

# Interval Versus Continuous Training With Identical Workload: Physiological and Aerobic Capacity Adaptations

G. G. DE ARAUJO<sup>1,2</sup>, C. A. GOBATTO<sup>1</sup>, M. MARCOS-PEREIRA<sup>3</sup>, I. G. M. DOS REIS<sup>1</sup>, R. VERLENGIA<sup>3</sup>

<sup>1</sup>Laboratory of Sport Applied Physiology, Campinas State University, UNICAMP – FCA, Limeira SP, Brazil, <sup>2</sup>Sports Science Research Group, Federal University of Alagoas, UFAL/PPGNUT/Physical Education – Maceio AL, Brazil, <sup>3</sup>Physical Education, Faculty of Sciences of Health, Methodist University of Piracicaba, São Paulo, Brazil

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## Summary

The interval model training has been more recommended to promote aerobic adaptations due to recovery period that enables the execution of elevated intensity and as consequence, higher workload in relation to continuous training. However, the physiological and aerobic capacity adaptations in interval training with identical workload to continuous are still uncertain. The purpose was to characterize the effects of chronic and acute biomarkers adaptations and aerobic capacity in interval and continuous protocols with equivalent load. Fifty Wistar rats were divided in three groups: Continuous training (GTC), interval training (GTI) and control (CG). The running training lasted 8 weeks (wk) and was based at Anaerobic Threshold (AT) velocity. GTI showed glycogen super-compensation (mg/100 mg) 48 h after training session in relation to CG and GTC (GTI red gastrocnemius (RG)=1.41±0.16; GTI white gastrocnemius (WG)=1.78±0.20; GTI soleus (S)=0.26±0.01; GTI liver (L)=2.72±0.36; GTC RG=0.42±0.17; GTC WG=0.54±0.22; GTC S=0.100±0.01; GTC L=1.12±0.24; CG RG=0.32±0.05; CG WG=0.65±0.17; CG S=0.14±0.01; CG L=2.28±0.33). The volume performed by GTI was higher than GTC. The aerobic capacity reduced 11 % after experimental period in GTC when compared to GTI, but this change was insignificant (19.6±5.4 m/min; 17.7±2.5 m/min, effect size = 0.59). Free fatty acids and glucose concentration did not show statistical differences among the groups. Corticosterone concentration increased in acute condition for GTI and GTC. Testosterone concentration reduced 71 % in GTC immediately after the exercise in comparison to CG. The GTI allowed positive adaptations when compared to GTC in relation to: glycogen super-compensation, training volume

performed and anabolic condition. However, the GTI not improved the aerobic performance.

## Key words

Anaerobic threshold • Glycogen • Testosterone • Corticosterone • Biomarkers • Endurance • Performance • Intensity

## Corresponding author

G. G. de Araujo, Sports Science Research Group – Federal University of Alagoas, UFAL – Maceio AL, Avenue Lourival Melo Mota, s/n, Tabuleiro dos Martins – Maceio – AL, CEP: 57072-900, Brazil. E-mail: [gusta\\_ef@yahoo.com.br](mailto:gusta_ef@yahoo.com.br)

## Introduction

The methodological diversity of training protocols, differences with regard to intensity, duration and frequency as well as the internal (i.e., genotype) and external (i.e., phenotype) factors, have impaired conclusions about the optimal training load to enhance the endurance performance (Booth *et al.* 2010). The physiological responses during chronic exercise are frequently questioned due to lack specific criteria for the program workload (Booth *et al.* 2010, de Araujo *et al.* 2012, 2013a,b). As an example, the interval model (i.e., repeated efforts and brief recovery periods within the session) has been more recommended than continuous to promote aerobic adaptations related to ventricular enlargement, mitochondrial biogenesis, capillarization, oxygen uptake, oxidative enzymes and endurance

performance (Billat 2001, Kubukeli *et al.* 2002, Daussin *et al.* 2008). For sure, the physical stress and as consequence the physiological adaptations are more pronounced in interval training due to recovery period that enables the execution of elevated intensity and as consequence, higher workload performed in relation to continuous (Tanisho and Hirakawa 2009). However, many studies have compared the interval and continuous protocol without identical training load in order to investigate the models responses (McKay *et al.* 2009, Tanisho and Hirakawa 2009, Hovanloo *et al.* 2013).

Among the studies with externally equated training load, no differences were found in aerobic and anaerobic performance between continuous and interval (Iellamo *et al.* 2012, Mador *et al.* 2009, Tuimil *et al.* 2011). Tuimil *et al.* (2011) evaluated the effect of interval and continuous training programs on the maximal aerobic speed and countermovement jump. The continuous and interval training consisted of 3 sessions per week during a period of 8 weeks with an identical external workload. The authors concluded that equated load led to similar improvements in the maximal aerobic speed without changing countermovement jump performance. Iellamo *et al.* (2012) reported that continuous and interval training with equated training load induced significant improvement in aerobic capacity in patients with postinfarction Chronic Heart Failure, without significant differences between the models.

