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Acute responses of high-intensity circuit training in women: Low physical fitness levels show higher muscle damage

Respostas agudas de um circuito de treino de alta intensidade em mulheres: Níveis baixos de aptidão física apresentam maior dano muscular

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Abstract – High-intensity intermittent exercise (HIIE) elicits large improvements in health and cardiorespiratory fitness (CRF). HIIE can be applied with calisthenics exercises to improve strength and endurance. The acute effects of high-intensity circuit training (HICT) considering different CRF on myological variables are unknown. The aim was measure acute effects of HICT in young women considering different levels of CRF. Twelve women were allocated in two groups, who achieve 41mLO_2•kg^{-1}•min^{-1} or more= High Physical Fitness (HPF, n=5) and who achieve less than 41mLO_2•kg^{-1}•min^{-1}= Low Physical Fitness (LPF,n=7). Protocol: 2x4 sets of 20 seconds at maximum intensity (all-out fashion) interspersed with 10 seconds of passive rest (jumping jacks, squat and thrust using 2kg dumbbells, mountain climber, and burpees). Blood samples were collected before, immediately after, 15minutes, 30minutes, one hour and 24 hours after. Heart rate, serum myoglobin, lactate, and creatine kinase (CK) concentration were analyzed. The HR achieved 94.1±3.7% of HR_{max} for LPF and 104.5±20.3% for HPF, p=0.03. The mean of delta lactate was similar between groups. The highest myoglobin has reached at 1h after the exercise protocol, with 50.0±30.2 ng/mL for LPF and 36.9±9.25 ng/mL for HPF. The delta of total CK before and after the exercise protocol shows that the serum CK level in LPF was significantly higher than HPF group (p=0.042). HICT composed by calisthenic protocol produced elevated and similar effects on HRmax, serum lactate and myoglobin in the woman with HPF and LPF. However, LPF group presented higher muscle damage inferred by serum CK concentrations.

Key words: Creatine kinase; High-intensity interval training; Myoglobin; Sodiun lactate.

Resumo – *O exercício intermitente de alta intensidade(HIIE) melhora a saúde e a aptidão cardiorrespiratória(CRF). HIIE pode ser aplicado com exercícios calistênicos para melhorar a força e resistência. Os efeitos agudos do treinamento de alta intensidade(HICT) considerando diferentes CRF em variáveis miológicas são desconhecidos. O objetivo foi medir os efeitos agudos do HICT em mulheres jovens, considerando diferentes níveis de CRF. Elas foram alocadas pelo nível de VO_2máx. em dois grupos, as que atingiram 41mLO2•kg^{-1}•min^{-1} ou mais= alta aptidão física(HPF,n=5) e menos de 41mLO2•kg^{-1}•min^{-1}= baixo aptidão física(LPF,n=7). Protocolo: 2x4 séries de 20s com intensidade máxima (all-out) intercalados com 10s de repouso passivo (jumping jacks, squat and thrust usando halteres 2kg, mountain climber e burpees). Sangue foi coletado antes, zero, 15, 30min, 1h e 24hs depois. Foram analisadas, freqüência cardíaca, mioglobina sérica, lactato e creatina quinase (CK). A FC alcançou 94,1±3,7% da FCmax para LPF e 104,5±20,3% para HPF, p=0,03. A média do delta lactato foi semelhante entre os grupos. O pico de mioglobina foi 1h após o protocolo de exercício, com 50.0±30.2ng/mL para LPF e 36.9±9.25ng/mL para HPF. O delta de CK total antes e depois do protocolo de exercício mostra que o nível sérico de CK no LPF foi significativamente maior do que o grupo HPF(p=0,042). O HICT com exercícios calistênicos produziu efeitos elevados e semelhantes sobre FCmax, lactato sérico e mioglobina nas mulheres com alta e baixa aptidão física. No entanto, o grupo LPF apresentou maior dano muscular inferido pelas concentrações séricas de CK.*

Palavras-chave: *Creatina quinase; Lactato de sódio; treinamento intervalado de alta inten-*

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INTRODUCTION

Interval training is characterized by relatively brief, intermittent bouts of intense exercise, separated by periods of lower intensity exercise or rest[1]. Chronically, high-intensity circuit exercise (HICT) elicits large improvements in health and cardiorespiratory fitness[2,3] and high-intensity circuit training (HICT) also elicit greater adherence than more traditional, longer duration, low-intensity training among women[4]. Despite the common use of running and cycling in HICT, calisthenics also can be applied to improve cardiorespiratory fitness (CRF), strength and endurance[4,5]. Previously, was found that CRF can contribute to intermittent performance on cycle ergometer, and higher levels are associated with better results in male athletes[6]. However, the effects of HICT considering different CRF on hemodynamical and myological variables in women are unknown.

