International Journal of EXERCISE SCIENCE

Original Research

Glycogen Kinetics of Wistar Rats: Different Exercise Intensities and Tissue Analyzed Influence

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ABSTRACT

International Journal of Exercise Science 15(2): 289-299, 2022. The aim of this study is to verify the influence of the intensity on muscle and hepatic glycogen depletion and recovery kinetics of Wistar rats, submitted to three acute training sessions with equalized loads. 81 male Wistar rats performed an incremental test to determine maximal running speed (MRS) and divided into 4 groups: baseline group (Control; n = 9); low intensity training session (G_{Z1}; n = 24; 48 minutes at 50% of MRS); moderate intensity group (G_{Z2}; n = 24; 32 minutes at 75% of MRS) and high intensity group (G_{Z3}; n = 24; 5x5 minutes and 20 seconds at 90% of MRS). Immediately after the sessions and after 6, 12 and 24 hours, 6 animals from each subgroup were euthanized for glycogen quantification in soleus and EDL muscles and liver. A Two-Way ANOVA and the Fisher's Post-hoc test was used ($p < 0.05$). Glycogen supercompensation occurred between 6 and 12 hours after exercise in muscle tissue and 24 after exercise in the liver. The muscle and hepatic glycogen depletion and recovery kinetics are not modulated by exercise intensity since the load was equalized, but effects were distinct in different tissues. Hepatic glycogenolysis and muscle glycogen synthesis processes seem to run in parallel.

KEY WORDS: Acute exercise, equalized load, recovery, supercompensation, running

INTRODUCTION

The human carbohydrate stores are predominantly present in glycogen form in the liver (approximately 100 grams) and muscle tissue (approximately 400 grams) (21, 22). Glycogen stores represent the primary fuel in the production of heat in the skeletal muscle (23) and maintenance of adenosine triphosphate (ATP) homeostasis during a moderate to intense exercise session (5). It is known that during a physical exercise session a decrease in muscle glycogen stores occurs and the rate of depletion of this substrate is modulated by the intensity of the exercise, which changes the muscle fiber types recruitment pattern (3, 21). During the

recovery period, after depletion of glycogen stores, following a subsequent carbohydrate intake, these stocks are restored and may even exceed the initial content (3, 19). This phenomenon is known as the "glycogen supercompensation" that Bergström and Hultman (3) observed for the first time, being attributed to the activation of the enzyme glycogen synthase (GS) and the increase in insulin sensitivity that lasts for some time after exercise (19, 21, 22, 25). This phenomenon has also been observed in the animal model after a high-volume training session (e.g. 4x30 min efforts) (25).

In addition, it is also generally accepted that besides muscle content, depletion of hepatic glycogen stores represents one of the major factors related to fatigue observed during an endurance exercise (21). Thus, a great deal of attention is given in research involving exercise and metabolism in muscle glycogen utilization during exercise, and little on the use of hepatic glycogen (18) and the possible association between the metabolic processes that occur in muscular and hepatic tissues during and after an exercise session.

From the physiological perspective, there are several factors that regulate the use of energetic substrates during exercise such as intensity, volume and practitioner's training status (21). The progression of moderate to intense exercise leads to an increase in carbohydrate metabolism (i.e. muscle and liver glycogenolysis and glucose consumption) to ATP resynthesis, with a consequent reduction of lipid oxidation (18, 35). In this context, the glucose availability to active muscle tissue increases as a result of an increased hepatic glycogenolysis rate (32).

It is also known that the same stimulus can cause different effects according to the typing of the predominant muscle fibers in different muscles recruited during exercise (33, 34), which is still little explored in the literature. Thus, we can observe a gap in the literature on the glycogen depletion and recovery kinetics, mainly related to the different induced responses: i) the volume and intensity of the exercise performed and ii) the biological tissue investigated (i.e., muscles with different fiber type composition and the liver). Therefore, the objective of the present study was to verify the behavior of muscular and hepatic glycogen depletion and recovery kinetics of Wistar rats, which were submitted to three acute training sessions with different relationships between volume and intensity. Considering the different distributions of muscle fibers and the physiology involved during the different exercise stimuli, our hypothesis is that exercise stimuli will induce the glycogen supercompensation phenomenon in all tissues analyzed, with different responses among them.