Although these studies in human beings have not found differences on endurance performance between interval and continuous training with equate load, the physiological adaptations (i.e., hormones, metabolites, glycogen stores, energy substrates) have not yet been investigated (Ferreira *et al.* 2007). Thus, some issues remain about the ideal training model to enhance aerobic performance and to reduce stress biomarkers considering an equivalent load. In this line, we designed a continuous and interval training with equivalent load to characterize the acute and chronic physiological responses as well as the performance between models in rodents.

Equalizing training load in two of the most common protocol (i.e., continuous and interval) in rodents may be important to: (1) standardize the acute and chronic responses due to better internal (i.e., same species, strain and age) and external controls (i.e., diet, time of manipulation, temperature, sleep) in comparison to human beings; (2) increase the number and frequency of invasive analysis; (3) experimental physiology area in which has applied interval and continuous exercise as a

therapeutic intervention, but without information and criteria about the intensity and duration prescription during physical stress and (4) provide comparative evidence among exercise systematization, physiological adaptations and performance.

Thus, the purpose of present study was to compare the aerobic performance as well as acute and chronic physiological responses measured by metabolites (lactate, creatinine, urea, uric acid), energy substrates (free fatty acids, triglycerides and glucose), hormones (corticosterone and testosterone), enzymes (creatine kinase) and glycogen stores (liver, soleus, red and white gastrocnemius) between continuous and interval training with identical workload. It was hypothesized that similar responses in aerobic performance, training volume performed and biomarkers are observed between interval and continuous training due to equivalent load.

## Materials and Methods

### Animals

All experiments involving the animals were performed in accordance to the specific Brazilian resolutions on the Bioethics in Experiments with Animals (no 93/08) that is in agreement with the guidelines of the European Convention for the Protection of Vertebrate Animals for research involving animals. Animal experimentation was approved by the Ethical Committed of Biomedical Institute of Sao Paulo University (ICB-I, USP).

Fifty male Wistar rats (*Rattus norvegicus*) (60 days old) were used. The animals were fed a commercial chow for rodents (23.5 % protein, 6.5 % fat, 70.0 % carbohydrate – Labina, Purina®) *ad libitum* and had free access to water throughout the experimental period. The animals were housed in collective cages (5 rats per cage) at 25 °C in a room with lights on from 6:00 to 18:00 h.

### Experimental design

The animals were acclimated during one month to treadmill at 13 m.min<sup>-1</sup>, 0 % grade, 10 min per day, 5 days per week (wk) before the experimental period. Training was performed on a motor-driven-rodent treadmill during 8 wk and 5 days per wk.

The animals were divided into trained (n=40) and untrained/control (n=10) groups. The trained group was subdivided into:

– *Continuous* (GTC, n=20). This protocol was designed

to the rats running continuously until 30 min at 95 % of the Anaerobic Threshold (AT);

- *Interval* (GTI, n=20). Three velocities were applied to elaborate the interval training: 85 %, 95 % and 105 % of the AT. The velocity equivalent to 85 % of AT was considered an active recovery session into the phases. The sessions were divided in 7 phases (first 5 phases with duration of 4 min; last 2 phases with duration of 5 min), totaling 30 min. The first session of the training was initialized with the sequence: 95 %; 105 %; 85 %; 95 %; 105 %; 85 % and 95 % of AT. The subsequent sessions were initialized from the last intensity of the previous day (i.e., 105 %; 85 %; 95 %;

105 %; 85 %; 95 % and 105 % of the AT) in accordance to Table 1. The total wk load (arbitrary unit – AU) of interval training was designed to be equal (14250 AU) to continuous protocol:

**Equation 1.** Continuous wk load (AU) = 30 min \* 95 % of AT<sub>day 1</sub> + 30 min \* 95 % of AT<sub>day 2</sub> + 30 min \* 95 % of AT<sub>day 3</sub> ... → 14250

**Equation 2.** Interval wk load (AU) = 30 min \* intensity average 95 % of AT<sub>day 1</sub> + 30 min \* intensity average 96 % of AT<sub>day 2</sub> + 30 min \* intensity average 94 % of AT<sub>day 3</sub> ... → 14250

**Table 1.** Distribution of training series for interval training.

Series	Day 1		Day 2		Day 3		Day 4		Day 5	
1	95 % of AT	4 min	105 % of AT	4 min	85 % of AT	4 min	95 % of AT	4 min	105 % of AT	4 min
2	105 % of AT	4 min	85 % of AT	4 min	95 % of AT	4 min	105 % of AT	4 min	85 % of AT	4 min
3	85 % of AT	4 min	95 % of AT	4 min	105 % of AT	4 min	85 % of AT	4 min	95 % of AT	4 min
4	95 % of AT	4 min	105 % of AT	4 min	85 % of AT	4 min	95 % of AT	4 min	105 % of AT	4 min
5	105 % of AT	4 min	85 % of AT	4 min	95 % of AT	4 min	105 % of AT	4 min	85 % of AT	4 min
6	85 % of AT	5 min	95 % of AT	5 min	105 % of AT	5 min	85 % of AT	5 min	95 % of AT	5 min
7	95 % of AT	5 min	105 % of AT	5 min	85 % of AT	5 min	95 % of AT	5 min	105 % of AT	5 min

