as well as performance of high intensity anaerobic exercise associated with the use of sports solution with maltodextrin caused increase in the hemoglobin content; however, both training patterns and the use of carbohydrate solution did not alter the blood concentrations of circulating leukocytes. Thus, the different kinds of exercise were not involved with the leucopenia which could lead to immunosuppression, hypoglycemia or anemia which may lead to early muscular fatigue and decrease in performance.

All authors have declared there is not any potential conflict of interests concerning this article.

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