Original research

The effects of cold water immersion with different dosages (duration and temperature variations) on heart rate variability post-exercise recovery: A randomized controlled trial

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ABSTRACT

Objectives: The aim of the present study was to investigate the effects of cold water immersion during post-exercise recovery, with different durations and temperatures, on heart rate variability indices.
Design: Hundred participants performed a protocol of jumps and a Wingate test, and immediately afterwards were immersed in cold water, according to the characteristics of each group (CG: control; G1: 5′ at $9 \pm 1\,°C$; G2: 5′ at $14 \pm 1\,°C$; G3: 15′ at $9 \pm 1\,°C$; G4: 15′ at $14 \pm 1\,°C$).
Methods: Analyses were performed at baseline, during the CWI recuperative technique (TRec) and 20, 30, 40, 50 and 60 min post-exercise. The average HRV indices of all RR-intervals in each analysis period (MeanRR), standard deviation of normal RR-intervals (SDNN), square root of the mean of the sum of the squares of differences between adjacent RR-intervals (RMSSD), spectral components of very low frequency (VLF), low frequency (LF) and high frequency (HF), scatter of points perpendicular to the line of identity of the Poincaré Plot (SD1) and scatter points along the line of identity (SD2) were assessed.
Results: Mean RR, VLF and LF presented an anticipated return to baseline values at all the intervention groups, but the same was observed for SDNN and SD2 only in the immersion for 15 min at $14\,°C$ group (G4). In addition, G4 presented higher values when compared to CG.
Conclusions: These findings demonstrate that if the purpose of the recovery process is restoration of cardiac autonomic modulation, the technique is recommended, specifically for 15 min at $14\,°C$.

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1. Introduction

Post-exercise recovery has been extensively investigated in the scientific scenario,[22] including techniques to accelerate the recovery process after exercise. Furthermore, studies investigating the effects of cold water immersion (CWI), which consists of body segments immersed in water at temperatures below $15\,°C$, have been encouraging.[7]

Analysis of heart rate variability (HRV) indices as indicators of autonomic nervous system (ANS) activity,[2,5,8] which performs a significant role in maintaining homeostasis, has been studied.[5,9] During exercise, sympathetic activity is increased, while during the

post-exercise period there is a concomitant progressive parasympathetic reactivation with a sympathetic withdrawal.[2] The balance between sympathetic and parasympathetic activity reflects autonomic recovery and restoration of cardiovascular homeostasis which is an important component of overall recovery.[20]

Considering that exercise acts as a stressor agent, which causes metabolic and autonomic changes,[4,12] it is important to understand the possible post-exercise effects of CWI on ANS. Studies[2,5,8] suggest that CWI results in better ANS activity post-exercise, anticipating the recovery of HRV indices. This can potentially improve the adaptation to training loads or at least reduce stress-related symptoms[23] leading to an imbalance stress-recovery[15] and can confer a great cardioprotective effect.[20]

Bastos et al.[5] observed higher cardiac autonomic modulation on post-exercise recovery after CWI application performed at $11 \pm 2\,°C$ for 6 min, with higher parasympathetic indices compared to other

recuperative techniques. The authors propose time and temperature application of this technique based on previous experience. This design is one of the various strategies commonly used. Pastre et al.,[22] in a systematic review, evidenced inconsistencies in studies using recovery techniques and attribute this to the variety of applications methods as immersion of members or total body, temperature and time of application used in CWI and the models of physical stress. The lack of the scientific literature refers to the absence of the association between dosages of intervention and the results. Therefore, it seems pertinent to investigate variables such as duration and temperature during CWI application.

The aim of this study was to investigate the effects of CWI on HRV indices as a post-exercise recovery technique, using different durations and temperatures. It was hypothesized that CWI, regardless of the application dosage, would be able to promote better autonomic modulation, thus anticipating the recovery moment. In addition it is believed that the immersion dosage is a decisive factor for optimizing the recovery of HRV indices, and CWI used for long durations and lower temperatures will present the positive effects already mentioned in previous studies.