From the cardiovascular and muscular point of view, calisthenic HICT increases the heart rate (HR) near to 87% of HR peak and blood lactate concentration higher than 8 mmol·L^{-1} in a mixed sample compose by male and female[5]. However, cardiorespiratory[7,8] and muscular[9,10] differences between genres in HICT are known[1], and studies involving women and separated analysis by sex are widely desirable[1,9].

Despite its aerobic-nature[11,12], HICT elicits high glycolytic activation, inferred by elevated lactate concentration[5] and glycogen depletion[12]. Additionally, the power output decrement[1], physiological demands[5,6,12] and muscle injury – traditionally analyzed by creatine kinase (CK) – are protocol dependent[13,14]. Considering well-trained male subjects (VO_{2max} = 58.3±5.9 mLO_2•kg^{-1}•min^{-1}), significantly higher CK values (from 300 U/L to more than 700 U/L) were observed 24 hours after 4 sets of six all-out sprints lasting 5 s, with 25 s between bouts and 5 min between sets in comparison to other longer sprints[14]. In highly trained female athletes, 15 x 30 m running resting 65 s generated an increase of creatine kinase (CK) higher than 400% (near to 300 U/L), with a peak in 24h post-HICT[13].

Both CK and Myoglobin (Mb) are released from the muscle and frequently are used as muscle damage markers[15]. CK blood concentration has higher values observed 24h to 72h after the exertion[13]. Myoglobin has faster transference to bloodstream after cell injury than CK and its peak is reached frequently earlier[16]. It is important to highlight that the level of fibers damage depends on physical fitness and exercise characteristics, as mode, intensity, and duration[17]. Specifically concerning the effects of physical fitness, seems that trained women show earlier increase in plasma glycerol concentrations, different profile in lactate concentration during and post-HICT, with no differences in HR and oxygen uptake[18], although CRF is related to HICT performance in cycle ergometer[19]. We expect a rise of lactate in both groups immediately after exercise, followed by decrease to basal level after one hour. For myoglobin concentration, our hypothesis is a rise one hour after exercise. We think that women with Low Physical Fitness (LPF) could have higher muscle damage, CK and myoglobin levels, than High Physical Fitness (HPF) after calisthenics HIIC, but it is necessary to measure and quantify its effects under muscular damage markers in the blood. Considering the lack of time for exercises in general population[1], the low complexity and adaptability of HICT[4], and the limited knowledge regarding the effects of CRF on physiological responses after high-intensity intermittent exercise with callisthenic protocol, the aim of this study was to measure and compare acute effects of a HICT session in young women with different levels of aerobic physical fitness.

METHODOLOGICAL PROCEDURES

Study design and participants

This research was an experimental trial and the sample size was calculated using the statistical program WinPepi11.65 considering the previous study that analyzed blood lactate concentration [Lac] in trained versus untrained women[18]. Assuming a [Lac] of 4.1±0.4 mmol.L^{-1} in the first and 5.2±0.8 mmol.L^{-1} in the second, an observed power of 0.8, and $p<0.05$ in a bi-tailed test, were needed 5 participants per group. The recruitment occurred on local Physical Education College. Were invited the healthy young women, from 18 to 35 years, free of chronic diseases with normal Body Mass Index (BMI), who were included[20]. Participants with some musculoskeletal limitation that disrupted exercise protocol performance and who had previously trained with this protocol were excluded. The final sample size had twelve young females, which signed an informed consent form and received previous guidelines for the intervention. The research was approved by Federal University of Pelotas Ethic committee under the number 004/2012.

The participants were submitted to three laboratories visits, firstly to an incremental test, the second to perform a single session of high-intensity intermittent exercise, before the session was performed a familiarization for all participants and the third to 24h-after blood collection. For CRF allocation, after the incremental test, women who achieve 41 mLO_2•kg^{-1}•min^{-1} or more were consider as High Physical Fitness (HPF, n = 5) and who achieve less than 41 mLO_2•kg^{-1}•min^{-1} were considered as Low Physical Fitness (LPF, n = 7) according percentile 80% of ACSM reference for female[21].