METHODS

Participants

Eighty-one male Wistar rats were used in the present study. The animals were 30 days old and weighed 70-90 g, when they were brought to the lab, where they were familiarized until they reach between 300 and 360 g at the experiment beginning. Throughout the experiment, the animals were kept in collective cages measuring 37 × 31 × 16 cm, not exceeding four rats per cage. The animals were subjected to a 12/12 hours' light / dark reversed cycle in an environment at 25°C. All the experiments were carried out in the morning by the same researchers who kept

the animals in the cages. All animals received commercial ration (NUVILAB) and water *ad libitum*. The animal's control of feed intake was done by weighing daily before each procedure. The animals belonging to the same group were kept in the same cage. All the procedures of the present study were submitted and approved by the Committee of Ethics and research on the Use of Animals of the School of Physical Education and Sports of Ribeirão Preto (protocol nº 2018.5.31.90.5). Authors confirm that this study meets the ethical standards of the journal (20).

Protocol

All procedures (evaluations and stimuli) were carried out on a treadmill (GESAM, São Paulo, Brazil), which had individual bays measuring 60 x 10 x 6.5 cm. The animals were adapted to exercise for 5 days, running 10 min.day^{-1} at different intensities (10 to 15 m · min^{-1}), continuously or intermittently. After 72 h of the last adaptation session, the animals underwent an incremental test to determine the maximum running speed (MRS).

The animals were randomly divided, by a simple draw, into four groups, according to the exercise stimulus performed. The first group did not perform any training stimulus, being euthanized 48h after the incremental test to obtain the values corresponding to the baseline (Control group; n = 9). The other groups (n = 24 rats each group) were submitted to training stimuli with i) high volume and low intensity (G_{Z1}, continuous effort of 48 min with intensity of 50% MRS), ii) moderate volume and intensity (G_{Z2}, continuous effort of 32 min with intensity of 70% MRS) and (iii) low volume and high intensity (G_{Z3}; intermittent effort consisting of five efforts of 5.3 min with intensity of 90% MRS separated by 2.4 min of a passive interval). The relationship between the volume and the intensity of the different stimuli were adjusted so that the exercise load [i.e. volume product (min) by running intensity (% MRS (1)] was the same for all the groups (2400 a. u.). The groups that were submitted to the different exercise stimuli were composed of 24 animals, which were subdivided again according to the moment of euthanasia. Thus, after the acute training session (G_{Z1}, G_{Z2} and G_{Z3}), six animals from each group were euthanized immediately (t0), six (t6), twelve (t12) and twenty-four hours (t24) after recovery, respectively. All exercise stimuli were performed 48h after the incremental test. All procedures and moments of euthanasia are shown in Figure 1.