2. Methods

Hundred young healthy male participants (aged 21.73 ± 2.92 years, height 1.75 ± 0.06 m, weight 74.22 ± 10.15 kg, BMI 24.15 ± 2.99 kg m^{-2}) were recruited, with a minimum classification requirement of being physically active according to the International Physical Activity Questionnaire (IPAQ). To be included, participants could not be smokers, alcoholics, using drugs that influenced cardiac autonomic activity or have cardiovascular, metabolic or endocrine diseases, as reported in a previous interview.[5]

To determine the sample size a priori knowledge was used, based on Bastos et al.[5] findings for SDNN, which represents the activity of both ANS branches, indicating the overall behavior of HRV.[29] A sample size of 18 participants per group was stipulated by a test of hypothesis (two-tail), with 5% level of significance and 80% power. The participants were randomized (*Microsoft Excel* software) and allocated into five groups, one control (CG—passive recovery), and four intervention (G1, G2, G3 and G4).

Following the analysis, there was some data loss due to the time series presenting less than 95% sinus beats. There was no loss in CG ($n = 20$), one loss in G1 ($n = 19$) and G2 ($n = 19$), two losses in G3 ($n = 18$) and no losses in G4 ($n = 20$).

All procedures were approved by the Research Ethics Committee of the Univ. Estadual Paulista (Protocol 51903/2012) and were performed in a laboratory with an average temperature of 22.0 ± 2.34 °C and relative humidity between 40% and 60%, between 5 and 10pm to avoid circadian variation.[5] Participants were advised not to perform vigorous exercise for either 24 h before and during the study, refrain from drinks and stimulants containing caffeine and any other supplements that might promote recovery. However, it was not carried out based on any monitoring beyond questioning the participants.

In order to capture the RR-intervals parameters, for further analysis of HRV, a chest belt was placed over the precordium region and a heart rate receiver (Polar RS800CX, Polar Electro-Oy, Finland) on the wrist.[12] During capture, the participants remained at rest in a sitting position breathing spontaneously.

The exercise protocol consisted of a jumping protocol and a short-duration maximal cycling test. The jumping protocol was composed of 10 sets of 10 maximal vertical jumps with a 1 min break between sets. Prior to starting the exercise, a maximal vertical jump was performed with a chalk mark made by the fingertips at the highest point of the jump. This mark was subsequently used as

a target height that the participant attempted to maintain for each jump. On landing, participants were instructed to position their hands on their hips and adopt a knee joint angle of approximately 90° to avoid compensation.[28]

Immediately after, participants performed the short-duration maximal cycling test, consisting of a Wingate Test, on a cyclo-ergometer (Biotec 2100-Cefise, Brazil), which triggers cardiac autonomic derangement.[18] The participants performed a 5 min warm-up, consisting of pedaling with a load of 1.0 kg at 60–90 rpm and sprints in the 2nd and 4th min, followed by maximum pedaling for 30 s with a load of 0.075 kp kg^{-1} of participant's body mass.[18] These stress protocols were considered to cause exhaustion in both musculoskeletal and metabolic systems.

Immediately after the stress protocol, an interval of 1 min was allowed for the transition to the recovery intervention and participants from the intervention groups were immersed up to the height of anterior–superior iliac spine, whilst remaining seated. The duration and water temperature of CWI were established in accordance with the characteristics of each group. G1 remained immersed for 5 min at 9 ± 1 °C; G2: 5 min at 14 ± 1 °C; G3:15 min at 9 ± 1 °C and G4: 15 min at 14 ± 1 °C. The immersion duration was controlled by a timer and the temperature was controlled by putting ice and stirring the water, and constantly evaluated by a thermometer with ± 0.3 °C accuracy. CG performed passive recovery seated for 15 min. These durations and temperatures of immersion were established in accordance with the most commonly used strategies in the literature[7,30] and those that allowed a more comprehensive assessment of CWI effects. Participants were not informed either which group they belonged to regarding intervention duration and temperature or which intervention was considered therapeutic.[11] After the recovery intervention, participants remained seated. The total recovery time was 75 min including the time the participant remained immersed.