Procedures

The participants received previous guidelines for the whole intervention, such as: being fed, having slept for at least 8 hours, not having consumed alcoholic beverages, stimulants or smoked for at least 2 hours before blood collections and did not perform vigorous physical exercises for at least 72 hours before the start of the exercise protocol.

At the first visit, the participants performed a maximum incremental cycle ergometer test (Ergo-FIT™, model Ergo 167 Cycle, Pirmasens, Germany) to measure the maximum oxygen uptake (VO_{2max}) and maximal heart rate (HR_{max}). Previously, they warmed-up during 5 minutes at self-selected intensity and rhythm[22]. The protocol adopted for evaluation of VO_{2max} consisted of an incremental test, in which the individual was instructed to pedal at a pre-established cadence in the range of 55 to 65 rpm (revolutions per minute), increasing by 25 watts every two minutes of the test, until exhaustion, or inability to maintain cadence. For VO_{2max} measure was used an open circuit gas analyzer (VO2000, MedicalGraph) during the test. It was calibrated with Brezee software (Medgraphics™, Minnesota, USA), using high flow pneumotachometer, medium size neoprene™ masks, and records of mean values for every three breaths. The test was finished when the participant was not able to maintain the cadence and we considered the highest oxygen uptake in the incremental test as VO_{2max}.

To measure the HR_{max} a cardiac monitor (Polar RS800CX™, Kempele, Finland) was used and records in internal memory of the own device during collection were saved, later the obtained data were downloaded in computer and analyzed in specific software (Polar Pro Trainer 5.0). The maximum, minimum and mean of HR measurements were obtained. For all visits, temperature (22-24^{o} C) and humidity (40%) were controlled.

After 72 hours of relaying the incremental test, women attended the laboratory for the second visit at same daytime. Initially, they performed each movement of the protocol for familiarization, then performed the HICT protocol adapted from McRae et al[4]. Briefly, they performed 8 sets of 20 seconds at maximum intensity (all-out fashion) interspersed with 10 seconds of passive rest the following exercises: jumping jacks, squat and thrust using 2 kg dumbbells, mountain climber, and burpees. The exercises were performed twice in this order, completing 4min session[4].

The blood samples were collected before, immediately after, 15 minutes, 30 minutes and one hour after the exercise protocol. In each time, 5 ml venous blood was collected and centrifuged immediately at 3000 rpm for 10 minutes in order to separate the serum. Serum was stored at -20C for further measurements of lactate, and myoglobin. At the third visit, performed 24 hours after, the same blood collection was performed, which was used to creatine kinase values, lactate and myoglobin analysis.

Before, during and after HICT the HR measurement also was done with cardiac monitor (Polar RS800CX™, Kempele, Finland). Samples each 1s were recorded, and the peak value was considered. HR was performed prior to performing the incremental test and the exercise protocol. Considering the CK time-course, only pre and post 24h measures were performed.

The electrochemiluminescence immunoassay was used for myoglobin analyses, serum samples were analyzed in Elecsys 2010 using the Roche® Kit -12178214 122. Serum total CK activity values were measured by UV assay using a Kit Labtest® (CK-NAC liquiform- 117), following the manufacturer's recommendations for use, with SpectraMax® 190 Microplate Reader. Serum lactate concentration was measured on the YSI 2300 STAT PLUS™ instrument.

Heart rate was analyzed with Student's t-test, comparing the mean of variation (delta) between HR post and pre incremental test or protocol exercise. For analyze Lactate concentration and CK in exercise protocol, were conducted two-way analyses of variance (ANOVA, group x moment) with repeated measures. The Mauchly test was used to analyze the sphericity and the Greenhouse-Geisser correction was employed when appropriated.

The mean of variation of post and pre values of lactate concentration in incremental test and protocol exercise was compared with Student's t-test and *Mann-Whitney U test* was used for CK (difference between CK pre and post 24h of exercise protocol). For myoglobin, due to the non-normal distribution and violation of the sphericity pointed by Levene's test, Friedman's analysis of variance was used, since repeated measurements occurred over time. As the study has repeated measures, trends were analyzed by ANOVA with repeated measures considering group Low Physical Fitness (LPF) vs High Physical Fitness (HPF) and moment. Bonferroni *post-hoc* was used considering different moments.