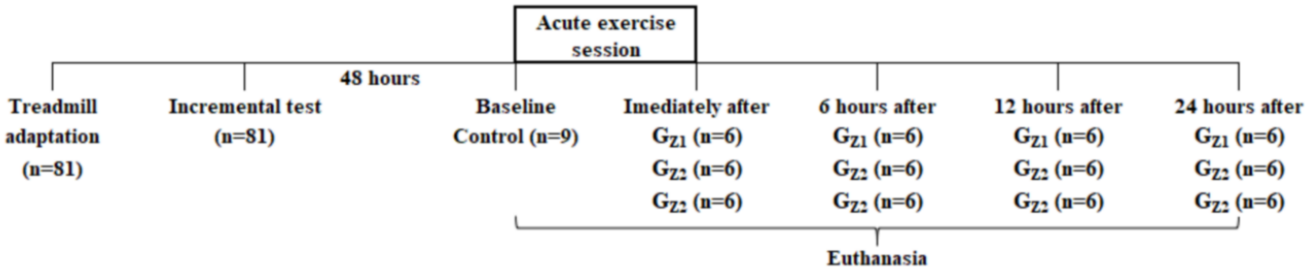

Figure 1. Schematic representation of experimental procedures involving the adaptation of all animals to the ergometer, incremental tests (Control, G_{Z1}, G_{Z2} and G_{Z3}), acute experimental training sessions and moments of euthanasia. G_{Z1}: Training characterized by low intensity and high volume; G_{Z2}: Training characterized by moderate intensity and moderate volume; G_{Z3}: Training characterized by high intensity and reduced volume.

Prior to the incremental test and the exercise stimuli, the animals remained for 10 min in the respective mat of the treadmill for acclimatization. The MRS was determined during an incremental test with an initial speed of 8 m · min^{-1} and increments of 3 m · min^{-1} every 3 min until exhaustion. The exhaustion criterion used was the loss of the running pattern (i.e. interruption of the locomotion pattern, even with the application of stimuli). The MRS was adjusted, considering the time of permanence in the last stage as proposed by Kuipers et al. (24).

All exercise stimuli were applied individually and monitored by the same researcher who performed the familiarization sessions and the incremental test. To characterize the stimuli, blood samples (25 µL) were collected from the distal end of the animals' tail to determine lactate concentrations ([La$^-$]) (YSI-2300; Yellow Springs Instruments®, Ohio, USA). These procedures were performed immediately before exercise, after 50% of the proposed volume and immediately after the end of the exercise. For each exercise stimulus, the rate of lactate accumulation was calculated by the equation:

Lactate accumulation rate = ([La$^-$]peak-[La$^-$]rest) · volume^{-1}, where the volume represents the running time.

The animals were anesthetized by intraperitoneal administration of xylazine (10 mg · kg body weight^{-1}) and ketamine (100 mg · kg body weight^{-1}). Anesthesia control was assessed by loss of foot reflex (28). Subsequently, soleus and EDL (*extensor digitorum longus*) muscles, in addition to the liver, were obtained for subsequent glycogen estimation analysis. The tissues were immediately frozen in liquid nitrogen and then stored at -80°C in specific cryotubes.

To determine the glycogen concentrations ([Glic]) of the different tissues' fractions weighing between 80-120 mg were used. Samples were digested in a bath at 100°C in 1 mL of KOH 1N for 20 minutes. 100 µL of saturated Na_2SO_4 solution was added and the glycogen was precipitated through the two passages of 3 mL of hot ethanol, followed by centrifugation in 4 mL of water and the colorimetric determination performed in 1 mM extract, 20 µL phenol to 80% and 2.0 mL of concentrated sulfuric acid after boiling for 15 minutes. The absorbance was measured in a spectrophotometer at 490 nm. Known solutions varying from 0 to 60 µg of glucose were used for the calibration curve (10,12).

Statistical Analysis

The data are presented in mean ± standard deviation and percent change. To verify if the data present normal distribution the Shapiro-Wilk test was used (IBM SPSS Statistics 20, São Paulo, SP). The comparison of the muscular and hepatic glycogen contents between the groups and moments of euthanasia was performed using Two-Way ANOVA, followed, when necessary, by Fisher's Post-hoc test (Statistica 7.0® Statsoft, Tulsa, OK). In all cases, the level of significance was set at $p < 0.05$. The effect size (ES; η^2) was calculated and interpreted as according to Cohen (7), where: negligible < 0.02; small = 0.02-0.13; medium = 0.13-0.26; large > 0.26 (13).

RESULTS

The daily food intake during all monitoring was not different between the moments of evaluation, training and euthanasia for all groups and was not different between the groups (p > 0.68) (Figure 2).