The volume of training performed in interval and continuous protocols was quantified in each training session for determination of the Week Volume:

**Equation 3.** Week Volume (min) =  $\sum$  daily minutes/ 5 days

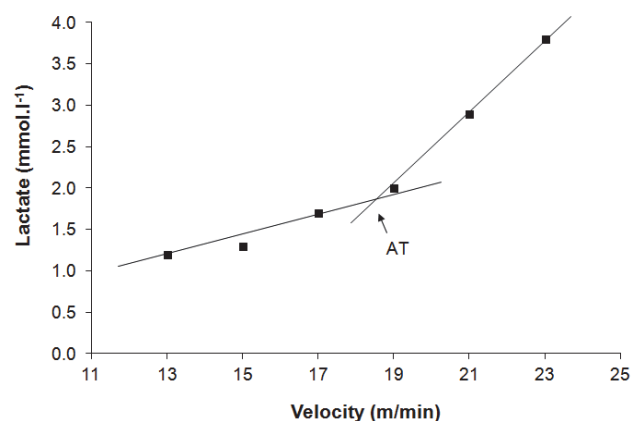
Ten rats of GTC and GTI were evaluated 48 h after the last exercise session and ten rats of GTC and GTI immediately after incremental exhaustive exercise (E) at the end of 8 wk.

During the 8 wk of experimental period, the control group (CG) was also accustomed to treadmill running: 1.0-1.2 km h<sup>-1</sup>, 10 min.day<sup>-1</sup>, 3 sessions in the wk.

#### Anaerobic threshold (AT)

The intensity of the training was based in AT test adapted to Wistar rats in accordance to Pilis *et al.* (1993). The AT was calculated by Linear adjusts (bi-segmentation) between lactate concentration and velocity (Fig. 1) and was applied at 0, 4 and 8 wk. The AT test was implemented in

the first day of training program, last day of the fourth wk and last day of the eighth wk of training.



**Fig. 1.** Bi-segmentation between lactate concentration and velocity. The anaerobic threshold (AT) was the linear regression intersection. The R<sup>2</sup> of the linear regressions should be between 0.80-1.00 to success of interpolation.

#### Collection samples

After 48 h of last session (8 wk), half of the trained animals (GTC, n=10; GTI, n=10) were euthanized in rest to verify the chronic adaptations (Non Exhaustive

– NE). The other half of trained groups (GTC, n=10 and GTI n=10), was euthanized immediately after the last session to analyze the acute effects of exercise. The CG group (n=10) was euthanized in the same period of trained group NE and in the same daytime.

The animals were euthanized with 20 % chloralhydrate (0.3 ml/100 g<sup>-1</sup> animal weight) for blood collection and tissue excision (soleus, white and red gastrocnemius and liver). Blood was collected *via* cardiac puncture after thoracotomy into EDTA tubes (plasma) or dry tubes (serum) in accordance with the analyses. The plasma and serum were separated into aliquots and analyzed using commercial kits.

### Biomarkers

Free fatty acids (FFA) were analyzed in accordance to Regow *et al.* (1971). The creatine kinase (Kit MPR3 CK NAC-active – Boehringer Mannheim<sup>®</sup>), glucose (Kit Laborlab<sup>®</sup>), triglycerides (Kit Laborlab<sup>®</sup>), creatinine (Kit Laborlab<sup>®</sup>), uric acid (Kit Laborlab<sup>®</sup>) and urea (Kit Laborlab<sup>®</sup>) were analyzed by Biochemical kits.

Muscles and liver samples were immediately digested in KOH 1 N (30 %) for 20 min. After this period, 20 µl of Na<sub>2</sub>SO<sub>4</sub> was added for glycogen precipitation using 2.5 ml of ethanol (5 min of centrifugation). The colorimetric assay method was performed using 20 µl of phenol (80 %) and 2.0 ml of sulfuric acid. After 15 min of boiling, the absorbance was determined at 490 nm (Dubois *et al.* 1956).

For the lactate concentration measurement, the blood samples (25 µl) were collected from the animals (tail) and placed in microtubes (1.5 ml) containing 400 µl of 4 % trichloroacetic acid, which were then stored at 8 °C. The samples were centrifuged for 3 min, and 100 µl of plasma was placed into fresh tubes containing 500 µl of the following reagent: glycine/EDTA, hydrazine hydrate 88 % (pH=8.85), lactate dehydrogenase and β-nicotinamide adenine dinucleotide. The homogenized sample and reagent were incubated at 37 °C for 20 min, and absorbance was determined at 340 nm.