HRV analysis was performed from RR-intervals series at a sampling rate of 1000 Hz analyzed using the software Polar Pro Trainer 5.0. Digital filtering was supplemented by manual filtering in order to remove the artifacts, ectopic and premature beats, and only series with more than 95% sinus beats were included.[12]

The extraction of each cardiac period (RR-interval) was analyzed using Kubios HRV Analysis Software 2.0 for Windows software (The Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland), and a fixed number of 256 consecutive RR-intervals[12] was considered at baseline (20 min with the participants at rest, of which the final 256 RR-intervals were selected), during the recuperative technique (TRec) (for intervention groups, the final 256 RR-intervals for 5 min for G1 and G2, for 15 min for G3 and G4, and for 5 or 15 min, for subsequent comparison with the appropriate intervention groups) and at 20, 30, 40, 50 and 60 min after exercise (128 RR-intervals before and after each time).

For analysis in the time domain (TD), Mean RR (average of all RR-intervals in each analysis period), RMSSD (square root of the average squared differences between adjacent normal RR-intervals) and SDNN (standard deviation of normal RR-intervals) indices were analyzed[26,29] expressed in milliseconds.

In the frequency domain (FD) the spectral components of very low frequency (VLF) (0.00–0.04 Hz); low frequency (LF) (0.04–0.15 Hz) and high frequency (HF) (0.15–0.4 Hz)[29] were utilized, expressed in ms^2, calculated using Fast Fourier Transform.[12] VLF assessed from short-term recordings, although tedious measure, was considered here for analysis.

For the quantitative analysis of the Poincaré plot (PP), SD1 (scatter of points perpendicular to the line of identity) and SD2 (scatter points along the line of identity) were utilized.[16]

To analyze the sample characteristics considering anthropometric and age data the descriptive statistical method was used and

the results were presented as mean values and standard deviations. Data normality was assessed (Kolmogorov–Smirnov test), considered normal and therefore the parametric analysis was used. Analysis of one-way variance (ANOVA) was performed, followed by the Tukey's test.

Sphericity of the data of HRV was tested by Mauchly's test. In case of violation of the sphericity assumption, the Greenhouse-Geisser corrections were used. Data were analyzed using Repeated Measures Analysis of Variance which provide information of time and group (Bonferroni's test was performed when required). The comparison between the moments was compared to baseline values for all groups (Baseline vs TRec, 20, 30, 40, 50 and 60). The significance level was set at $p < 0.05$.

3. Results

Anthropometric characteristics and age are presented in Table 1. No significant differences were observed between groups in any variables.

For the TD (Fig. 1), FD (Fig. 2) and PP (Fig. 3) a better recovery was observed, favoring the CWI interventions ($p < 0.05$). For MeanRR (Fig. 1a), VLF (Fig. 2a) and LF (Fig. 2b), there was an earlier recovery between 10 and 20 min for the CWI groups compared to CG. For SDNN (Fig. 1b) and SD2 (Fig. 3b), G4 presented an earlier recovery (10 min) compared to the other groups. For RMSSD (Fig. 1c), HF (Fig. 2c) and SD1 (Fig. 3a) there was no difference in recovery time for any groups.

In the groups comparison, G4 presented statistically greater ($p < 0.05$) results compared to CG in most indices evaluated between 40 and 60 min post-exercise, with the exception of VLF, which was statistically higher in G1 ($p < 0.05$) at 60 min post-exercise.

In the relationship between time and intervention, there was a significant interaction for MeanRR ($p < 0.001$), SDNN ($p = 0.001$), RMSSD ($p = 0.003$), VLF ($p = 0.025$), SD1 ($p = 0.014$) and SD2 ($p = 0.045$).

4. Discussion

When considering the return to baseline values, 60 min was sufficient in any situation, regardless of the use or not of CWI for recovery of HRV indices and in overall variability there was a return to baseline values at between 10 and 20 min when CWI was applied. The principal findings were related to the CWI for 15 min at 14 °C.