RESULTS

The sample consisted of 12 women (7 LPF and 5 HPF), there were no differences between groups for age, height, body mass, BMI, resting heart rate (RHR) and maximal heart rate achieved in incremental test (HR_{max}). Regarding VO_{2max}, there were differences between groups, and data are shown in Table 1.

Table 1. Subjects characteristics, according to physical fitness level (N=12).

	Physical Fitness Level		
	Low (n=7)	High (n=5)	p-value
Age (years)	23.0± 4.7	23.6± 2.9	0.80
Height (cm)	164.0±6.9	161±7.8	0.50
Body mass (kg)	164.0±6.9	161±7.8	0.50
BMI (kg/m^{2})	63.3±7.9	58.5±4.9	0.50
RHR (bpm)	76.8± 9.3	80.2± 8.3	0.50
HR_{max} (bpm)	184.8±9.2	172.0±24.0	0.22
VO_{2max} ($mLO_2•kg^{-1}•min^{-1}$)	37.9 ± 1.5	48.9 ± 5.5	0.001

Note. BMI = Body Mass Index; RHR = Resting Heart Rate; HR_{max} = Maximal Heart Rate in incremental test; VO_{2max} = Maximum Oxygen Uptake.

No differences were found between the groups for delta HR (HR post – HR pre) during the incremental test (LPF = 108.0±13.1 bpm; HPF = 91.8±26.4 bpm; p = 0.18) and exercise protocol (LPF = 89.4±13.7 bpm; HPF = 97.4±20.3 bpm; p = 0.43). However, figure 1A shows that delta HR in the incremental test was different from the delta in exercise protocol only in LPF group (p = 0.005; p = 0.76 for HPF). Considering HR_{max}, the highest HR in the incremental test, in the end of HICT protocol the HR achieved 94.1±3.7% of HR_{max} for LPF and 104.5±20.3% for HPF (p= 0.03). When comparing the means of the delta lactate (Lactate post 0' - Lactate pre) between the incremental test and the exercise protocol (figure 1B), both groups showed significantly higher values after the exercise protocol. The mean delta lactate for LPF group was 10.7 ± 2.7 mmol•L^{-1} in the incremental test and 17.3 ± 4.7 mmol•L^{-1} in exercise protocol (p = 0.003). Also, for the HPF group, the exercise protocol obtained higher lactate means compared to the incremental test (respectively 16.9 ± 4.6 mmol•L^{-1} and 10.0 ± 2.3 mmol•L^{-1}; p = 0.03).

The analysis of the serum lactate concentration during exercise protocol (figure 2) showed differences between moments (F = 98.2, p <0.001, η^2_p = 0.91, power = 1), but not between groups (F = 0.66; P = 0.8, η^2_p = 0.07, power = 0.05), or interactions (F =0.97; p = 0.99; η^2_p = 0.01, power =0.07). The repeated measures ANOVA identified a significant cubic trend curve, with initial elevation followed by a decrease in subsequent moments (F = 165.1, p <0.001, η^2_p = 0.94, power = 1). The highest values were observed immediately after HICT, with 19.9 ± 4.5 mmoL^{-1} for LPF and 19.3± 5.2 mmol.L^{-1} for HPF.

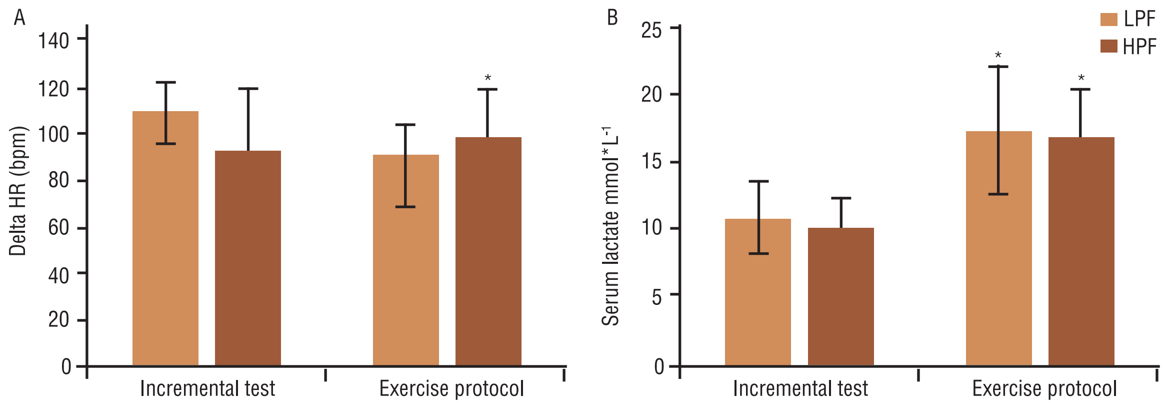

Figure 1. A) Delta HR (difference between HR post minus pre) in incremental test or exercise protocol), considering low (LPF) or high physical fitness (HPF). B) Comparison of delta lactate concentration between incremental test and exercise protocol, according fitness level. *Statistical difference comparing with LPF in incremental test. # Statistical difference comparing with HPF in incremental test.