Corticosterone and testosterone concentrations were analyzed using a Coat-A-Count Kit from Diagnostic Products Corporation-DPC<sup>®</sup> and Cayman Chemical, Testosterone ELISA Kit<sup>®</sup> respectively.

### Statistical analyses

All of the dependent variables were subjected to the normality test using the Shapiro-Wilk W-test. All analyses were conducted with a statistical software

package (Statistica, version 7.0, Tulsa, OK), and data are presented as the mean ± standard deviation (SD). An analysis of variance (ANOVA repeated measures) was used to examine changes inter and intra-groups (GTC = Exhaustive x Non-Exhaustive x Control *vs.* GTI = Exhaustive x Non-Exhaustive x Control) within the glycogen stores (soleus, liver, white and red gastrocnemius) and biochemical analyses (corticosterone, testosterone, glucose, uric acid, creatine kinase, urea, creatinine, FFA and triglycerides) after 8 wk of experimental period. When a significant interaction effect was found, a Tukey *post-hoc* test was used to identify where the difference existed among groups. The AT differences inter-groups (GTI *vs.* GTC) at 0, 4 and 8 wk were tested by Student's t-test for independent samples. The AT differences intra-groups at 0, 4 and 8 wk were analyzed by ANOVA one-way. When a significant interaction effect was found, a Tukey *post-hoc* test was used to identify where the difference existed among the periods. Also, the effect size, Cohen's d, was calculated for AT velocity among the periods (0, 4 and 8 wk) for GTC and GTI. The thresholds for small, moderate, and large effects were 0.20, 0.50, and 0.80, respectively. Effect sizes (ES) were determined by the formula: (mean<sub>1</sub> – mean<sub>2</sub>)/pooled SD (Cohen 1988). The significance level was set *a priori* p≤0.05.

## Results

### Body weight

The body weight (g) intra-group increased after 4 wk (GTC=374.7±38.4; GTI=379.5±38.4; CG=389.3±39.5) in relation to 0 wk (GTC=232.6±4.9; GTI=234.5±33.2; CG=248.3±25.5); after 8 wk the body weight (GTC=425.1±41.8; GTI=432.2±43.8; GC=430.2±44.6) was higher in comparison to 0 and 4 wk.

### Aerobic performance and training volume

No differences were found in AT velocity (m/min) between continuous and interval groups after 8 wk (Table 2). The AT velocity in continuous was higher than interval group after 4 wk (Table 2). Therefore, the aerobic capacity in continuous group showed a moderate/high effect size (0.67) in comparison to interval group after 8 wk when compared to 4 wk, but these averages were not significantly different. Although the AT velocity did not show differences in interval training after 8 wk in comparison to 0 wk, the aerobic capacity increased 14 % (effect size = 0.51) when

compared to 4 wk and 11 % in relation to continuous group (effect size = 0.59) at 8 wk (Table 2).

The AT lactate concentration did not show statistical differences inter-groups in baseline and after 8 wk, but increased 29 % in interval group when compared to continuous (Table 2).

The total volume (min) performed in interval

group among 5-8 wk was higher than 0-4 wk (effect size = 0.97) and different when compared to continuous group at 0-4 wk (effect size = 1.12) and 5-8 wk (effect size = 1.39) (Fig. 2A). On the other hand, the average volume (min) did not show differences in interval training group during experimental period in comparison to continuous group (Fig. 2B).

**Table 2.** Values of anaerobic threshold (AT) velocity and lactate concentration during 8 weeks of interval and continuous training.

	AT velocity (m.min <sup>-1</sup> )			AT lactate concentration (mmol.l <sup>-1</sup> )		
	0 week	4 week	8 week	0 week	4 week	8 week
<i>Continuous</i>	19.59 ± 5.39	20.20 ± 5.05*	17.69 ± 2.49	2.37 ± 1.16	2.11 ± 0.93*	3.25 ± 0.82
<i>Interval</i>	19.15 ± 5.59	17.39 ± 4.92	19.87 ± 4.84	2.60 ± 0.97	2.96 ± 0.66	2.73 ± 0.80
<i>% differences inter-group</i>	2	16	11	9	29	19
<i>Effect size inter-group</i>	0.08	0.56	0.59	0.22	1.07	0.64

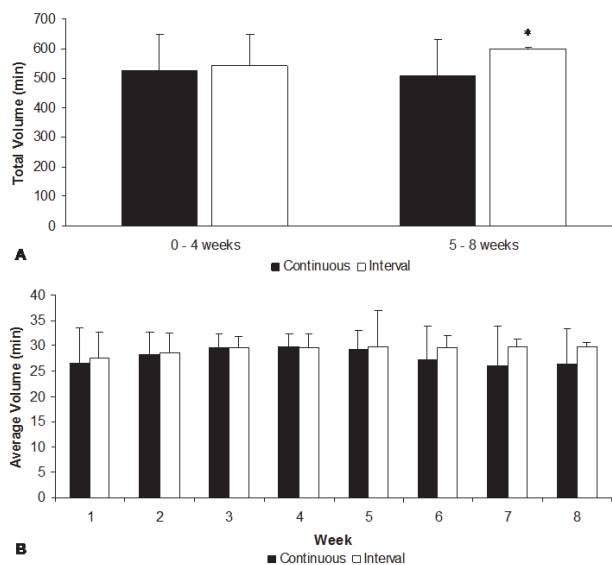
\* Significantly different in relation to Interval Group (p<0.05)

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The AT lactate concentration did not show statistical differences inter-groups in baseline and after 8 wk, but increased 29 % in interval group when compared to continuous (Table 2).