The analysis performed during TRec demonstrated similar behavior for all groups. CWI performed at any duration or temperature did not influence the behavior of the ANS, when compared to passive recovery, and all indices presented a significant decrease when compared to baseline, reflected by the autonomic derangement caused by the exercise protocol.

Studies comparing the effects of CWI with other recuperative techniques have found better results during the TRec.[3,8] The discrepancy between the findings could be related to the stress model used. This study used a supramaximal exercise model,[13,18] which may have triggered an intense autonomic derangement in

an attempt to meet the metabolic needs.[17] However, a gradual increase in HRV indices presented 20 min into the recuperative period demonstrated an attempt to return to baseline values, and 60 min time was sufficient for physically active subjects to receive a new stimulus from a cardiac autonomic point of view.

The application of CWI, principally performed for 15 min at 14 °C, presented the greatest effect on the sympathetic branch of ANS, so as to reduce the modulation in the subsequent moments of immersion. Such behavior was evidenced in the indices that represent the action of both branches, which clearly demonstrated better recovery in all groups that performed CWI for the MeanRR, VLF and LF indices and only in G4 for the SDNN and SD2 indices, which demonstrated an anticipated time of return to baseline values.

However, there were no differences in the recovery times between groups for the indices that represent parasympathetic modulation. Although, when comparing the groups, better results were found for the immersion performed for 15 min at 14 °C compared to CG in the closing moments of recovery. In the present study we have not investigated parasympathetic reactivation from CWI action as suggested by Buchheit et al.[8] Regardless of occurrence of this phenomenon, there is no significant increase in parasympathetic tone in the first analysis intervals. There is, however, a significant rebound after 50 min post-exercise on RMSSD, HF and SD1, referring to the parasympathetic modulation for groups at 15 min of immersion. One hypothesis to explain these results may be related to the duration of dosing that reflects later its impact on the system.

Some studies[2,7,8] have indicated the occurrence of a cascade of responses promoted by the hemodynamic system after CWI, including flow redirection from the periphery to the central regions of the chest, increased venous return and cardiac output, contributing to higher cardiac autonomic modulation,[2,8,25] which can be reflected, as in the findings of the present study, by anticipated values to baseline in the global indices.

The hypotheses for the described outcomes may be related to two main factors. Although an immediate response was not observed during the TRec, there was a response in baroreceptors stimulus and a temperature reduction through thermoregulation in the subsequent moments. Baroreceptors are sensitive to changes in blood volume. Thus, the consequent increase in blood volume in the chest region due to hydrostatic pressure possibly induced the reduction in the sympathetic modulation. In case of temperature reduction, the thermoreceptors located in the skin, subcutaneous tissue and blood vessels are stimulated, increasing flow redirection to protect the vital organs.[3,19]

These findings agree in part with Buchheit et al.,[8] Al Haddad et al.,[2] Stanley et al.[25] and Parouty et al.[21] who also noted faster HRV recovery using CWI, but over a shorter duration. Although the time of immersion was less than the 15 min, the temperature of 14 °C was used in the above studies where CWI also promoted better results when compared to control condition. Al Haddad et al.[2] emphasized that CWI performed at 14 °C can stimulate cold receptors and promote a cumulative effect of hemodynamic responses through the thermoregulatory system, favoring better autonomic control.

Table 1
Mean and standard deviation of anthropometric variables and age.