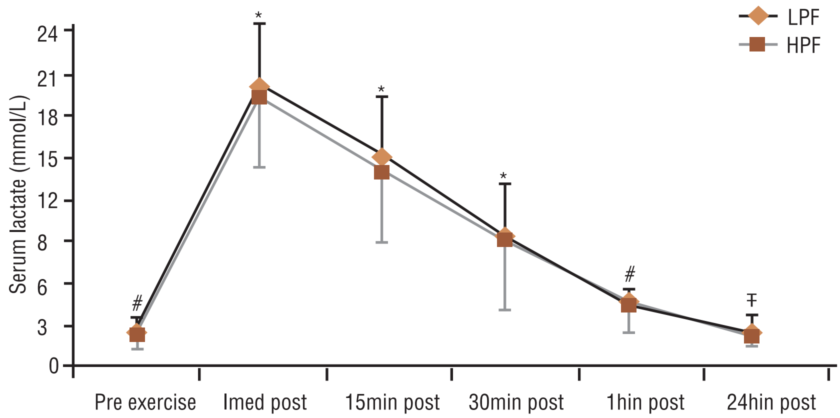

Figure 2. Lactate serum concentration during exercise protocol. LPF: Low Physical Fitness; HPF: High Physical Fitness. * = Different of all others moments; # = different of time 0'post, 15'post and 30'post; Ŧ = different of time 0'post, 15'post and 30'post and 1h post.

In the analysis of the serum myoglobin concentration (figure 4), differences between moments (F = 4.3; p = 0.3; η^2_p = 0.3; power = 0.63) were observed, with no differences between groups (F = 0.45; p = 0.5, η^2_p = 0.43, power = 0.09), or interactions (F =0.9; p = 0.3; η^2_p = 0.8; power =0.17). A cubic tendency was identified by repeated measures ANOVA, with elevation followed by a decrease in subsequent moment (F = 5.0; p= 0.04; η^2_p = 0.33; power = 0.52). The highest values were observed at 1h after the exercise protocol, with 50.0± 30.2 ng/mL for LPF and 36.9± 9.25 ng/mL for HPF.

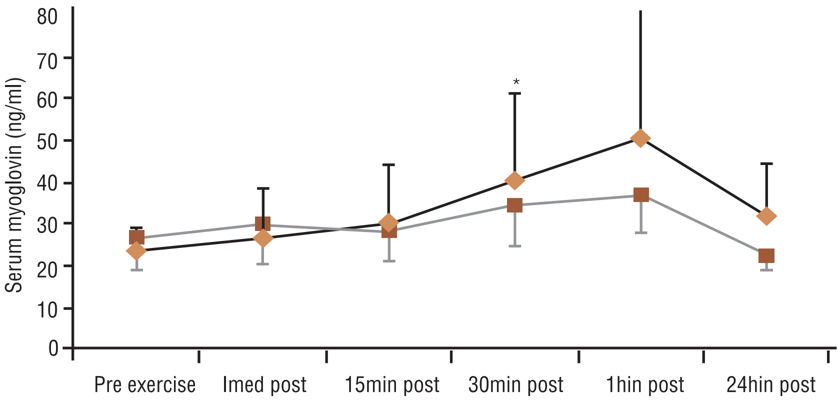

Figure 3. Myoglobin serum concentration during exercise protocol. LPF (Low Physical Fitness) and HPF (High Physical Fitness). * = Different of 15 min post in LPF.