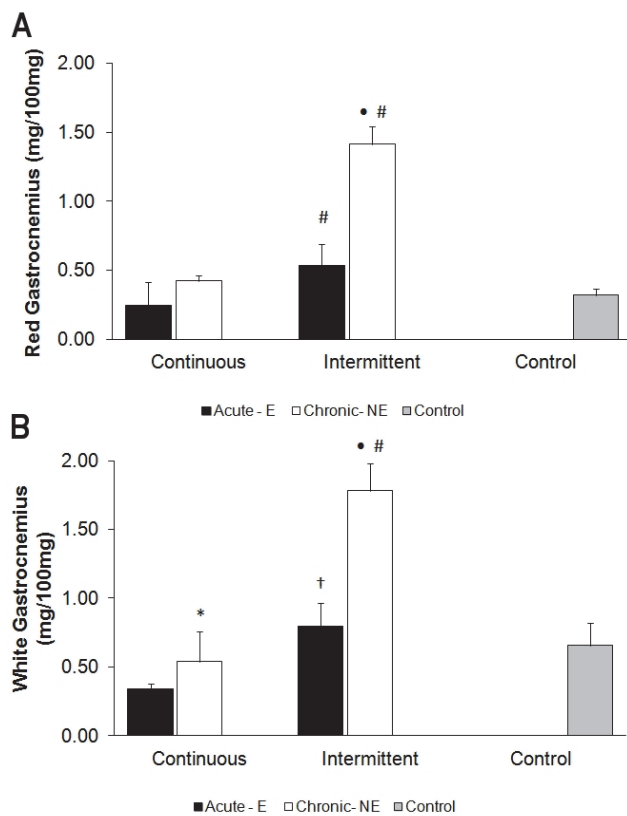
The total volume (min) performed in interval group among 5-8 wk was higher than 0-4 wk (effect size = 0.97) and different when compared to continuous group at 0-4 wk (effect size = 1.12) and 5-8 wk (effect size = 1.39) (Fig. 2A). On the other hand, the average volume (min) did not show differences in interval training group during experimental period in comparison to continuous group (Fig. 2B).



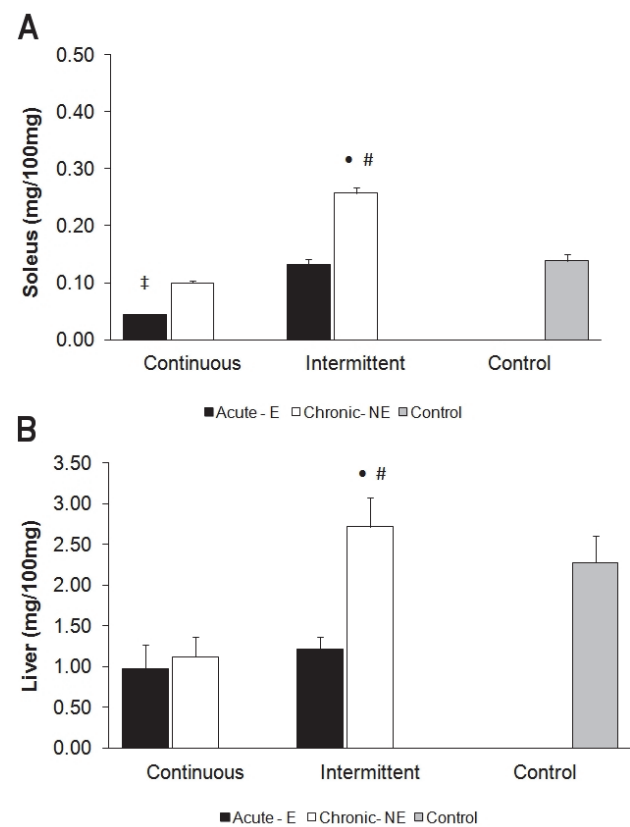
**Fig. 2.** Total volume performed among 0-4 wk and 5-8 wk for interval and continuous group. \* Significantly different in relation to continuous (0-4 and 5-8 weeks) and interval (0-4 weeks) group ( $P \leq 0.05$ )

#### Glycogen stores

Figure 3 showed the glycogen concentration in red gastrocnemius and white gastrocnemius. The glycogen stores in red gastrocnemius in interval group, for acute and chronic conditions, were significantly higher than GTC (acute and chronic) and CG (Fig. 3A). Also, the glycogen super-compensation in GTI after 48 h was 165 % higher in comparison to GTI acute (Fig. 3A).