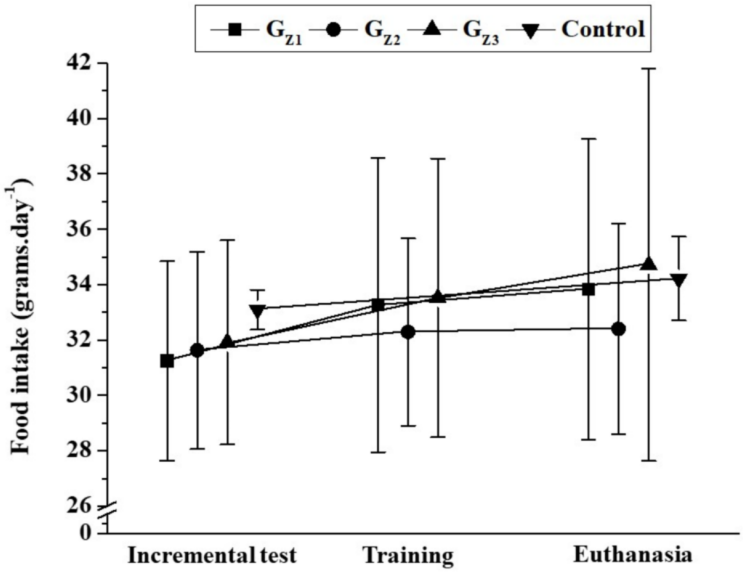

Figure 2. Mean ± standard deviation of daily feed intake for the entire evaluation period. G_{Z1} = low intensity training group; G_{Z2} = moderate intensity training group; G_{Z3} = high intensity training group.

Values of 1.28 ± 0.28, 1.82 ± 1.02 and 2.57 ± 1.61 mM were observed for the [La^-] in the intensities of Z1, Z2 and Z3 respectively, with no statistical difference between the intensities. However, the accumulation rates of [La^-] were significantly higher in Z3 compared to Z1 (p < 0.038; ES = 0.409; large) (Figure 3).

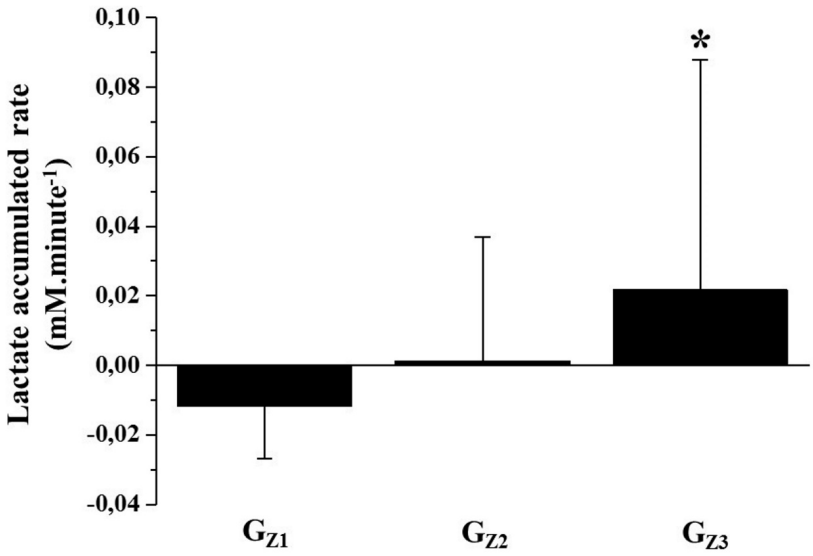

Figure 3. Mean ± standard deviation of lactate accumulation rate of the three acute training sessions. G_{Z1} = low intensity training group; G_{Z2} = moderate intensity training group; G_{Z3} = high intensity training group; * = significant difference compared to G_{Z1}.