The median (25-75% percentile) of total CK pre exercise protocol for LPF was 48.5 (16.5-52.3) U•L^{-1} and for HPF 79.9 (22.0-97.9) U•L^{-1} and post 24hs was 95.0 (55.10-131,0) U•L^{-1} for LPF and 76.3 (21.3-116.2) U•L^{-1} for HPF, figure 4A. We compare the delta of total CK (difference between CK pre and post 24h of exercise protocol) with *Mann-Whitney U test* that indicated, on average, that the serum CK level in LPF (Mean Rank= 8.29 U•L^{-1}, n=7) was significantly higher than HPF group (Mean Rank = 4.0 U•L^{-1}, n=5), U=5.0, z = -2.03, p=0.042, figure 4B.

DISCUSSION

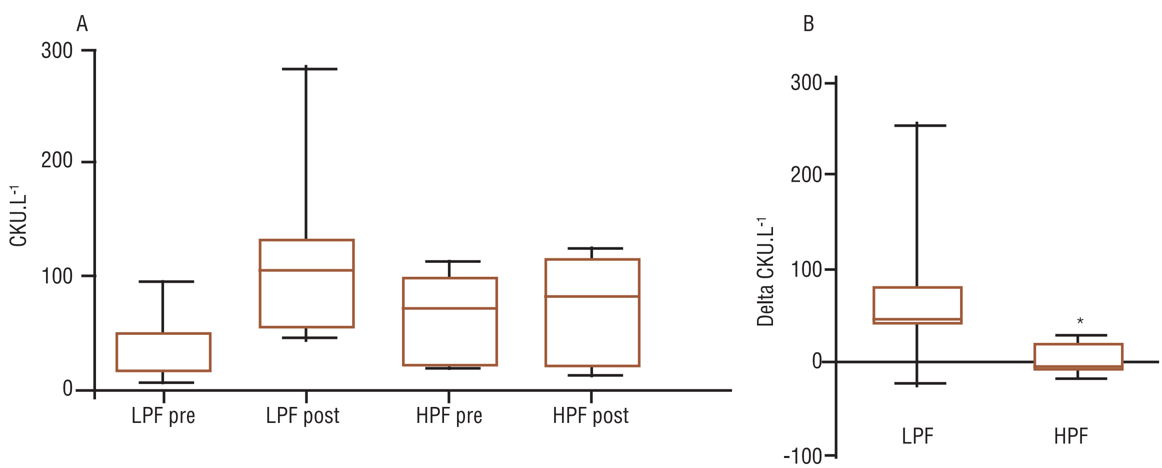

Figure 4. Panel A) Median, minimum and maximum of CK according physical fitness level, pre and post HICT. Panel B) Delta CK with median, minimum and maximum. LPF (Low Physical Fitness) and HPF (High Physical Fitness); * = different of LPF.

The present study aimed to compare the acute physiological effects of an HICT session in women with different levels of physical fitness. The analyzed groups were similar for the anthropometric variables, RHR, RPE, and HR_{max}, while VO_{2max} were higher for HPF group. We did not find differences between LPF and HPF subjects for the variation of the HR, serum lactate and myoglobin after HICT. However, the main finding was that the serum CK level in LPF was significantly higher than HPF group.

Exercise-induced muscle damage occurs following unaccustomed and/or very vigorous exercise and the increased levels of CK and myoglobin rank among those signs of exercise-induced muscle damage[23]. The high-intensity character of HIIE can potentially lead to muscle fiber impairment which can be manifested by increases in of CK and myoglobin concentration in plasma[24,25]. To our knowledge, this is the first study that analyzed the acute effects of a calisthenics HICT on muscle injury markers such as myoglobin and CK. For myoglobin concentrations, we did not find differences between the fitness levels, it was only observed that the concentration increases 30 min after HICT, achieve the highest values 1 h after and returns to the baseline values after 24 hours. This result is similar to the finding of Joo[16], that analyzed the myoglobin concentration in ten healthy active men underwent a HIIE protocol (eight 3-min bouts at a running velocity corresponding to 90% of maximal oxygen uptake interspersed with 3-min active recovery periods). Cipryan[25] also presented similar results to ours regarding concentrations and curve of myoglobin, with its peak in one hour after short HIIE completed by endurance and sprint athletes. Another study, involving professional female basketball players, the myoglobin curve was similar to Joo[16] after a match, and the highest levels were around 100-200 ng•mL^{-}1[27]. In the present study, the highest levels of myoglobin were lower, probably for the low volume of exercise. In our study, CK concentrations showed an increase 24 h post exercise, and the LPF group had higher increase than HPF. We believe that exercise-induced muscle damage occurs more intensely in LPF probably for lower muscular fitness caused by low physical fitness of this group[26,27]. As a limitation, we did not measure CK and myoglobin points after 24hs, but previous studies shown that concentrations returning to pre-exercise levels after 48 h[13,28].