**Fig. 3.** Glycogen concentration (mg/100 mg) in red gastrocnemius (A) and white gastrocnemius (B) after 8 wk of training. White bar = chronic effect (48 h after the last session); Black bar = acute effect (immediately after exercise); Gray bar = control group. # Significantly different in relation to CG and GTC acute and chronic ( $P \leq 0.05$ ). \* Significantly different in relation to CG ( $P \leq 0.05$ ); • Significantly different in relation to GTI acute ( $p \leq 0.05$ ); † Significantly different in relation to GTC acute ( $P \leq 0.05$ ); ‡ Significantly different in relation to CG and GTI acute and chronic ( $P \leq 0.05$ )



**Fig. 4.** Soleus (A) and liver (B) glycogen concentration (mg/100 mg) after 8 wk of training. White bar = chronic effect (48 h after the last session); Black bar = acute effect (immediately after exercise); Gray bar = control group. # Significantly different in relation to CG and GTC acute and chronic ( $P \leq 0.05$ ); • Significantly different in relation to GTI acute ( $p \leq 0.05$ )

**Table 3.** Values of triglycerides, free fatty acid, glucose, urea, testosterone, corticosterone and ratio testosterone/corticosterone in Interval and Continuous Group at Exhaustive and Non Exhaustive Conditions.

	Control	Interval Group		Continuous Group	
		Exhaustive	Non-Exhaustive	Exhaustive	Non-Exhaustive
Triglycerides (mg/dl)	68.9 ± 9.0	61.8 ± 11.7	91.0 ± 11.6	37.5 ± 9.8*	116.6 ± 23.2
Free fatty acid (nEq/l)	331.4 ± 19.4	328.5 ± 36.9	324.2 ± 68.1	324.1 ± 80.4	348.8 ± 73.3
Glucose (mg/dl)	275.4 ± 21.9	271.0 ± 27.2	304.6 ± 22.8	235.7 ± 28.0	333.6 ± 16.0
Urea (mg/dl)	47.6 ± 1.3	54.1 ± 2.1	59.2 ± 2.4*	54.5 ± 2.0	53.6 ± 1.9
Testosterone (ng/dl)	457.7 ± 102.2	324.7 ± 29.1	303.1 ± 67.3	132.9 ± 27.4*	274.5 ± 68.1
Corticosterone (ng/dl)	21.1 ± 1.7	33.0 ± 2.9*	26.9 ± 1.1	29.4 ± 2.1*	25.8 ± 1.5

\* Significantly different in relation to CG ( $p < 0.05$ )

The glycogen concentration in white gastrocnemius enhanced in GTI chronic when compared to GTC (acute and chronic) and CG. This muscle showed

an improved of 136 % in glycogen concentration for GTI acute when compared to GTC acute (Fig. 3B). Also, the GTC had significant super-compensation after 48 h in

relation to GTC acute (Fig. 3B).

GTC acute was significantly lower than CG and GTI acute and chronic for soleus muscle (Fig. 4A). Figure 4 showed that interval training increased the glycogen concentration at chronic condition in relation to GTI acute, CG and GTC (acute and chronic).

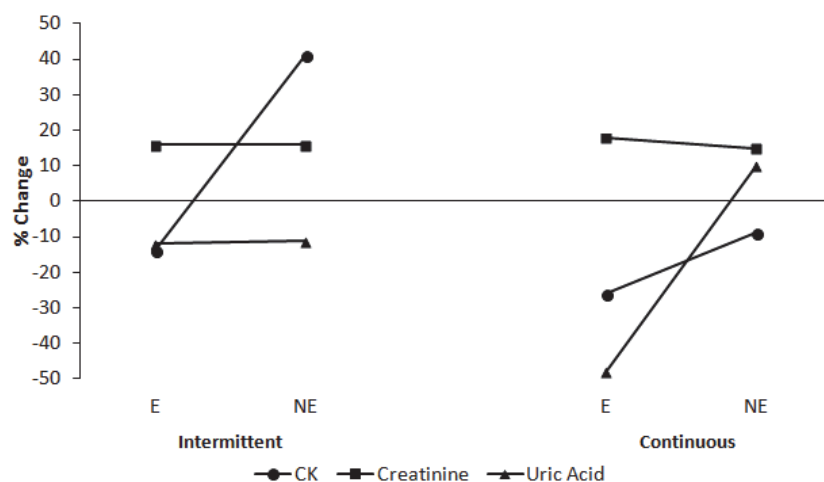
The liver glycogen concentration was significantly higher in GTI chronic than GTI acute and GTC (acute and chronic), but indifferent when compared to CG (Fig. 4B).