It was found effect from recovery time in soleus (ES > 0.323; large) and EDL (ES > 0.133; medium) muscles and in the liver (ES > 0.448; large). In the soleus muscle the [Glic] were higher in t6 and t12 compared to G_{Base}, t0 and t24 for G_{Z1}, G_{Z2} and G_{Z3} ($p < 0.024$) and in t24 were smaller in comparison to t12 only for G_{Z3} ($p = 0.038$) (Figure 4A). In the EDL muscle, the [Glic] were higher only in t6 compared to G_{Base} and t0 in G_{Z1} ($p < 0.046$) (Figure 4B). In the liver, the [Glic] were lower in t6 compared to G_{Base} only for G_{Z3} and were higher in t24 compared to G_{Base}, t0, t6 and t12 for G_{Z1}, G_{Z2} and G_{Z3} ($p < 0.032$) (Figure 4C).

Figure 4D shows the comparison between the percent change from the baseline of the three analyzed tissues. The percentage change in t6 differed between the three tissues ($p < 0.001$; ES = 0.421; large); in t12 for soleus compared to the other tissues ($p < 0.001$; ES = 0.453; medium) and in t24 for EDL compared to the other tissues ($p < 0.009$; ES = 0.323; large).

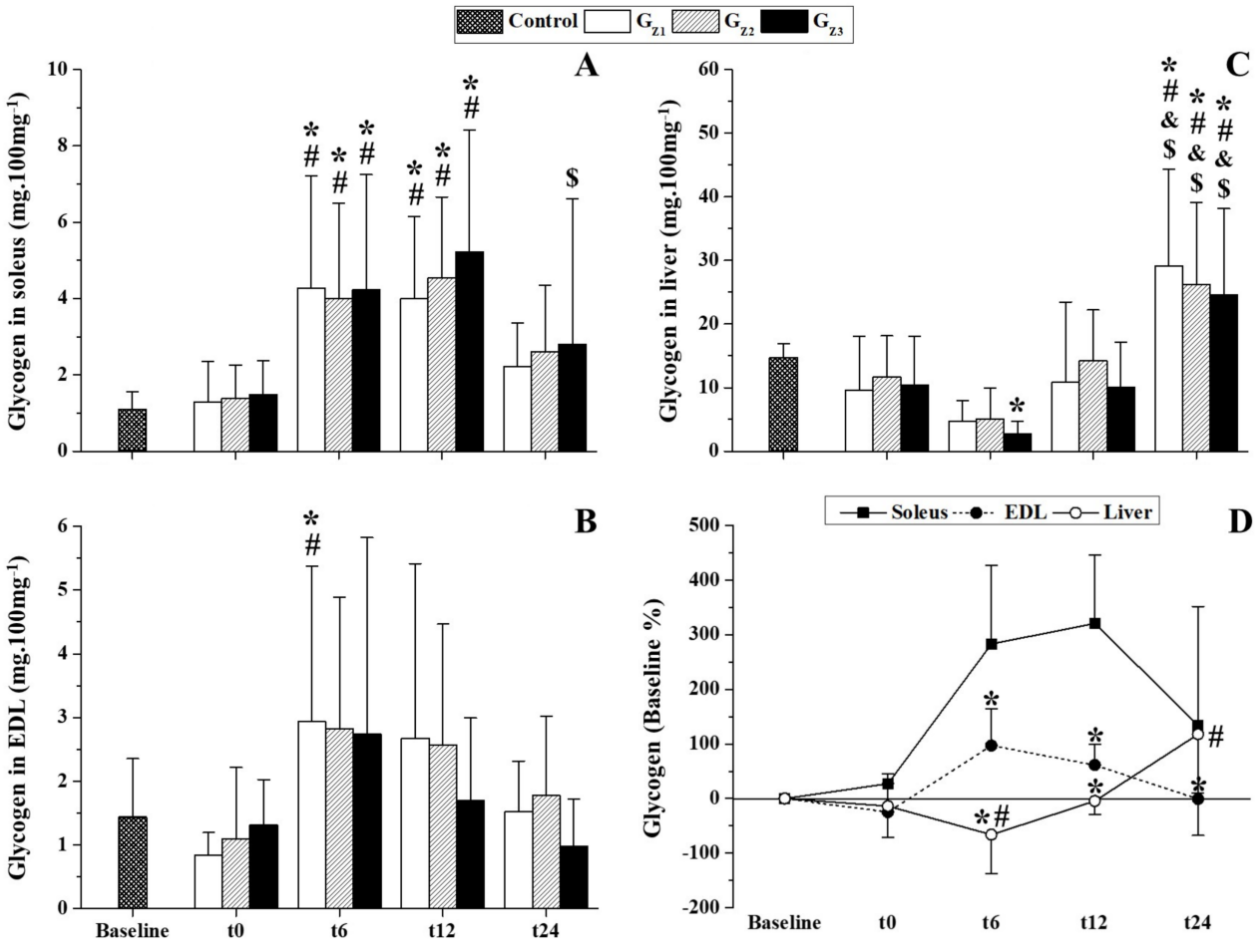

Figure 4. Mean ± standard deviation of glycogen in the soleus (A) and EDL (B) muscles and in the liver (C); and percentage variation comparison of glycogen depletion and recovery kinetics in the three tissues analysed (D). G_{Z1} = low intensity training group; G_{Z2} = moderate intensity training group; G_{Z3} = high intensity training group; * = statistical difference in relation to the baseline group (A-C), statistical difference in relation to the soleus muscle (D); # = statistical difference from the group immediately after (A-C), statistical difference in relation to the EDL muscle (D); $ = statistical difference in relation to group 12 hours.

DISCUSSION

In the present study, we tested the hypothesis that training sessions would induce the glycogen supercompensation phenomenon in all tissues analyzed, with different responses between muscles and for the liver. We did not verify the glycogen depletion in any of the tissues analyzed immediately after exercise, but we observed the supercompensation of this substrate, which occurred between 6 and 12 hours after the exercise stimulus, with a greater response magnitude in the soleus muscle, whereas in the hepatic tissue occurred only 24 after exercise.