Variables	Groups					p value
	CG (n = 20)	G1 (n = 19)	G2 (n = 19)	G3 (n = 18)	G4 (n = 20)	
Age (years)	21.48 ± 2.64	21.56 ± 3.42	22.19 ± 3.15	20.73 ± 2.49	22.00 ± 2.85	0.557
Weight (kg)	76.60 ± 13.41	71.32 ± 9.84	73.86 ± 9.46	71.62 ± 6.69	75.50 ± 11.17	0.845
Height (m)	1.74 ± 0.06	1.75 ± 0.07	1.75 ± 0.06	1.77 ± 0.07	1.77 ± 0.07	0.363
BMI (kg/m²)	25.19 ± 4.08	24.08 ± 3.60	24.23 ± 2.61	23.04 ± 2.35	24.09 ± 2.57	0.460

CG: control group; **G1:** 5′ at 9 ± 1 °C; **G2:** 5′ at 14 ± 1 °C; **G3:** 15′ at 9 ± 1 °C; **G4:** 15′ at 14 ± 1 °C.
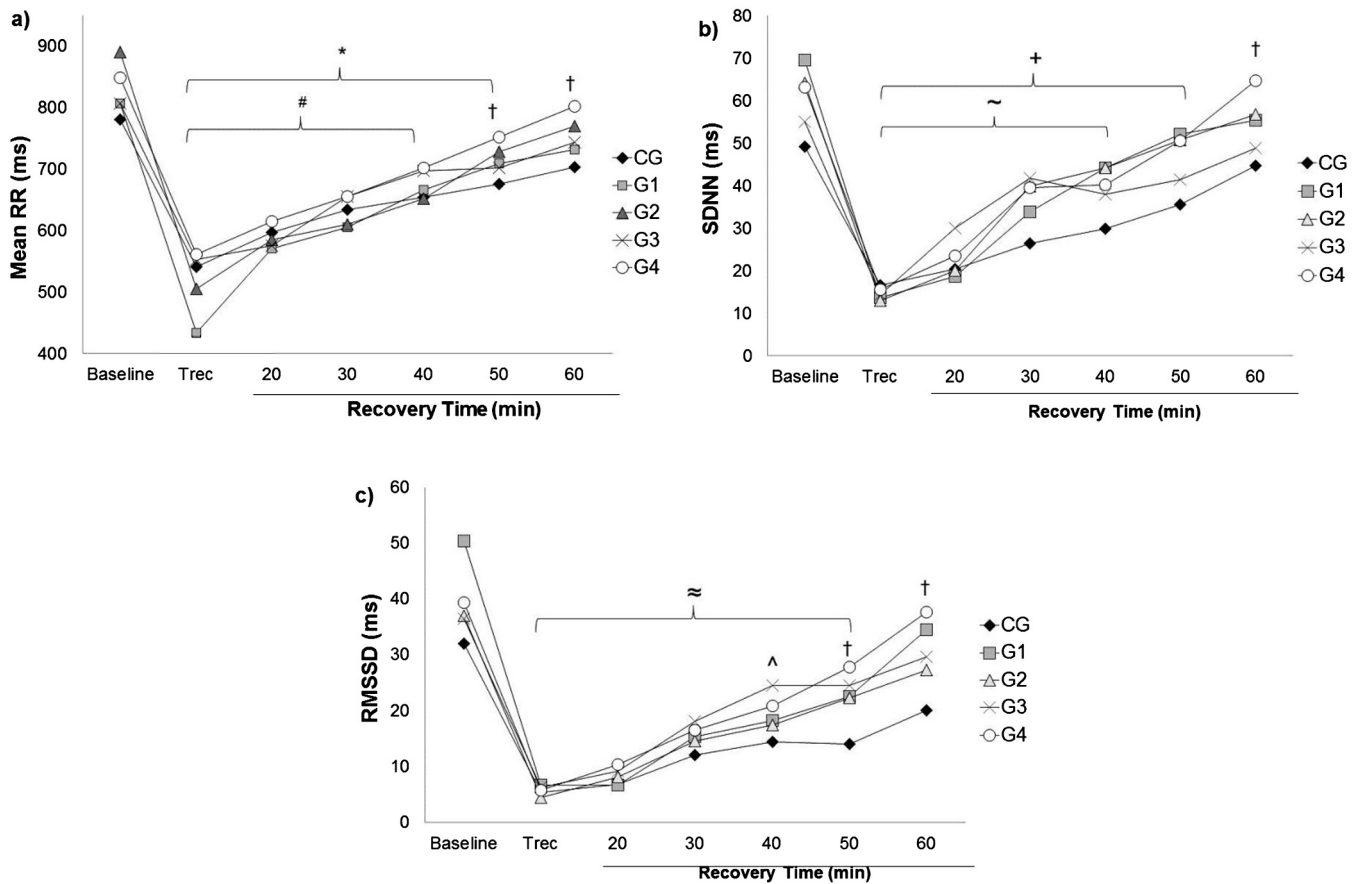
Fig. 1. Median values of the indices Mean RR (a), SDNN (b) and RMSSD (c) in ms. (*): Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for CG. (#) Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for G1, G2, G3 and G4. (+): Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for CG, G1, G2 and G3. ($\sim$): Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for G4. ($\approx$): Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for all groups. (†): Statistically significant difference ($p < 0.05$) in the comparison between the G4 and CG groups. ($\hat{}$): Statistically significant difference ($p < 0.05$) in the comparison between G3 and G4 in relation to CG. CG: control group; G1: 5′ at $9 \pm 1\,^\circ C$; G2: 5′ at $14 \pm 1\,^\circ C$; G3: 15′ at $9 \pm 1\,^\circ C$; G4: 15′at $14 \pm 1\,^\circ C$.