#### Blood determinations

There were no significant differences in FFA and glucose concentration among the groups, but triglycerides reduced 54 % in GTC immediately after the exercise in relation to CG (Table 3). Urea concentration

increased 20 % after 48 h of recovery when compared to CG (Table 3). Significant increases were found in corticosterone concentration in acute condition for GTI and GTC. Testosterone concentration reduced 71 % in GTI immediately after the exercise in comparison to CG (Table 3).

The creatinine concentration in both groups (GTC and GTI) was not different in acute and chronic conditions when compared to CG (Fig. 5). The CK concentration increased by 38 % at chronic condition in GTI but showed values next to the CG for acute situation in GTI and GTC as well as chronic condition in GTC (Fig. 5). The uric acid reduced by 49 % immediately after exercise for continuous group, but in other conditions the values were near the CG (Fig. 5).



**Fig. 5.** Percentage changes from the control group in creatine kinase, creatinine and uric acid. NE = chronic effect (48 h after the last session). E = exhaustive exercise (immediately after the exercise)

## Discussion

To the best of our knowledge, this is the first study to investigate the effects of continuous and interval model equating the training load and evaluating the acute and chronic biomarkers and performance adaptations. Our results are innovative since it enabled an acute and chronic physiological characterization as well as performance response. This characterization is important to elucidate a paradigm in relation to interval and continuous training adaptations (Daussin *et al.* 2008). The intensity prescription was identical (95 % of Anaerobic Threshold), but the real volume performed at this intensity was lower in continuous group than interval group. The aerobic performance in interval group was not superior to continuous after 8 wk due to matched overload. This data corroborated with our hypothesis that expected similar responses between models due to

correspondent load. On the other hand, different physiological adaptations occurred in different times contradict our initial opinion. Although training models have been designed to achieve the same load, the interval protocol showed better adaptations than continuous in relation to: (1) glycogen super-compensation, (2) volume performed and (3) anabolic condition.

The AT test have been an interesting protocol to evaluate the aerobic capacity in different experimental approaches (Pilis *et al.* 1993, Gobatto *et al.* 2001, de Araujo *et al.* 2007, Ferreira *et al.* 2007). Among several training regimens used for improving performance, AT workload is considered the best marker of aerobic endurance capacity (Gobatto *et al.* 2001). Our results showed that the aerobic capacity evaluated by AT was sensible to measure the training effects, but the training protocol was insufficient to improve significantly this parameter. The AT in GTI was 11 % higher than

GTC after experimental period due to GTC reduction, but this difference was not significant (moderate effect size = 0.59). Daussin *et al.* (2008) reported in human beings that interval training increased the peripheral muscle and central cardiorespiratory adaptations in relation to continuous protocol with identical workload in sedentary subjects. However, we cannot affirm that GTI was better than GTC due to aerobic capacity fall after 8 wk.

The monotonous training, without intensity variation, can exceed the individual physiology capacity to exercise, promoting a disruption of homeostasis, muscle fatigue, depletion of energy substrates and performance loss (Fry *et al.* 1992, Halson and Jeukendrup 2004). Similar phenomenon may be attributed to continuous model that did not alter the intensity and volume after 8 wk in comparison to 0 wk (Kuipers 1998). de Araujo *et al.* (2013b) reported that monotonous training in swimming rats at intensity correspondent to 100 % of AT, decreased the training volume and increased serum CK concentration; suggesting that exercise at constant intensity cannot be applied in long-term training to improve the aerobic performance. Although these authors found glycogen super-compensation, the stores were not mobilized to increase the aerobic performance and to sustain the estimated volume. Our data showed that continuous training after short period of training (first 4 wk) increased the aerobic performance in relation to interval protocol, but reduced the aerobic capacity with the advancement of monotonous training. A similar data was found by de Araujo *et al.* (2013b) that described a significant training volume reduction after 7 wk of continuous training but not after 4 wk. Probably, the animals of interval group developed overreaching symptoms earlier (4 wk) than continuous (8 wk) considering the AT values (Carvalho *et al.* 2005). Carvalho *et al.* (2005) reported that continuous training at anaerobic threshold during 4 weeks increases the aerobic capacity. In association with de Araujo *et al.* (2013b), the physiological effects of monotonous training seem to appear after 4 weeks at 100 % of anaerobic threshold intensity. However, due to aerobic capacity decreased and AT reassessment after 4 wk, the interval group during the second period (5-8 wk) performed lower intensity than continuous. The control group was used only to refer to the biochemical analysis. This is a study limitation since it AT evaluation in this group could improve the aerobic capacity interpretation.