A factor that may influence glycogen metabolism is caloric intake before and after exercise (21). In the present study, we found no difference in daily food intake between the groups at any time, which ensures that the differences found result only from the exercise stimuli manipulation and the analyzed tissues. In addition, we found significant differences in the lactate accumulation rate between groups Z1 and Z3, indicating that exercise-induced physiological stress had a satisfactory increase concomitant with the stimuli intensity.

First, it is important to consider that there is no consensus in the literature on muscle glycogen content in different muscle fibers types (21). From histochemical techniques in men, it has been reported that there is no difference in [Glic] between type I and II fibers types (14, 30). However, from biochemical quantification also in men (more quantitative measure), [Glic] appear to be larger in type II fibers compared to type I fibers (33, 34). in rabbits it was shown that glycogen was higher in EDL than in soleus (9). In the present study, we found no difference in [Glic] between the two G_{Base} muscles, indicating that the animals presented similar amounts of this substrate in muscles with different fiber compositions.

Evidence indicates that glycogen depletion as a result of physical exercise first occurs in type I (oxidative) fibers and, as exercise expands, glycogen depletion progressively occurs in type II (glycolytic) fibers (17). Therefore, glycogen supercompensation is expected to be higher in type I fibers. However, in the present study glycogen depletion was not observed in the analysed muscles immediately after the sessions, but the [Glic] in the soleus were higher than EDL' 6 hours after the sessions, indicating that in different muscle tissues intensity modulation does not alter the depletion kinetics, but the recovery and supercompensation of glycogen, since the load be equalized between the different exercise sessions.