The present study indicates that temperature and duration of immersion can be determining factors to optimize ANS response. Speculations about possible stress caused by immediate contact with CWI could justify the smaller effect on the recovery of G1 and G3 compared to the other groups.[14] However, in VLF better recovery was observed in G1 compared to the control condition. It is known that VLF suffers, among other systems, influence of the thermoregulatory system,[26,27] which due to lower temperatures may have promoted greater stimulation of thermoreceptors due to the sharp decrease in temperature after immersion[2,8,25] even if observed for a short duration (5 min).

From a physiological point of view, the thermoregulation process is related to autonomic activity. Although this process is well established, detailed descriptions of the vagal afferents, its exact location and morphological type are uncertain.[6] Schlader et al.[24] corroborates these statements but describes the direct relationship between sensation/thermal comfort and behavior of the thermoregulatory system. In this case, the skin temperature has an important role considering the stimulation of its receptors with consequent thermal response. However, just as nerve conduction velocity[1], the answers of ANS after small temperature variations are unknown.

Based on the statements described, it can be inferred about the possibility of different responses on changes in technical application doses. The findings of this study reinforce the need for investigations aimed at a better understanding of the thermoregulation process and its relationship with the ANS.

Another aspect that also should be discussed concerns the physiological deleterious effects of CWI. Datta and Tiptom[10] describe the mechanism of "cold-shock" as probable disturbing homeostasis, reflecting in cardiovascular risks. This condition, however, did not happen in the autonomic scope even in temperatures of 9 °C. Thus, all groups tested in this trial responded within safe limits at least on the basis of HRV.

Therefore, if the objective is cardiac autonomic modulation restoration, it can be inferred that the best application strategy of CWI is 15 min at 14 °C. Such strategies can promote an anticipated recovery in HRV indices, and are easily reproducible due to the lower thermal discomfort that, maintaining temperatures at around 14 °C can promote compared to lower temperatures often used in sports and scientific fields.