Regarding the heart rate, there were no differences between groups, and applied protocol can be considered intense and reached 94-104% HR_{max}. Previously, Gist et al[5] involving trained subjects (VO_{2peak} = 54.1 ± 5.4 mLO_2•kg^{-1}•min^{-1}) in all-out calisthenics exercises composed by 4 sets of 30s of burpee with a rest of 4 minutes between bouts, found values of 84% of HR_{max}. Additionally, with active women, McRae et al[4] using a similar exercise protocol similar applied here (8 sets, 20s:10s), found averages of 92 ± 4% HR_{max}. These differences can be due to the nature of applied HICT because Gist et al[5] used an effort: pause relationship similar to Sprint Interval Training, while we and McRae et al[4] applied short-time HICT[28]. In the HICT protocol, the LPF group reached a lower variation (delta) of the HR compared to the incremental test. Perhaps this finding can be explained by the fact that LPF responds less intensely to the all-out stimulus, probably because they have a lower neuromuscular response, thus achieving a lower HR[2].

The serum lactate concentration behaved similarly between the two groups after calisthenic HICT. This protocol was able to rapidly raise the serum lactate of both groups to values above 19 mmol•L^{-1}, which leads to a high anaerobic contribution[5]. When comparing calisthenic HICT with the incremental test, we found significant differences with higher serum levels for HICT. Smilios et al[28] studied responses of eleven moderately trained man, who executed on three separate sessions, 4x4-min runs at 90% of maximal aerobic velocity (MAV) with 2-min, 3-min, and 4-min of active recovery. They found lactate levels around 7-8 mmol.L^{-1} for 2 min recovery and that the use of a short compared to longer recovery interval during the execution of HICT does not substantially affect the exercise time performed at near maximal rate of oxygen consumption, but results in greater cardiovascular stress, as evident by higher heart rates, and activates more the anaerobic glycolysis[28]. Our protocol had only 10s of rest between the bouts, which caused intense effects on HR and anaerobic glycolysis, evidenced by high HR and measured lactate levels. It is important to point that this kind of protocol, lasting only 4 min, when applied four times per week during one month, was able to improve maximal oxygen uptake and muscular endurance in a similar sample of women[4]. Recently, other investigation found that this same protocol, made thrice during 16 weeks (total volume accumulated = 384 min), was sufficient to improve VO_{2peak}, intensity associated to VO_{2peak}, as well as body fat in healthy males similar to 30 min/day of moderate-continuous aerobic running (total volume = 1,440 min)[29]. Considering the low complexity and short time during this workout, new studies should investigate the chronic effects of whole body high intensity interval training on body composition of female population.

The present study has interesting points, as the comparison of physiological responses between incremental test and HICT, and the analysis considering the cardiorespiratory fitness. Additionally, other useful aspect was the inclusion of McRae´s recommendation[4], mixing different calisthenic exercises in the same workout. Finally, we must to highlight the absence of RPE after HICT as a limitation. However, it is important to point that similar sample previously evaluated performing similar protocol reported values near from 17 arbitrary units in 6-20 scale[4]. Furthermore, our sample reached a blood lactate concentration near from 20 mmol.L^{-1}, and HRmax of 94.1±3.7% for LPF and 104.5±20.3% for HPF, confirming the high intensity nature of this HICT.

CONCLUSION

In summary, we found that HICT protocol produced similar effects under HRmax, serum lactate and myoglobin in the woman with high and low physical fitness, but LPF presented more muscle damage marked for an increase in serum CK concentrations. A high-intensity calisthenic protocol can achieve elevated values of HR_{max} and produce higher lactate concentrations than an incremental test.

COMPLIANCE WITH ETHICAL STANDARDS

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Ethical approval
Ethical approval was obtained from the local Human Research Ethics Committee – Federal University of Pelotas, and the protocol was written in accordance with the standards set by the Declaration of Helsinki.

Conflict of interest statement
The authors have no conflict of interests to declare.

Author Contributions
Conceived and designed the experiments: FBDV and ABAN Performed the experiments: ABAN, RBO, LDC and AF. Analyzed the data: RBO, ABAN and FBDV. Contributed reagents/materials/analysis tools: FBDV and ABAN. Wrote the paper: ABAN, RBO and FBDV.

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