In the comparison between muscles, the percentage variation of glycogen showed a more pronounced supercompensation 6 hours after sessions in the soleus muscle compared to EDL. Fiber type predominance is different from soleus (i.e. slow-twitch oxidative; type I) and EDL (i.e. fast-twitch glycolytic; Type II) (5, 31). Thus, we observed that the muscle tissue was most responsive to training sessions is that of type I fibers (i.e., soleus muscle in the present study), which is expected considering that the predominant metabolism in the present study efforts was aerobic. A possible mechanism that explain this finding may be the activity of glucose transporters through the cell membrane, especially the translocation of GLUT4 from the cytosol to the sarcolemma (16) and also from the intracellular phosphorylation of the hexokinase

enzyme (3, 28), which plays an important role in the modulation of muscle-stimulated glucose consumption by physical exercise (16), more markedly in tissues with type I fibers (3, 28). We could speculate that soleus contribution to running exercise is higher than EDL, but there is no any study that brings this information that could corroborate this speculation.

In the liver, unlike muscle tissue, glycogen depletion was observed 6 hours after the session. Conlee et al. (7) observed hepatic glycogen depletion immediately after the end of a 90-minute swimming exercise session in rats. We probably did not observe in the present study the depletion after exercise due to the duration of the sessions that did not exceed 48 minutes (Z1). In addition, the supercompensation phenomenon was observed 24 hours after the session for all sessions, which is expected, considering the depletion of [Glic] in t6.

It is known that the processes of liver glycogenolysis and muscle glycogen synthesis occurs simultaneously (18). Therefore, it is plausible to expect these processes to occur simultaneously during the physical exercise session recovery period. In the present study, considering the comparison of all sessions, hepatic depletion of [Glic] was observed 6 hours after, which is the same moment when the highest [Glic] in the soleus and EDL muscles, evidencing a probable association between these findings, reinforced by the significant difference between the percentage variation of the liver and the muscle tissues 6 hours after the training sessions.

According to the general theory of adaptation currently accepted and proposed by Selye (29), the supercompensation of energy substrates should occur only after their depletion induced by physiological stress (8), but in the present study we observed that the exercise did not cause depletion of [Glic], probably due to the fact that the animals did not reach exhaustion (2, 26), but the phenomenon of glycogen supercompensation occurred. There may be three theoretical explanations for these findings: i) the lactate produced during exercise was converted to glucose and then to glycogen, since it is known that this can occur in low glycogen situations (15), but this is only speculation since we did not observe elevated [La^-]; ii) the exercise was sufficient stimulus for GS activation and allied to *ad libitum* food consumption after exercise [ingestion of carbohydrate sources after exercise is a strategy commonly used by athletes (11)] caused an increase in [Glic] hours after exercise, corroborating with Nakatani et al. (25), which showed in animal model, increased GS activity after swimming exercise, without depletion of [Glic] and a subsequent increase of glycogen between 4 and 48 hours after exercise and iii) the induced-exercise AMP activated protein kinase (AMPK) activation increased at the recovery period leading to an increase in the [Glic]. This last two corroborate with Hingst et al. (22) that demonstrated that both GS and AMPK are key regulators of glycogen supercompensation following a single bout of exercise in skeletal muscle of both man and mouse, and according with these same authors the exercise results in GS activation by the allosteric activator glucose-6-phosphate (22), therefore our results shows that maybe there is no need glycogen depletion for that the supercompensation occurs.

Although the extrapolation of animal models to human physiology is limited, the results of the present study point to a [Glic] recovery differentiation according to the studied muscle and not by the intensity performed, which seems to be related to the different fiber distributions (i.e.

type I, predominant in soleus and type II, predominant in EDL), whereas, in hepatic tissue, glycogen supercompensation is observed only later in comparison to muscle tissue, so that hepatic glycogenolysis may be related to muscle glycogen synthesis.

In conclusion, the present study shows that muscle and hepatic glycogen depletion and recovery kinetics are not modulated by exercise intensity when the load is equalized, but the effects are different in the different tissues. The processes of hepatic glycogenolysis and muscle glycogen synthesis seems to be related.

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