One limitation is worth mentioning. Although all participants were classified as physically active, according to IPAQ, it was impossible to ensure the specificity of the activities that the participants performed, and performance capacity was also not controlled, which constitutes a limitation, as the level and type of physical activity influence ANS.[17] Specifically for this study, no statistical differences in baseline and during the application of CWI ensure that at least the same response profile for the primary variable HRV. To ensure more complete participants descriptions to the exercise
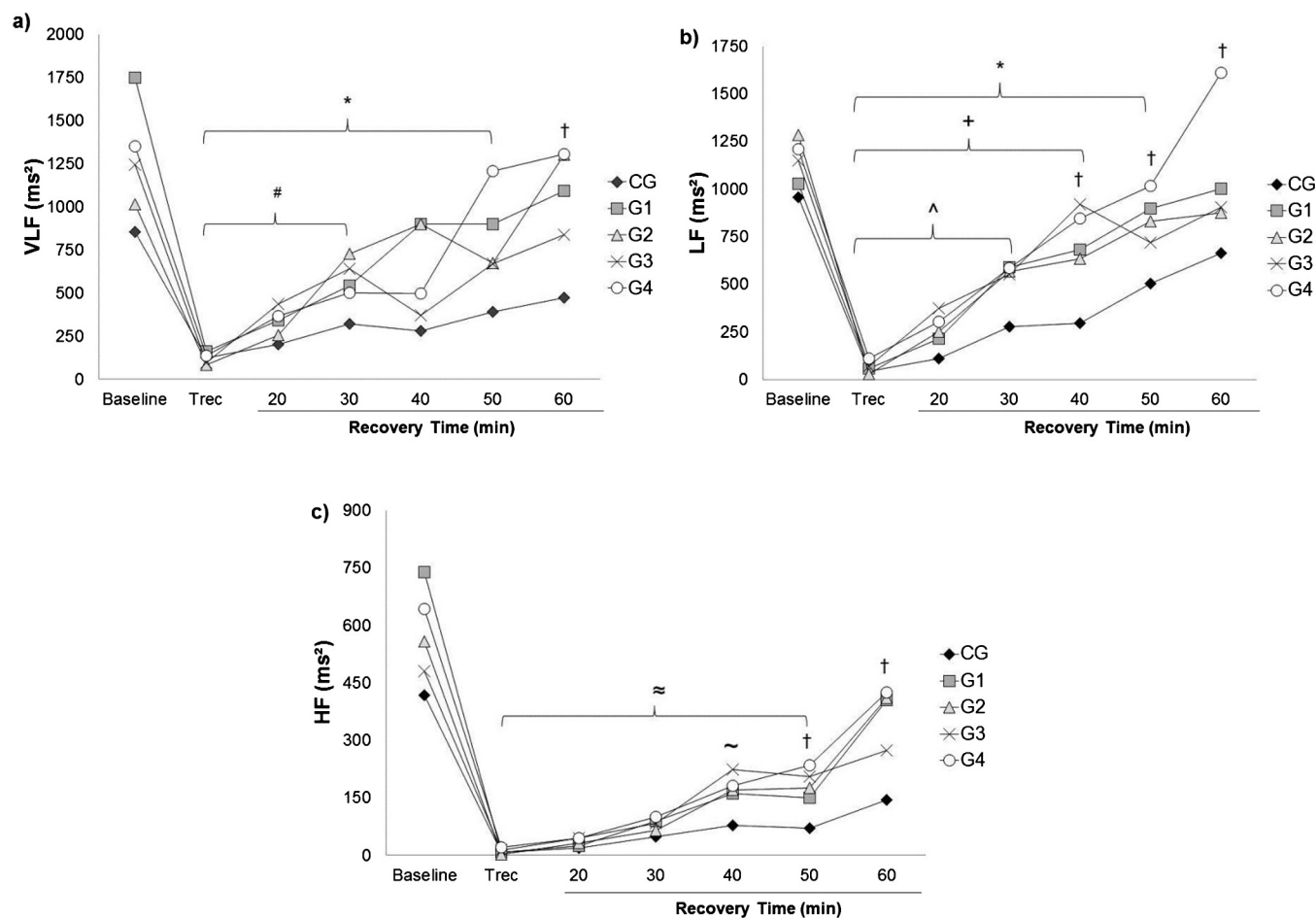
Fig. 2. Median values of VLF indices (a), LF (b) and HF (c) in ms^2. (*): Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for CG. (#): Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for G1, G2, G3 and G4. (+): Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for G1 and G2. ($\hat{}$): Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for G3 and G4. ($\approx$) Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for all groups. (†): Statistically significant difference ($p < 0.05$) in the comparison between the G4 and CG groups. ($\sim$) Statistically significant difference ($p < 0.05$) in the comparison between the G3 and CG groups. CG: control group; G1: 5′ at 9 ± 1 °C; G2: 5′ at 14 ± 1 °C; G3: 15′ at 9 ± 1 °C; G4: 15′at 14 ± 1 °C.
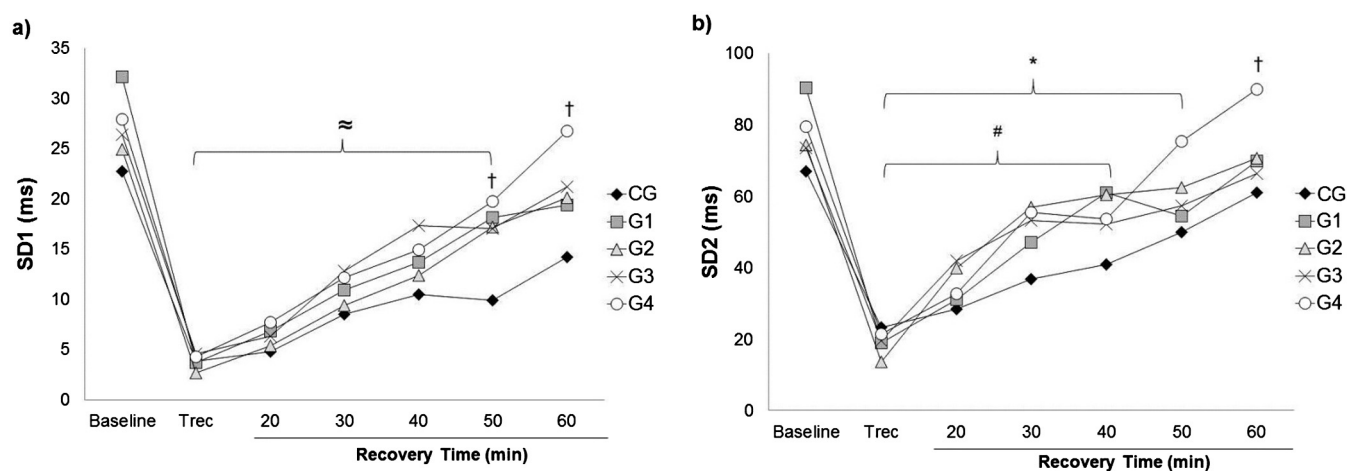