In another study, de Araujo *et al.* (2013a)

verified the aerobic performance and biomarkers adaptations in rats submitted to continuous training at intensities correspondent to 80 and 90 % of the AT. These authors reported that continuous training did not cause significant enhance in stress biomarkers. In addition, chronic exercise at 80 and 90 % of the AT enhanced glycogen super-compensation in soleus and gastrocnemius muscles in order to increase the aerobic performance in comparison to baseline. In present study, the continuous training at 95 % of AT reduced the training volume in comparison to GTI. Furthermore, the monotonous effects in GTC may be visualized by reduced glycogen stores in relation to GTI at acute and chronic conditions. The continuous group showed a significant glycogen depletion in soleus, liver, red and white gastrocnemius after exercise and as consequence impaired repletion after 48 h of recovery. The association among volume reduction, performance decline and anabolic impairment suggests an overreaching state in GTC caused by monotonous load (Kuipers 1998). Thus, the continuous training at intensities correspondent to 95 and 100 % of AT, independently of ergometer (i.e., swimming or treadmill), it seems inappropriate intervention to laboratory rats (Ferreira *et al.* 2007, de Araujo *et al.* 2012, 2013a,b). Therefore, the intensities between 80-90 % of AT may be more interesting to continuous protocol in order to increase the aerobic performance without enhances in stress biomarkers (de Araujo *et al.* 2013a).

On the other hand, the interval training showed glycogen super-compensation after 48 h in all tissues. The high glycogen values after 48 h showed that this recovery period was important to promote super-compensation in GTI, but not in GTC (Vandenbergh *et al.* 1995, Nakatani *et al.* 1997, Hargreaves 2004). Despite the glycogen super-compensation, the aerobic performance did not increase in relation to the beginning of the training, but showed moderate effect size (0.59) in comparison to the GTC after 8 wk. The AT test replaced the last training session; in this line, the AT performance in both groups was influenced by previous training days. Thus, if the AT test had been performed after 48 h of the last training session, the aerobic performance in interval group could be enhanced in relation to GTC and 0 wk.

The CK has been related to muscle injury and may be utilized as fatigue biomarker (Totsuka *et al.* 2002). Our CK values were obtained immediately and 48 h after last training session. In this context, the chronic period (48 h) was sufficient to identify the peak



concentration or the time to CK shift from the intracellular for plasma (Totsuka *et al.* 2002, Banfi *et al.* 2012). The CK showed raised values in GTI, indicating a prominent effort during AT test when compared to GTC. Also, the CK values after 48 h may indicate high muscle injury during GTI when compared to GTC, probably due to high volume performed.

The urea has been used to measure the protein metabolism and can indicate indirectly a decomposition of muscle cell (Wyss and Kaddurah-Daouk 2000, Kelly and Jones 2013). The elevated uremia may indicate catabolism phase (Phillips *et al.* 2009, Kelly and Jones 2013). Despite of high values of uremia in GTI chronic, this singly results was insufficient to characterize a catabolism effect because the testosterone concentration (anabolic hormone) did not alter in relation to CG in same period.

Testosterone participates in several metabolic processes, increasing the protein synthesis and deposition of glycogen in the muscles (Kelly and Jones 2013). This hormone concentration reduced significantly in GTC showing a deficiency in anabolic process. Thus, the reduced glycogen stores in GTC (acute and chronic) and reduced testosterone concentration shows an impaired anabolic condition (Kelly and Jones 2013). In association, the corticosterone is a catabolic hormone with lipolysis and proteolysis functions as well as hepatic glycogenesis (Halson and Jeukendrup 2004). In the present study, corticosterone increased in GTC and GTI after exhaustive exercise showing an expected acute stress of the session. However, corticosterone concentration after 48 h showed a controlled stress during recovery period (Halson and Jeukendrup 2004).

In this context, our results can be important either to experimental researches that use the physical stress as a therapeutic intervention or studies with human beings that still need to establish the reference values in relation to physiological responses during interval and continuous training. It is known that because of individual/internal differences (i.e., training status, injury, sleep, nutritional condition, psychological factors, genetic

and others) and external factors (i.e., culture of athletes and coaches, nationality, temperature, humidity, economic factors, sponsorship, different number of competitions, calendar, social and others), the performance responses varies among humans (de Araujo *et al.* 2012). These internal and external factors influence physiological adaptations during the training protocol, hindering the reproduction of the models by coaches, athletes and researchers.

The rats may be important to study training models due to better internal (i.e., same species, strain and age) and external controls (i.e., diet, time of manipulation, temperature, sleep) in comparison to humans. Furthermore, it becomes possible to increase the number and frequency of invasive and biochemical analysis. Thus, research methods/models of training on these animals is essential: 1) to improve the understanding on physiological responses at different methods with same workload; 2) to analyze the reliability of the training model; 3) to improve the knowledge about the manipulation of intensity and volume. Studying the training protocols in rats improves the chronic exercise prescription for clinical, pharmacological, dietetic and other interventions in experimental physiology (Booth *et al.* 2010).

In summary, the interval model even with an equivalent load to continuous protocol allowed positive physiological adaptations in relation to: glycogen super-compensation in soleus, gastrocnemius and liver; training volume performed; and anabolic period after 8 wk. Despite this adaptations, the interval model was insufficient to enhance significantly the aerobic performance measured by anaerobic threshold in relation to training begin and continuous protocol.

### Conflict of Interest

There is no conflict of interest.

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