Fig. 3. Median values of SD1 (a) and SD2 (b) in ms. ($\approx$): Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for all groups. (*): Statistically significant differences in the comparison between the moments for CG, G1, G2 and G3. (#) Statistically significant differences ($p < 0.05$) in the comparison between the moments when compared to baseline values for G4. (†): Statistically significant difference ($p < 0.05$) between G4 and CG in the comparison between groups. CG: control group; G1: 5′ at 9 ± 1 °C; G2: 5′ at 14 ± 1 °C; G3: 15′ at 9 ± 1 °C; G4: 15′at 14 ± 1 °C.

profile, it's suggested, in future studies, the control of performance during the exercise protocol.

Thus, future studies are necessary addressing the duration and temperature application of CWI on other variables, such as clinical, biochemical and functional, in addition to considering the stress models and population investigated. Extrapolation of findings on CWI without considering such factors could promote bias in interpretation by readers who use science as means of investigating performance on a practical level in the field.

5. Conclusion

This study demonstrated the positive effects of CWI on the restoration of cardiac autonomic modulation, implying that CWI applied for 15 min at 14 °C is the most effective dosage for this purpose. It is worth noting that the period of 60 min post-exercise was sufficient to restore the values of the indices to pre-exercise conditions in physically active subjects, regardless of the technique applied.

6. Practical implications

The results of this study provide important information for clinical practice in sports medicine and exercise, considering the post-exercise recovery, suggesting that:

(1) Sixty minutes is enough for physically active people to recover their autonomic functions after high-intensity exercise.
(2) CWI can be indicated to accelerate the cardiac autonomic recovery.
(3) CWI best strategy considering temperature and duration is 15 min at 14 °C.

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